Innovations in Companion Animal Nutrition

Washington, DC, USA

September 15-18, 2005
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The WALTHAM International Nutritional Sciences Symposium

Co-sponsored by the University of California, Davis, and Cornell University

September 15-18, 2005, Washington, DC, USA

INNOVATIONS IN COMPANION ANIMAL NUTRITION

WEDNESDAY, 14 September

18:00 -20:00  WELCOME RECEPTION  - Registration, cocktails and hors d’oeuvres

THURSDAY, 15 September

07:00 - 08:30  Breakfast

08:30 - 09:00  Welcome and Introductions: Helen Munday, Head of Research, WALTHAM

Dean Smith, Cornell University

Dean Osburn, University of California, Davis

Morning session:

NUTRITIONAL EVOLUTION: WILD TO DOMESTICATION – EXPLORING BEHAVIORAL AND NUTRITIONAL ADAPTATIONS

09:00 - 09:50  FORAGING AND FEEDING ECOLOGY OF WOLVES: LESSONS FROM YELLOWSTONE

Mr. Dan Stahler, Project Biologist, Yellowstone Gray Wolf Restoration Project, Yellowstone National Park

09:50 - 10:30  EVOLUTIONARY BASIS FOR THE FEEDING BEHAVIOR OF DOGS AND CATS

Dr. John Bradshaw, Senior Lecturer in Companion Animal Welfare, Director of the Anthrozoology Institute, University of Bristol

10:30 - 10:50  Cats lack sweet taste receptor

X. Li, W. Li, H. Wang, Legrand-Defretin, V., Beauchamp, G., Brand, J.

10:50 - 11:20  Coffee break

11:20 - 12:00  NUTRITIONAL AND METABOLIC ADAPTATIONS OF DOGS AND CATS

Dr. Andrea Fascetti, Associate Professor of Nutrition, Service Chief for the Nutrition Support Service in the Veterinary Medical Teaching Hospital, University of California, Davis

12:00 - 12:20  In vivo taurine biosynthesis is greater in small dogs than large dogs

Ko, K., Backus, R.C., Berg, J.R., Lame, M.W., Rogers, Q.R.

12:20 - 12:30  Discussion and Close Of Session

12:30 - 13:30  Lunch

Afternoon session:

OBESITY AND WEIGHT MANAGEMENT: CURRENT STATE OF PROGRESS IN HUMAN AND ANIMAL OBESITY RESEARCH

13:30 - 14:30  ADIPOSE TISSUE AND ADIPOKINES - ENERGY REGULATION FROM THE HUMAN PERSPECTIVE

Prof. Paul Trayhurn, Chair of Nutritional Biology at the University of Liverpool, Director of the Neuroendocrine and Obesity Biology Unit and the Liverpool Centre for Nutritional Genomics
14:30-15:30 **OBESITY IN CATS AND DOGS: HOW BIG IS THE PROBLEM?**  
Dr. Alex German, Head of the Internal Medicine/Gastroenterology Services, Lecturer in Small Animal Medicine, Director of the Royal Canin Weight Management Clinic, University of Liverpool, Merseyside, UK

15:30 - 15:50 Coffee break

15:50 - 16:10 Human-animal-relationship of owners of normal and overweight cats  
Kienzle, E., Bergler R., Ziegler, D.

16:10 - 16:30 Risk factors for obesity and owner’s perception of dog’s body condition in France  
Colliard, L., Ancel, J., Benet, J.J., Paragon, B.M., Blanchard, G.

16:30 - 16:50 Post-prandial plasma lipolytic activities and lipid and carbohydrate responses of dogs fed diacylglycerol-rich meals containing high and low glycemic index carbohydrates  
Porterpan, B., Nagaoka, D., Bigley, K., Umeda, T., Otsuji, K., Bauer, J.E.

16:50 - 17:00 Discussion and Close Of Session

17:30 - 19:30 **POSTER SESSION** - Drinks and hors d’oeuvres

Dinner on your own in DC - lists of local eateries provided

**FRIDAY, 16 September**

07:00 - 08:30 Breakfast

Morning session: **OBESITY: CHALLENGES IN MEASURING ENERGY EXPENDITURE IN COMPANION ANIMALS**

08:30 - 09:00 **CHALLENGES – A RESEARCHER’S PERSPECTIVE**  
Dr. Jon Ramsey, Associate Professor, Department of Molecular Biosciences, University of California, Davis, School of Veterinary Medicine

09:00 - 09:30 **CHALLENGES – A CLINICIAN’S PERSPECTIVE**  
Dr. Richard Hill, WALTHAM Associate Professor in Small Animal Internal Medicine and Clinical Nutrition, University of Florida, Member National Research Council

09:30 - 09:50 Retrospective study on the energy requirements of adult colony cats  
Edtstadtler-Pietsch, G., Rudnick, R., Kienzle, E.

09:50 - 10:10 Standardised positioning is essential for precise determination of body composition using dual-energy x-ray absorptionmetry  
Raffan, E., Holden, S.L., Cullingham, F., Hackett, R., Rawlings, J.M., German, A.J.

10:10 - 10:30 Energy expenditure and body composition of dogs from four different breeds  
Nguyen, P., Leray, V., Hernot, D., Dumon, H., Martin, L., Siliart, B., Sergheraert, R., Biourge, V.

10:30 - 11:00 Coffee break

11:00 - 11:20 The effect of feeding frequency on growth of goldfish  
Priestley S.M., Stevenson, A.E.

11:20 - 11:40 No effect of conjugated linoleic acid or Garcinia cambogia on body composition, and energy expenditure in non-obese cats  
Leray, V., Dumon, H., Martin, L., Siliart, B., Sergheraert, R., Biourge, V., Nguyen, P.

11:40 - 12:00 Diabetes in dogs; feeding, exercise and weight as possible predisposing factors  
Hedhammar, A., Sallander, M., Klinkenberg, H.

12:00 - 12:15 Discussion and Close of Session
SUNDAY, 18 September

07:00 - 08:30 Breakfast

Special session: BEYOND THE CANINE GENOME, see page 87
The WALTHAM International Nutritional Sciences Symposium

Innovations in Companion Animal Nutrition

PLENARY SESSIONS
**Speakers at the Plenary Sessions**

**Daniel Stahler**

BS, MS  
Project Biologist, Yellowstone Gray Wolf Restoration Project, Yellowstone National Park

Daniel Stahler has been the project biologist for the National Park Service, Yellowstone Gray Wolf Restoration Program in Yellowstone National Park since 2002. From 1997-2000, Daniel worked as a field technician and graduate student with the Yellowstone Gray Wolf Restoration Program and earned a Master of Science in Ecology, Evolution, and Behavior from the University of Vermont studying the predator-scavenger relationship of wolves and ravens. From 2000-2002, Daniel worked as a biologist for the Wildlife Conservation Society, Yellowstone Cougar Project studying cougar predation, population dynamics, and interactions with other carnivores in Yellowstone. He first studied wolves in Minnesota with Dr. L. David Mech in 1996. He received his Bachelor's degree in Conservation Biology from Middlebury College in 1996. Daniel Stahler, along with project leader Dr. Douglas Smith, is responsible for coordinating all aspects of the Yellowstone Wolf Project's research program which focuses on wolf population dynamics, predation and feeding patterns, social dynamics, and ecological and behavioral interactions with ungulate, scavenger, and other carnivore species. Stahler has produced publications and contributed many hours of lecture time on these topics and has devoted the last ten years of his life to the study of carnivores and their role in ecosystems. Daniel lives in Gardiner, Montana.

**J.W.S. Bradshaw**

BA, PhD  
Senior Lecturer in Companion Animal Welfare, Director of the Anthrozoology Institute, University of Bristol


**Andrea J. Fascetti**

VMD, PhD, Dipl. ACVIM, ACVN  
Associate Professor of Nutrition, Service Chief for the Nutrition Support Service in the Veterinary Medical Teaching Hospital, University of California, Davis

Andrea Fascetti graduated from the University of Pennsylvania School of Veterinary Medicine. Following graduation she completed an internship and medicine residency at The Animal Medical Center in New York City. She then became an adjunct instructor in physiological chemistry in the Department of Molecular Biosciences in the School of Veterinary Medicine at the University of California, Davis. She also started a graduate degree in nutrition and a clinical nutrition residency. Three years later Andrea became a Hill’s Fellow in Clinical Nutrition. Her doctoral research investigated copper nutriture in the queen and its influences on reproduction and cuproenzyme activities. She is a diplomate of the American College of Veterinary Internal Medicine and the American College of Veterinary Nutrition. Andrea is currently an Associate Professor of Nutrition at the University of California, Davis. She is also the service chief for the Nutrition Support Service in the Veterinary Medical Teaching Hospital of the University of California, Davis. Her current research interests are trace mineral metabolism in dogs and cats, improvement of pet foods and taurine bioavailability and metabolism in the dog.

**Paul Trayhurn**

DSc DPhil FRSE  
Chair of Nutritional Biology at the University of Liverpool, Director of the Neuroendocrine and Obesity Biology Unit and the Liverpool Centre for Nutritional Genomics

Professor Paul Trayhurn (DPhil DSc, Oxford) holds the Chair of Nutritional Biology at the University of Liverpool and is Director of the Neuroendocrine & Obesity Biology Unit and the Liverpool Centre for Nutritional Genomics. His previous appointments
include a NATO European Research Fellowship in Strasbourg and Professorships at the Universities of Alberta (Heritage Scholar), Aberdeen and Oslo; in 1997 he was elected a Fellow of the Royal Society of Edinburgh. He has published extensively on energy balance and adipose tissue biology, and is currently Editor-in-Chief of the British Journal of Nutrition and chairs the Awards Committee of the International Association for the Study of Obesity.

Alex German  BVSc (Hons), PhD, CertSAM, DipECVIM-CA, MRCVS
Head of the Internal Medicine/Gastroenterology Services, Lecturer in Small Animal Medicine, Director of the Royal Canin Weight Management Clinic, University of Liverpool

Alex German qualified, with honours, from the University of Bristol (UK) in 1994. He then worked for two years in mixed practice before returning to Bristol to undertake a PhD in gastrointestinal immunology, and then a residency in small animal internal medicine. He was awarded the Royal College of Veterinary Surgeons certificate in small animal medicine in August 2001. In October 2002, he moved to The University of Liverpool (UK), and is currently the Royal Canin Lecturer in Small Animal Medicine, and head of the Internal Medicine/Gastroenterology referral service. He is also Head of the Royal Canin Weight Management Clinic, which was established at Liverpool University in 2004. In September 2004 he became a Diplomat of the European College of Veterinary Internal Medicine. His current research interests include obesity biology, metabolomics and small animal gastroenterology.

Jon Ramsey  PhD, BSc
Associate Professor, Department of Molecular Biosciences, University of California, Davis, School of Veterinary Medicine

Dr. Ramsey is Associate Professor in the Department of Molecular Biosciences at the UC Davis School of Veterinary Medicine. He received his B.S. degree in Agricultural Biochemistry from Iowa State University and his PhD in Animal Sciences from Colorado State University. He completed post-doctoral training at the Wisconsin National Primate Research Center and University of Wisconsin School of Medicine. His research interests focus on energy metabolism as it relates to aging and obesity.

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 England in 1980 and spent 5 years in the small animal hospital a large (18 veterinarian) mixed practice north of London. He completed a residency in small animal internal medicine at the University of Pennsylvania and a PhD at the University of Florida. He is currently the WALTHAM Associate Professor of Clinical Nutrition at the University of Florida College of Veterinary Medicine and a diplomate of the American College of Veterinary Internal Medicine. His clinical responsibilities include teaching clinical small animal medicine and nutrition, and running the small animal nutrition service at the University of Florida. He is conducting research in gastroenterology, primarily colonic physiology, and nutrition. The nutrition research has mostly been to establish the nutritional requirements of racing greyhounds, particularly of antioxidants, using a training track at the University of Florida. He is currently a member of the Subcommittee on Dog and Cat Nutrition of the National Research Council Committee on Animal Nutrition with responsibility to be primary author on a Chapter on Physical Activity and Environment and its Effects on Nutrient Requirements.
FORAGING AND FEEDING ECOLOGY OF WOLVES: LESSONS FROM YELLOWSTONE

Stahler, D.R.1, Project Biologist BS MS, Smith, D.W.1, Project Leader and Guernsey, D.S.1

Yellowstone Gray Wolf Restoration Project, Yellowstone National Park National Park Service

Since the 1920s, when the last of the wolves (Canis lupus) in Yellowstone National Park (YNP) were killed, these predators were absent as an ecological force in the Greater Yellowstone Ecosystem (GYE), one of the last great wildernes in the contiguous United States. This ecosystem, however, had evolved in the presence of large carnivores, as did most of the Northern Hemisphere. Wolf-like canids have been around for about 40 million years, making them the most ancient of all carnivores. The first modern wolf, Canis lupus, appeared during the Pleistocene, about one million years ago. Despite this rich evolutionary history and ecological importance, European settlers to North America eliminated wolves from most of the United States by the early 1900s. Since that time, most of our ecosystems have had to function with the top level of the food chain lopped off. Recent studies have indicated that these large carnivores played highly important roles in the original structuring of ecosystems, acting as a top-down influence.

The effort to restore them took decades, involved many, and culminated with the reintroduction of wolves to Park in 1995 and 1996. By 2005, wolves were fully restored to the GYE and ten years of intensive research has begun to unravel some of the mysteries associated with the ecological ramifications of large predators and their impacts to one of the last great intact ecosystems in the continental United States. In YNP, research on wolves has focused largely on their role as a predator. Being a highly adaptive and behaviorally flexible carnivore, wolves have evolved to hunt prey ranging in size from 1kg-1,000kg prey, although they are most commonly categorized as cursorial hunters of large ungulates.

In Yellowstone, elk (Cervus elephus) have been the primary prey of wolves since the reintroduction. With eight Yellowstone-area ungulate species present, elk consist of 90% of the documented prey taken by wolves. The proportion of elk calves, cows, and bulls killed on the northern range on YNP by wolves is 38%, 36%, and 26%, respectively (10% unknown). There is strong selection for calves, which comprised 18% of the available elk between 1995 and 2000. It also represents strong selection against cows, as they comprised roughly 60% of the available prey for the same time period. Wolves killed bulls in proportion to their availability. Young-of-the-year, no matter what the species, are always a large part of any carnivore’s diet as they are the easier to kill compared to the older, larger, and more experienced ones. In addition, Yellowstone wolves also select for the older, senescent ones; the average age of wolf-killed adult cow elk is 14.

Wolves do not attack prey at random because the risk of injury and death is too high. Therefore, as selective foragers, wolves search for vulnerable prey and must be risk-aversive. In early winter, prey are in good condition and more difficult to kill, resulting in an estimated kill rate per individual wolf of 1.4 elk over 30 days. In contrast, as winter progresses, prey get weaker and easier to kill resulting in an estimated kill rate of 2.2 elk per wolf during a 30-day period. This was the pattern for the first five years (1995-2000) of wolf restoration, but kill rate has decreased significantly since 2000, possibly due to changing patterns of prey vulnerability, social competition, and effects of drought. The pattern of strong selectivity for elk also holds true for the more formidable bison (Bison bison), another prominent YNP ungulate. Bison comprise only 4% of the wolves’ diet each year, likely due to the greater risk involved in killing them.

As most of our information on wolf kills comes from winter data, kill rates and prey selection are less known in summer. The combination of divided hunting units, smaller prey, more widely distributed prey on their larger summer ranges, and greater activity at night, makes understanding summer predation difficult. However, preliminary evidence indicates that wolves may eat as much as 30% less in the summer. Summer diets are more diverse as and include smaller prey species such as rodents and ungulates otherwise absent in the winter. Analysis of summer scats in YNP show that mule deer (Odocoileus hemionus) can make up to 25% of some wolves’ summer diet.
In addition, plant matter consistently shows up in scats, and wolves have been observed consuming plant material, especially grass (Graminae). These food types may be an added source of vitamins or may aid in ridding intestinal parasites.

Much of the foraging and feeding patterns seen in wolves are influenced by their degree of sociality. Wolves are territorial mammals that establish firm boundaries that they defend against other wolves. These territories are defended by a family of wolves, a pack, which is the basic structure of wolf society. There are two kinds of pack structures in YNP: simple and complex. Simple packs are made up of a breeding pair with pups; a complex pack is a breeding pair with several generations of offspring or unrelated pack individuals. In Yellowstone, feeding largely on mid-sized elk, the average pack size over the first 10 years has been 11 wolves, but the range of pack sizes was 2–37. Complex packs have more experienced individuals, which aids in hunting success and food provisioning to pups in the summer. Multiple litters are not uncommon in YNP due to high prey densities and frequency of complex packs.

With respect to the food requirements for wild wolves, a minimum daily energy requirement of 3.25/kg/wolf/day (5 x daily basal metabolic rate) has been estimated for a 35kg wolf. For wolves in YNP (mean weight 45kg), estimated mean food consumption rates based on early and later winter kill rates is 5.7kg/wolf/day and 10.4kg/wolf/day, respectively. This however does not include biomass lost to scavengers or inedible rumen or bone, as consumption rates are based on live weights of respective age and sex class of ungulates killed. The loss to scavengers is significant in YNP. It is largely dependent upon pack size, with large packs losing less, while small packs may lose half or more of their kill, as they can not consume it all in one sitting.

Immediately after killing an elk, wolves open the body cavity using their canines and incisors, removing and consuming the larger internal organs. Organs such as the heart, lungs, liver, spleen, and kidneys are high in essential nutrients required for maintenance, growth, and reproduction. Next consumed are the large muscle masses of each leg. Wolves consume up to 10 kg of meat during initial feeding bouts, and then rest in close proximity to the carcass for several hours before feeding again. Some hair is ingested along with meat, which may aid in faster passage through the intestinal tract. With organs and major muscle mass consumed, wolves then pick the remaining tissue off ribs, leg bones, and hide. Using their carnassials to shear remaining meat and crush hard to chew materials such as bone, tendon, cartilage, and hide, wolves continue to obtain nutritional derivatives from virtually all parts of ungulate carcasses. Rumen contents, large unbreakable bones, and some hide are often the only things remaining when wolves and associated scavengers are done. In YNP, where prey and scavenger density is high, carcasses rarely last more than 48 hours on the landscape. The degree of utilization depends on variables such as prey vulnerability and pack size.

Wolves are adapted to a feast-or-famine diet and can overcome periods without fresh carcasses through food caching, reduced activity, and scavenging old prey carcasses. A lone wolf in YNP was followed for 10 weeks without any evidence of making a kill, but survived by scavenging over 10 carcasses ranging from 2 weeks to 4 months old, most of which consisted of only bone and hide.

Previous wild and captive studies suggests a high degree of competition and feeding hierarchy at carcasses with dominant male and female wolves feeding first and squabbles breaking out. In Yellowstone, feeding opportunity seems only restricted to the availability of a position to obtain meat from a carcass, and we see little social tension between age and sex classes at carcasses. This is likely due to the fact that the ecosystem still offers a high prey density and little food stress at this time. We predict foraging and feeding behavior to change with decreasing prey vulnerability, increasing wolf density, and food shortages.
THE EVOLUTIONARY BASIS FOR THE FEEDING BEHAVIOUR OF DOGS AND CATS
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Both the domestic dog and the domestic cat are members of the family Carnivora, which also includes hyaenas, bears, seals and weasels. Within this family, there is considerable variation between species as to the extent to which they require a carnivorous diet. Some, such as the bears, are omnivores, while others, notably the cats and the seals, are obligate carnivores. The dogs, genus Canis, fall towards the middle of this spectrum. The diet of the modern-day wolf Canis lupus consists largely of prey and carrion, but this may be partly a reflection of the habitats in which it is tolerated by man. The more widely distributed coyote and jackal can subsist on fruit and other plant materials, and can therefore be classed as omnivores. Other adaptations to feeding behaviour within Canis tend to confirm this. Their jaws and dentition, while specialised for prey-killing, are hinged in a way that allows prolonged chewing of plant material. Their taste-buds not only respond to amino-acids and other compounds characteristic of flesh, but also to sugars and “fruity” compounds such as methyl maltol and furaneol, found in plant materials. One aspect of the feeding behaviour of the domestic dog which is most analogous to that of the wolf is the tendency to take large meals. Wolves can consume up to 16% of their bodyweight at a single meal, presumably an adaptation to competition with other members of the pack at the carcass of a large animal, such as a caribou. Some breeds of dog retain this habit, and become obese if offered food ad libitum, but others do not, suggesting effects of domestication on satiation and meal-pattern; the latter is also apparent in the division of breeds into those which will take meals during the night, and those that will not (Bradshaw & Thorne, 1992).

The importance of learning as a factor determining food selection in dogs has not been studied in depth, and there may again be substantial differences between breeds. To the extent that it is possible to generalise, it appears that dogs may be less neophobic than some other species, including the cat and the rat, and also less ready to learn aversions to foods that make them ill. Rather than being adaptive, this may be an example of relaxed selection due to domestication, since for millennia much of the food eaten by dogs has been selected by man. The role of genetics and early experience in influencing differences in food preferences have also not been studied in depth in the dog, but similar mechanisms may apply to those operating in the cat, as discussed below.

The domestic cat is descended from the African wildcat Felis lybica, a more specialised predator than the wolf. Moreover, it is a solitary, not a co-operative hunter, and its jaw and dentition are adapted to take small vertebrate prey, especially small mammals. Obligate carnivores, like the members of the cat family, essentially decide what they wish to eat by choosing what they are going to hunt, since energy expended on hunting inedible prey would be energy wasted. It is therefore perhaps surprising that cats have quite narrow requirements in terms of the flavour of the foods provided by man, and also are able to modify their preferences on the basis of experience.

The domestic cat, along with all the members of the cat family, are “trapped” into obligate carnivory by the loss, several million years ago, of several of the standard mammalian metabolic capabilities, in their common ancestor. Thereafter, only a diet that consisted largely of flesh could be sufficiently complete nutritionally to permit successful breeding (Zoran 2002). Scavenging food from human habitation is therefore a much less productive option for the cat compared to the dog, and this may explain why to this day, even with the wide availability of nutritionally complete foods, most domestic cats still retain both the ability and the inclination to hunt (Bradshaw et al., 1999). Their innate food preferences are also strongly focussed on meat and fish, and their taste system, which is unresponsive to both sugars and salt, but highly responsive to amino-acids, nucleotides and other compounds characteristic of flesh, is well adapted to this end.

The ability to adapt preferences in response to experience may therefore have been refined by the
process of domestication, even in the cat. As adults, cats can form rapid aversions to toxic or severely nutritionally deficient foods (Bradshaw et al., 1996). Nutrient-specific hungers, for example for key vitamins such as thiamine, or amino-acids such as taurine, have been postulated but have received little study. A further strategy towards nutritional balance is anti-apostatic selection, an enhanced preference for food items that are known to be safe but have not formed part of the diet in the past few days (Church et al., 1996); in pet cats this is sometimes referred to as the novelty effect, or ‘neophilia’. Cats can also be neophobic towards foods they have not encountered before, even those, such as raw offals, which they might be expected to have built-in preferences for (Bradshaw et al., 2000).

Studies of the role of early experience in determining adult food preferences have produced conflicting results. Initially it was thought that if kittens were fed a single food, particularly if it was also eaten by the queen, it would come to be preferred over all other potential foods; only kittens fed a variety of food types went on to develop broad tastes (Stasiak 2002). However, it now appears that this only occurs under extreme experimental conditions, such as hand-rearing, and that kittens weaned naturally on to a single food show a powerful novelty effect when they are offered choices between palatable foods a few weeks later. In practice, most cat owners wean kittens on to a mixture of foods, so in the domestic situation the novelty effect may be less apparent. In a study of ten domestic litters, preferences for various foods (raw kidney, fish, and a commercial meat-based tray product) a few weeks after weaning showed some similarities within litters, and in seven cases out of the ten, the preferences of the kittens were similar to those of their mothers. However by four months of age most of these similarities had disappeared, although patterns of change in individual preferences were food-specific. The role of experience therefore appears to be cumulative, with no obvious ‘sensitive period, and in pet cats must be a product of an interaction between the consumer – the pet - and the provider – the owner.

References
Both dogs and cats are members of the biological order Carnivora. Scientific observation and research support that differences in their metabolism and nutritional requirements exist. The differences in nutritional requirements likely correlate with the evolution of these two species. Nutritionally and metabolically, dogs and other members of Canidea are generally considered omnivores, whereas cats and other members of the family Felidea are regarded as carnivores. However, there exist nutritional and metabolic examples that are not consistent with the view that the cat is a strict carnivore and the dog is simply an omnivore.

The only member of the family Felidea whose nutritional requirements have been studied extensively is the domestic cat (Felis catus). Scientific research has shown that cats have obligatory requirements for nutrients that are not essential for many other mammals. The high protein requirement of cats is due to their high requirement for nitrogen. This appears to be because cats have a limited ability to control the activity of their aminotransferases and urea cycle enzymes. Conversely, cats are able to control the activity of enzymes in the first irreversible step of essential amino acid degradation to some extent; explaining why they do not have a high requirement for essential amino acids. The lack of down-regulatory control over aminotransferases and urea cycle enzymes renders cats immediately able to metabolize and use amino acids for gluconeogenesis and as an energy source. Additional benefits of this ability are realized in times of starvation; carnivores are better able to immediately maintain blood glucose concentrations compared to omnivorous species.

There are five other nutrients, considered essential in feline diets that are not recognized as essential in most other species due to the low activities of enzymes in their synthetic pathways. Two of these nutrients are the amino acids arginine and taurine. The low activities of ornithine aminotransferase and pyrroline-5-carboxylate result in the minimal production of citrulline in the gastrointestinal tract. As a result, the cat is completely dependent upon dietary arginine to meet its needs for this amino acid. The endogenous synthesis of taurine is limited by the low activities of cysteine dioxygenase and cysteinesulphinic acid decarboxylase. The low activity of these enzymes in the synthetic pathway, coupled with the low affinity of N acyltransferase for glycine for bile acid synthesis, results in the depletion of body taurine stores. The remaining three nutrients are niacin and vitamins A and D. The cat has a dietary requirement for niacin and vitamin D because of the high activity of the enzymes picolinic carboxylase and 7-dehydrocholesterol-δ7-reductase respectively, that result in the degradation of precursors for their synthesis. Vitamin A must be supplied pre-formed in the diet because cats lack the enzyme β-carotene 15, 15’-dioxygenase, needed to cleave β-carotene.

Consistent with their classification as obligate carnivores, cats have a reduced number of carbohydrate metabolizing enzymes compared to omnivores. Cats lack glucokinase in their livers. However in contrast to certain others carnivores, and not at all consistent with the cat being a strict carnivore, cats can efficiently digest uncooked starch and do so better than dogs.

Nutritionally and metabolically many consider the dog an omnivore. However there are nutritional and metabolic characteristics that dogs share with the cat. One veterinary nutritionist (D. Kronfeld) has suggested the term adaptive carnivore when referring to the dog. In contrast to the cat, and similar to other omnivores, the dog has the ability to make taurine from the sulfur amino acid precursors methionine and cysteine, as well as vitamin A from β-carotene. However, unlike many other omnivores and more like the cat, the dog conjugates bile acids only with taurine and can not make vitamin D, an animal product. Like cats, dogs require a source of dietary arginine to maintain nitrogen balance in adults and puppies. The dog’s requirement for arginine...
is less than that of the cat but greater than the rat, positioning it between carnivores and other omnivores nutritionally.

Over time, evolution rendered some of the metabolic pathways and enzymes present in omnivores redundant in the cat. These pressures likely resulted in changes in biochemical pathways and nutritional requirements more suited to the cat’s metabolism. Although nutritional requirements of the cat differ from that of the dog, scientific findings and observations are not fully consistent with the cat being a strict carnivore and the dog a simple omnivore.
ADIPOSE TISSUE AND ADIPOKINES – ENERGY REGULATION FROM THE HUMAN PERSPECTIVE
Trayhurn, P., DSc, DPhil, FRSE
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There has been a rapid rise in human obesity over the past two decades. In the UK, for example, we have moved from a position in the early 1980s where just 6% of men and 8% of women were clinically obese (BMI > 30) to the current situation where 24% of adults are now obese. Obesity is, of course, a consequence of changes in lifestyle (diet and activity levels), but its rise has provided considerable impetus for the investigation of the fundamental mechanisms involved in the control of energy balance. In the genomic (or nutrigenomic) era, this has included a search for those genes, or gene polymorphisms, which are linked to body weight. At a mechanistic level, important developments have occurred recently in the identification of novel factors involved in the control of appetite, such as CART (cocaine- and amphetamine-regulated transcript) and the endogenous cannabinoids, while the discovery of new uncoupling proteins (UCP2, UCP3...) was thought initially to provide a locus for adaptive thermogenesis in organs outwith brown fat.

A radical change in perspective on energy balance has come from new understanding of the function of white adipose tissue. The traditional view of this tissue as simply a highly efficient system for fuel storage has been supplanted by the recognition that it is an important endocrine organ which communicates extensively both with the brain (hypothalamus) and with peripheral organs through the release of leptin and other hormones. Leptin, which is secreted principally from adipocytes, acts as a critical signal in energy balance (and other physiological processes such as reproduction), and its discovery was the pivotal event in changing perspectives on white fat. Leptin is in practise but one of a rapidly expanding list of protein factors and signals released by white adipose tissue, now generally referred to as adipokines. These adipokines include the hormones adiponectin and resistin (implicated in insulin resistance), classical cytokines (such as IL-6, TNFα), as well as proteins involved in vascular hemostasis (e.g. plasminogen activator inhibitor-1), lipid metabolism (e.g. cholesteryl ester transfer protein), the complement system (e.g. adipsin) and angiogenesis (e.g. vascular endothelial growth factor). Of particular note is the range of cytokines and other inflammation-related proteins secreted by white fat, particularly as tissue mass rises. Indeed, obesity is now recognised as being characterised by chronic mild inflammation with increased circulating levels of several inflammatory markers (e.g. C-reactive protein, haptoglobin, IL-6). Recently identified adipokines which are linked to inflammation include the neurotrophin, nerve growth factor.

Overall, the adipokines provide an extensive network of communication between adipose tissue and other organs, and some, such as plasminogen activator inhibitor-1 and pro-inflammatory cytokines, are directly implicated in the pathologies associated with obesity, particularly the metabolic syndrome. White adipose tissue has now moved centre stage in obesity research as a consequence of its major role in metabolic homeostasis and the growing direct association between the tissue and the diseases associated with being obese.
OBESITY IN CATS AND DOGS: HOW BIG IS THE PROBLEM?
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Obesity is defined as an accumulation of excessive amounts of adipose tissue in the body, and has been defined as a greater than 15% increase above the ideal body weight for the individual. In humans, strict definitions of the degree of adiposity exist, and these are based upon epidemiological data. At the current time, such epidemiological data are not available for companion animals. There is a need for further work, which can associate body composition with mortality and morbidity data.

The importance of determining body composition is demonstrated in humans; with the most common measure of composition, body mass index (BMI; weight [kg] divided by height^2 [m]), Caucasian individuals can either be defined as underweight (BMI<18), normal (BMI=18-25), overweight (BMI=25-30), obese (BMI=31-40) and morbidly obese (BMI>40). A multitude of epidemiological data exists for humans, demonstrating increases in morbidity and mortality if BMI differs from normal. The data from companion animals is more limited, although some studies do suggest and increase in morbidity in sick patients with poorer body condition. Evidence in cats also suggests increase health risks in obese individuals.

Measurement of obesity in companion animals
All measures of adiposity involve defining body composition, which is the ‘relative amounts of the various biological components of the body’. The main conceptual division of importance is between fat mass (FM; the triglyceride component in adipose tissue) and lean body mass (LBM). The various techniques differ in applicability to research, referral veterinary practice and first-opinion practice. Whatever method is used, investigators should be aware of both the precision and accuracy of the chosen method. Ideally, a test which is both accurate and precise should be chosen; however, many tests for body composition are precise but not accurate, whilst some are neither! Other important aspects of a test are cost, ease of use, acceptance by veterinary surgeons and clients, and invasiveness. At the current time, there is no method which cannot be criticised and, therefore, the perfect method for analysis does not yet exist.

Potential research techniques include chemical analysis, densitometry, total body water measurement, absorptionometry (including dual-energy X-ray absorptionometry – DEXA), ultrasonography, electrical conductance, and advanced imaging techniques (CT and MRI). In the clinical setting there is more of a need for quick, cheap and non-invasive methods of body composition measurement. The most widely adopted quantitative procedures include measurement of body weight and morphometry.

Morphometry is defined as the measurement of ‘form’ and, in relation to body composition analysis, refers to a variety of measured parameters that are used to estimate body composition. The three main approaches are measurement of skinfold thickness, dimensional evaluations (where various measures of stature are combined with weight) and body condition scores.

Dimensional evaluations are usually performed by tape measure, and a number have been reported in dogs and cats. Measurements of ‘length’ measurements (e.g. head, thorax and limb) are correlated with lean body components, whilst measures of ‘girth’ are more often associated with BF. By combining more than one measure (usually one that correlates with FM, and one correlating with LBM), equations can be generated to predict different body components. The best example of this is the WALTHAM Feline BMI™ e.g.: Body fat (%) = ( [ {ribcage/0.7067} – LIM] / 0.9156 ) – LIM.

Body condition scoring is a subjective, semi-quantitative method of evaluating body composition.
A number of schemes have been devised, with 9-point scheme being the most widely accepted. All systems assess visual and palpable characteristics which correlate subcutaneous fat, abdominal fat and superficial musculature (e.g. ribcage, dorsal spinous processes, and waist). Unfortunately, all current systems remain subjective, training is required, between-operator variability can be problematic, and breed variability (especially in dogs) can present a major problem in interpretation. In light of such concerns, a new 7-point algorithm-based approach, designed to minimise inter-operator variability, and expertise required. A recent study has demonstrated good correlation between the system and body fat measurements made by DEXA and excellent agreement between experienced operators. Most importantly, good agreement was found between measurements by the experienced operators and the owners, suggesting that the method is reliable when used without prior training.

Incidence of obesity in companion animals
We are all now well-aware of the ever-growing problem of obesity in people, with current estimates in the UK suggesting that over 55% of adults are overweight and a further 22% are obese. As in humans, obesity is one of the most important medical problems in the pet population. Studies, from various parts of the world, have estimated the incidence of obesity in the pet population to be between 22% and 40%. The most recently published data comes from a large study in Australia, where 33.5% of dogs were classed as overweight, whilst 7.6% were judged to be obese. These figures are similar to recent observations from the UK, where 43% of dogs, and 36% of cats were found to be obese.

Causes of obesity
Although some diseases (e.g. hypothyroidism and hyperadrenocorticism in dogs), pharmaceuticals (e.g. drug-induced polyphagia caused by glucocorticoids and anti-convulsant drugs) and rare genetic defects (in humans) can cause obesity, the main reason for development of obesity is getting the ‘energy balance equation’ wrong. In this respect, either excessive dietary intake or inadequate energy utilisation can lead to a state of positive energy balance; numerous factors may be involved including genetics, amount of physical activity, and the calorific content of the diet.

The effect of genetics is illustrated by recognised breed associations (e.g. Labrador retriever, Cairn terrier, cavalier King Charles spaniel, Scottish terrier, Cocker spaniel for dogs; domestic shorthair for cats). Neutering is an important risk factor in both species, whilst gender is a predisposing factor in some canine studies, with females over-represented. Other recognised associations in dogs include indoor lifestyle, inactivity, middle age, neutering, have all been associated with obesity in dogs. In cats, middle age, male gender, neutering, and apartment dwelling are possible risk factors.

Dietary factors can also lead to the development of obesity in both species. For instance, in dogs, the number of meals and snacks fed, the feeding of table scraps, and the animal being present when owners prepared or ate their own meal. However, the price of the pet food does have a significant effect, where obese dogs are more likely to have been fed cheaper rather than premium brand foods. Behavioural factors may also play a part; the development of obesity in cats may be caused by anxiety, depression, failure to establish a normal feeding behavior, and failure to develop control of satiety.

The pathological importance of obesity
In humans, the medical importance of obesity lies in the effect on mortality and morbidity of associated diseases. Obese humans, on average, do not live as long, and are more likely to suffer from diseases such as type II diabetes mellitus, hypertension, coronary heart disease, certain cancers (e.g. breast, ovarian, prostate), osteoarthritis, respiratory disease, and reproductive disorders. Similarly, obesity has detrimental effects on health and longevity of dogs and cats. Problems to which obese companion animals may be predisposed include orthopaedic disease, diabetes mellitus, abnormalities in circulating lipid profiles, cardiorespiratory disease, urinary disorders, reproductive disorders, neoplasia (mammary tumors, transitional cell carcinoma), dermatological diseases, and anesthetic complications.
Treatment of obesity

In humans, current therapeutic options for obesity include dietary management, exercise, psychological and behavioural modification, drug therapy, and surgery. Many of these options are available for companion animals, although it is not ethically justifiable to consider surgical approaches. Further, no pharmaceutical compounds have, as of yet, been licensed for weight loss in dogs and cats.

Dietary therapy forms the cornerstone to weight management in dogs and cats, but increasing exercise and behavioural management form useful adjuncts. It is recommended that the weight reduction protocol is tailored towards the individual patient, and use of a purpose-formulated diet is essential. These diets are designed to be low in fat and calories, whilst being supplemented in protein and micronutrients. Additional dietary factors that may be of benefit for weight loss include L-carnitine supplementation (to maintain lean mass), and use of high-fibre diets (to provide satiety).

Increasing physical activity is a useful adjunct to dietary therapy in a weight management regime, since it promotes fat loss, and may help to preserve lean tissue during weight loss. The exact programme must be tailored to the individual, and take account of any concurrent medical concerns. Suitable exercise strategies in dogs include lead walking, swimming, hydrotherapy, and treadmills. Exercise in cats can be encouraged by increasing play activity, using cat toys (e.g. fishing rod toys), motorised units and feeding toys. Cats can also be encouraged to 'work' for their food by moving the food bowl between rooms prior to feeding, or by the use of feeding toys.

In addition to the above strategies, it is essential that the whole weight reduction regime be supervised. This is labour intensive, requires some degree or expertise and training in owner counselling, and often requires a dedicated member of staff. Nevertheless, in the author’s opinion, this is the single most important component to the weight loss strategy. A recent study has demonstrated that weight loss is more successful if an organised strategy is followed, which includes owner education.

References

References available on request.
Energy expenditure has been measured in dogs and cats for over 100 years, and data from these studies has been used to develop a number of equations to predict the energy requirements for these animals. Despite continuing efforts to improve these equations, predicting the energy requirements of an individual animal are still difficult and subject to potentially large errors. The ability to successfully prevent or treat obesity is hampered by our inability to precisely predict energy requirements in all individuals. To better understand and treat obesity, two key questions need to be addressed. First, what is the cause of the individual variability in animal energy requirements? Second, does restriction of energy intake induce energetic adaptations that oppose weight loss (i.e., is mass-adjusted energy expenditure altered by level of energy intake?) Work combining whole animal, organ, and cellular energy expenditure measurements have increased current knowledge about the factors regulating energy expenditure and will hopefully lead to improved methods for predicting individual energy expenditure.

Several methods have been used to determine energy expenditure in dogs and cats. These methods include extrapolation of energy expenditure from energy intake measurements (feeding experiments) or calculation of energy expenditure from measurements of respiratory gases (indirect respiration calorimetry). If performed properly, these methods are capable of adequately providing measurements of energy expenditure (kcal or kJ/unit of time). However, energy expenditure must be adjusted for differences in body weight or composition when making comparisons between individual animals, and this adjustment is the subject of continuing debate. Energy expenditure is typically divided by either lean body mass or an exponential function of total body mass (e.g., kg^0.67 or kg^0.75) to adjust for differences in body size. These adjustments for energy expenditure are primarily derived from statistical regressions and often lack the theoretical basis to adequately explain individual deviations from predicted values. Also, many current methods to adjust energy expenditure for body size were not derived for comparisons with obese or chronic calorie restricted animals, and therefore may not be appropriate for use in obesity or weight loss studies.

The problem with existing methods to adjust energy expenditure for body size is that they frequently assume either all lean tissues have uniform rates of energy expenditure, or organ mass shows little variation for a given body weight. Neither of these assumptions is correct. Lean body mass consists of high (internal organs; >1,000 kJ/kg/d) and low (skeletal muscle and bone; <100 kJ/kg/d) energy expenditure compartments. The metabolically active internal organ comprise less than 10% of body weight but are responsible for greater than 50% of resting energy expenditure. Therefore, measures of total body weight or lean body mass may not adequately indicate the size of the metabolically active internal organs. This may be especially important with dietary energy restriction in adult animals. Dietary energy restriction does not result in uniform changes in the sizes of all tissues, with some tissues showing no change (brain) and other tissues (adipose, liver) showing rapid decreases in weight following energy restriction. Therefore, energy expenditure adjustment methods that take organ size into consideration will be useful in determining the energetic response to dietary energy restriction and may also explain some of the factors contributing to variation in energy expenditure between individual animals.

The use of MRI to quantify organ size has produced promising results in human and animal energy expenditure studies, and these techniques may prove useful in dogs and cats.

Ultimately, however, energy expenditure is determined by the cellular energy expending processes.
Protein turnover, mitochondrial proton leak, and Na+-K+-ATPase are the primary processes contributing to whole animal resting energy expenditure, and these processes together may account for up to 80% of resting energy expenditure. A few studies have investigated the role these biochemical processes play in conditions which alter whole animal energy expenditure. However, few studies combining measures of whole animal energy expenditure and cellular energy expending processes have been completed in dogs and cats. Such studies may prove useful in identifying factors that will improve our ability to predict energy requirements.

In recent years, studies involving whole animal, organ, and cellular energy expenditure measurements have increased current understanding of the factors regulating energy expenditure. Further studies in this area will hopefully lead to improved methods for predicting individual energy requirements in dogs and cats.
In dogs and cats, energy is required to maintain metabolic processes within the body and for activity. If this balance is not maintained, dogs and cats tend to gain or lose body mass. Deciding how much to feed a patient to provide the energy it needs to maintain normal body condition is the first step in any nutritional recommendation. Subsequent recommendations as to how nutrient dense the food should be must then to be adjusted based on this initial assessment of energy requirement. Nutrient recommendations provided by the National Research Council and the American Association of Feed Control Officials are based on the requirements of average laboratory dogs and cats undertaking modest amounts of exercise, so the nutrient density of foods may need to be adjusted up or down subject to whether the energy needs of the patient are below or above the energy requirements of an average laboratory dog or cat.

Unfortunately, determining the energy needs of any individual animal is not easy because there is no point of care test available that enables the clinician to determine energy expenditure. Experimental studies have mostly focused on the energy needs of groups of animals and how the mean of these groups varies with body weight and various other parameters, but all such experiments, even those attempting to measure basal metabolic rate under strict physiological conditions have shown wide variability even when taking account of variations in body weight with whichever exponent. Dr. Ramsay has highlighted some of the problems with these types of measurement and some novel methods of trying to solve them but no calculation based on experimental studies is even close to providing an accurate estimate of the energy needs of a particular individual patient. Furthermore, even using the methods available to us, remarkably little is known about the energy requirements of dogs or cats undertaking different types of activity or under different environmental conditions or even the amount and type of activity undertaken by free-living pet or working animals. Adjusting mean estimates of energy consumption for any number of factors such as body weight, breed, life stage, body condition, activity or environment, therefore, results in no more than an educated guess as to the requirements of an individual.

Given this situation, how does a clinician make a recommendation? Obtaining an accurate diet history remains the best and currently the only method of estimating the energy needs of an individual patient but obtaining such a history is difficult and time consuming. Diet histories obtained from human patients are notoriously inaccurate and are probably equally so when obtained from the owners of dogs and cats. Most owners feed treats and supplements which makes the diet complex to assess. Most owners do not initially confess or remember all that they have fed and most do not accurately measure how much is given. Assumptions made when estimating the energy content of both human and animal feeds may be inaccurate also. Obtaining an accurate diet history and calculating the energy content of a complex diet can take several hours which makes the process prohibitively expensive for all but the most committed of owners.

Various alternative methods have been used in humans to solve this dilemma. Small single use indirect calorimeters have been used to measure resting energy expenditure in human patients but have not to date been adapted for use in dogs and cats. Indirect calorimetry can also be markedly affected by stress which makes its use unreliable in dogs and cats which are unused to wearing a mask. Indirect calorimetry collecting expired gas is also impractical in free-living animals. The double-labelled water method (as discussed by Dr. Ramsay) is prohibitively expensive for use in individual animals and requires a mass spectrometer for analysis. A radio-labelled bicarbonate method has been used to measure energy expenditure both at rest and during short bouts of exercise in a number of species but the radioactivity of the marker makes this method impractical. One report describes using a continuous infusion of bicarbonate labelled with stable isotope in dogs but continuous infusion is impractical in free-living animals. A number of other method-
ologies that have been used in humans as indirect measures of energy expenditure, such as heart rate monitors and pedometers, need to be assessed in dogs and cats. Some method of estimating activity may lead to a markedly better estimate of energy requirements based on the population mean. Nevertheless, in the interim, assessing energy consumption in free-living patients remains a major problem for each clinician.
The WALTHAM International Nutritional Sciences Symposium

Innovations in Companion Animal Nutrition

ORAL ABSTRACTS
CATS LACK SWEET TASTE RECEPTOR
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Although domestic cats (*Felis silvestris catus*) possess a functional sense of taste, they, unlike most mammals, do not prefer and may not detect the sweetness of sugars. One explanation is that cats lack the sensory receptors needed to taste sugars. To test this possibility, we characterized the sweet receptor genes of domestic cats and those of other members of the *Felidae* family of obligate carnivores. The mammalian sweet taste receptor is formed when two proteins (T1R2 and T1R3; gene symbols *Tas1r2* and *Tas1r3*) dimerize. By screening a feline genomic BAC library and performing PCR with degenerate primers on cat genomic DNA, we identified and sequenced both genes. Gene expression in taste tissue was assessed by *in situ* hybridization and immunohistochemistry and in taste papillae and other organs of the gut by RT-PCR. The cat *Tas1r3* gene shows high sequence similarity with functional *Tas1r3* genes of other species. Expression of *Tas1r3* in taste buds was detected by RT-PCR, *in situ* hybridization and immunohistochemistry. Cat *Tas1r2* gene shows a 247 bp microdeletion in exon 3 and stop codons in exons 4 and 6. Expression of this gene was not detectable, neither by RT-PCR nor by *in situ* hybridization, nor immunohistochemistry. *Tas1r2* in tiger and cheetah and in six additional healthy adult domestic cats all show the same deletion and stop codons. Several visceral organs expressed T1R3, but not T1R2. As cat *Tas1r2* is an unexpressed pseudogene, a functional sweet taste receptor heteromer cannot form. Lacking this receptor, the cat cannot detect sweet stimuli. This molecular change was very likely an important event in the evolution of the cat’s obligate, carnivorous behavior.
IN VIVO TAURINE BIOSYNTHESIS IS GREATER IN SMALL DOGS THAN LARGE DOGS
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Recently, diet-induced, taurine deficiency, dilated cardiomyopathy has been reported in large dogs (LDs) but not small dogs (SDs). The reason for the body size difference in incidence has not been elucidated. We hypothesize that taurine biosynthesis rate (TBR) is lower in LDs than SDs. TBR of 6 LDs (37.85±2.10kg mongrels) and 6 SDs (12.81±0.37kg Beagles) were measured using a modification of the stable isotopic taurine (d4-tau) dilution technique used in cats by (Fay et al., 1998).

All dogs were adapted to a diet of 15.6% protein and 0.60% sulfur amino acids (SAA) for three months with sufficient food to maintain an ideal body condition score. The SDs and LDs were paired by order of their SAA intake to make 6 pairs. The dogs in each pair were given the same amount of diet/kg BW^{0.75} for 3 weeks, followed by a single oral dose of d4-tau. D4-tau to taurine ratio in urine collected daily for 6 days was measured by GC-MS to determine TBR. Plasma, whole blood (WB) and urinary taurine concentrations were measured.

TBR of SDs vs. LDs were 80±8.9 vs. 50±12 mg/kg BW^{0.87}/day (kg BW^{0.87} = relative metabolic liver weight, mean±SEM, p<0.05) respectively. Plasma, WB and urine taurine concentrations of SDs and LDs were 86±10 vs. 40±14 nmol/mL (p<0.05), 232±27 vs. 151±19 nmol/mL (p<0.05), and 1016±337 vs. 357±298 nmol/mg creatinine (p>0.05), respectively.

These results support the hypothesis that LDs have lower TBR than SDs when fed diets near-limiting in dietary SAA, but adequate to maintain body weight. The lower TBR of LDs probably underlies the more frequent observation of taurine-deficiency dilated cardiomyopathy in LDs relative to SDs.

References
In the present investigation 60 cat owners with overweight cats and 60 cat owners with normal cats were interviewed by a standardized questionnaire. Body condition of cats was determined by body weight and body condition scoring. Cats in the respective groups were either unequivocally lean or overweight. The questionnaire contained a part on psychological balance of cat ownership (advantages and drawbacks), another part on time spent with the cat, a part on feeding of the cat, behavior of the cat, health and eating behavior of the owner and demography. Most answers were scaled from one (not important, not true) to seven (very important, exactly true). Means of both groups were compared (criteria of practical significance). A cluster analysis was carried out on the questions on psychological balance of cat ownership with the factors enjoyment and relaxation, social support, agreeable, esthetic companion, tenderness, easy to take care of pet, part of nature, usefulness, activity.

Human-animal-relationship of owners of overweight cats differed significantly from human-animal-relationship of owners of normal cats. Owners of overweight cats tended more to overhumanization of the cat and they felt closer to their cats than owners of normal cats. The role of the cat as a substitute for human companions was more important for owners of overweight cats. The cat was rather frequently acquired as a consolation in a personal crisis. Several items indicate that feeding the cat was an important stimulant for communication with the cat. The owners of overweight cats played less with their cats. With regard to feeding habits, the most important difference was that overweight cats had more often free choice of food intake, and they were more frequently given meat and kitchen scraps on top of their usual diet. The majority of owners of overweight cats did not perceive their cat as overweight. Preventive health behavior for the cat (health checks, watching digestion) was more important to the owners of normal cats than to those of overweight cats.

By contrast the personal health and eating behavior of the owners was not markedly different between the groups. There was no difference in the environment of the cat or the number of cats in the household. Age distribution of the cats was different: middle aged cats were overrepresented in the overweight group. Six types of cat owners were identified by cluster analyses. Two types of cat owners had a rather weak bond to their cats, one appreciated mainly the easy to care for quality of the cat as a pet, and the other could be described as mutual tolerance. In both types of cat owners the risk of overweight of the cats was below average. A third type of cat owner had a strong bond to their cats but no increased risk of overweight for the cat. Owners of this type had a strong interest in playing with the cat. The other three types had strong human-animal bonds and an increased risk of overweight for the cat. The role of the cat differed somewhat ranging from undemanding listener, friend, child substitute to family member.
RISK FACTORS FOR OBESITY AND OWNER’S PERCEPTION OF DOGS’ BODY CONDITION IN FRANCE

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Different studies, but none in France, reported from 18% to 44% of overweight dogs, with gender, sexual status, age, breeds suggested as risk factors.

The purpose of our study was to assess the prevalence rate and risk factors of overweight in dogs, and to investigate the owner’s perception of their dogs’ body condition (BC).

Between February and May 2003, dogs’ owners coming for vaccination at ENVA were questioned by a veterinarian. Owners and veterinarians evaluated dogs’ BC by verbal appreciation and by comparison with a legend-free visual scale.

Agreements between weights and between estimations of BC were measured by correlation coefficient and kappa coefficient respectively.

Univariate analysis and stratified analysis by Mantel-Haenszel test on neutering, dogs’ or owners’ age were performed. Multivariate logistic regression analysis was used for variables significantly associated with overweight and strongly estimated as risk factors.

We found 38.8% overweight (33.8%) and obese (5%) dogs. Females (OR 1.54), neutered dogs (OR 2.23), Retriever breed (OR 2.25) and increasing age (OR 2.81 at 2-3yrs, 11.75 over 12 years old) appeared of higher risk.

The owners underestimated their dog’s BC compared with veterinarian’s assessment. Estimation of overweight by the owner was better with the visual than with the verbal scale.

Conclusion
This was the first French study on dog’s BC and overweight with such a sample (616 dogs) and the first one since Mason (Vet. Rec.1970; 86: 612) to compare the owner’s and the veterinarian’s views of the same dogs. It confirmed that females, neutering, age and Retriever breed were risk factors of overweight in dogs, and that the owner underestimated his dog’s BC.
POST-PRANDIAL PLASMA LIPOLYTIC ACTIVITIES AND LIPOID AND CARBOHYDRATE RESPONSES OF DOGS FED DIACYLGLYCEROL-RICH MEALS CONTAINING HIGH AND LOW GLYCEMIC INDEX CARBOHYDRATES.

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Digestion and assimilation of dietary fatty acids is a complex post-prandial (P-P) process involving triacylglycerol (TG)-hydrolysis, fatty acid absorption, re-synthesis of TG in intestinal mucosal cells, and chylomicron secretion.

This study provides comparative information in dogs regarding P-P effects of meals enriched in diacylglycerol (DAG) with varying glycemic-index carbohydrates on plasma lipase activities, lipid, and carbohydrate metabolism. Normal adult Beagles were fed meals containing vegetable-oils enriched in either DAG or the more typical triacylglycerol (TAG). Two carbohydrates with different glycemic-indices (GI) were also evaluated. Four meals were fed containing cooked chicken breast plus either DAG or TAG and either high- (HGI, waxy corn starch) or low- (LGI, high amylose corn starch) GI carbohydrates.

A crossover design and two week wash-out period between meals was used. Meals were readily consumed providing discrete start-points for sampling. Blood was collected via jugular catheter at 0, 0.5, 1, 2, 3, 4, and 6 hours and plasma harvested. TG, non-esterified fatty acids (NEFA), β–hydroxybutyrate (βHB), glucose, and circulating insulin concentrations were determined. At hour-6, post-heparin plasma was collected 15 minutes after intravenous injection of 100 I.U. Na heparin/kg body-wt. Lipoprotein lipase (LPL) and hepatic lipase (HL) of post-heparin plasma were assayed using radiolabeled substrates.

Significant differences were found for the peak and duration of hypertriglyceridemia with DAG independent of GI. LGI-carbohydrate caused peak NEFA responses at three hours P-P. A transient decrease of βHB occurred early on but returned to pre-meal values with all meals. Glucose and insulin concentrations demonstrated significantly greater peak elevations/areas under the P-P curve with HGI-starch.

No significant differences in LPL were found but HL was greater with LGI. DAG meals decreased P-P hypertriglyceridemia vs TAG while maintaining similar NEFA and glucose responses although glucose and insulin response were modified by GI. Increased HL with LGI-DAG vs LGI-TAG suggests that increased HL-mediated hepatic lipoprotein uptake may more effectively remove circulating TG.
RETROSPECTIVE STUDY ON THE ENERGY REQUIREMENTS OF ADULT COLONY CATS
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Energy intake during periods of weight constancy longer than four weeks was retrospectively evaluated in 138 adult colony cats. Body weight of male cats averaged 5.09kg. There was no effect of neutering in males. By contrast neutered females were significantly heavier than intact queens (mean body weight of 4.09kg and 3.64kg, respectively, p<0.05).

Weight distribution also varied according to age: Middle-aged cats were heavier than young (< 5 years) and very old cats (> 10 years.). The mean energy requirement of adult cats was 251kJ ME/kg BW.

Maintenance energy requirements per body mass unit decreased with increasing body weight. Mean energy requirements of cats with a body weight of up to 3kg were of 319kJ ME/kg BW, while heavy cats with a body weight of over 5kg needed only 209kJ ME/kg BW for weight maintenance. Many heavier cats were overweight and probably had a reduced percentage of fat-free body mass. Energy requirements of this cat population were best described by a function with an exponent for metabolic body mass, which was considerably lower than 0.75 (maintenance energy requirements= 544kJ ME/kg BW⁰.⁴).

Similar results were reported by Earl and Smith. 1991 (J. Nutr., 121: S45-S46). The mean energy requirements of neutered cats was lower than that of intact cats (286kJ ME/kg BW vs. of 231kJ ME/kg BW; p<0.05). When lean intact cats were compared with lean neutered cats and overweight intact cats were compared with overweight neutered cats there were no longer clear-cut effects of neutering in female cats.

While young adult cats of one to five years of age needed an average of 313kJ ME/kg BW, mean maintenance energy requirements of cats between 6 and 10 years of age only came up to 231kJ ME/kg BW. Older cats had slightly higher energy requirements than middle aged cats. Age effect interacted with the effect of overweight. When only data of lean cats were evaluated there was a gradual decrease of energy requirements with age.

The results demonstrate the importance of taking into account overweight when interpreting effects of sex, neutering or age on energy requirements. The study is, however, a retrospective evaluation of data on cats which became spontaneously overweight or stayed lean under similar conditions, an experimental design which obviously differentiates between animals who are predisposed to overweight and animals which are not.
STANDARDISED POSITIONING IS ESSENTIAL FOR PRECISE DETERMINATION OF BODY COMPOSITION USING DUAL-ENERGY X-RAY ABSORPTION METRY

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Dual-energy X-ray absorptionometry (DXA) is a practical, non-invasive technique to measure body composition. Accuracy has previously been demonstrated, but limited data exist on both precision and on the effects of position on the measurements obtained.

Ten canine cadavers of various breeds, ages and genders were scanned using a fan-beam DXA (Lunar Prodigy Advance; GE Lunar; Madison, USA). All dogs were euthanised for medical reasons unrelated to the study, and scanned prior to onset of rigor mortis. Subjects were scanned six times, alternating between dorsal (D) and lateral (L) recumbency. Data analysis used pre-specified protocols with purpose computer software (enCORE 2004, 8.70.005; GE Lunar), and analysis was repeated by three operators.

Precision of body composition analysis was excellent and marginally better in dorsal recumbency (CVs 0.5% [total tissue], 3.6% [fat] and 1.0% [lean] for D; CV 0.7% [total tissue], 4.2% [fat] and 1.0% [lean] for L), with excellent correlation between results (Rp >0.99 for all, P<0.0001).

Although total body mass correlated with true mass, both methods marginally under-estimated the true value (mean true mass 14.54kg; 14.20kg for D, 14.07kg for L; P≤ 0.001). Composition results differed between positioning methods; dorsal scanning read more fat and less lean tissue (mean fat 2240g [D] vs. 1890g [L], P=0.018; mean lean tissue 11421g vs. 11681g, P=0.04). Inter-operator variability was minimal (CVs 0.05% [total tissue], 1.5% [fat] and 0.5% [lean]) with near-perfect correlation (Rp ≥ 0.999, P<0.0001), although a difference in absolute results was evident (mean results for two operators: total tissue mass 13455g vs 13467g; fat mass 2506g vs. 2409g; lean mass 10948g vs. 11038g; P<0.005).

In conclusion, whole body DXA shows excellent precision but significant operator and body positioning effects are evident. The marginal inter-operator difference is unlikely to be of clinical significance. Position discrepancy is greater and should be standardised; dorsal recumbency is recommended.
ENERGY EXPENDITURE AND BODY COMPOSITION OF DOGS FROM FOUR DIFFERENT BREEDS
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Great variation in body weight, morphology, temperament, etc. induces wide differences in the energy expenditure and requirement of dogs. It has also been shown in different species that neutering is responsible for a decrease in basal metabolism, and physical activity. The aim of this study was 1) to determine the energy expenditure of dogs from different size, and to relate it to body composition, 2) to assess the effect of ovariectomy on these parameters.

Twenty female dogs (5 Miniature Poodle MP, Medium Schnauzer MS, Giant Schnauzer SG, Great Dane GD). Dogs lived in an outdoor kennel and were fed the same dry food. Dietary allowance was adjusted to body maintenance but no adjustment was made following ovariectomy. Body composition was determined using deuterium oxide dilution, and energy expenditure by gas exchange monitoring. Measurements were done in SG and GD before ovariectomy, and then 6 months later, but only 6 months after neutering in MP and MS.

The energy expenditure calculated from all VCO2 and VO2 records was 129±19% (mean±SD) of the lowest observed 30-min value (least observed metabolism, LOM). Ovariectomized MP were fatter than ovariectomized SM and GD (26±3 % body fat vs. 18±3%, p < 0.05). When expressed according to BW the LOM was similar in MS, GS and GD (38±4 kcal/kg/d) while it was higher in MP (55±7, p = 0.0001). Similar differences were observed according to fat-free mass FFM (47±4vs.76±11 kcal/kg FFM/d, p = 0.001). On metabolic BW (BW0.75) as well as on metabolic fat-free-mass (FFM0.75) basis the LOM of GD was higher (104 ± 10kcal/kg BW0.75 vs. MP: 80±8, MS: 74±2 and GS: 84±9; 120±10kcal/kg FFM0.75 vs. MP: 102±9, MS: 85±2 and GS: 101±12). Ovariectomy induced a slight increase in body weight (p < 0.05) while there was no difference in LOM expressed according either to BW, metabolic BW or FFM.

These data confirm firstly it is not possible to extrapolate short-term measurements to daily energy expenditure. Secondly they confirm breed differences in LOM that are not related to body weight or body composition. Thirdly they do not show, in the conditions of the experiment, any effect of ovariectomy.
The frequency at which a fish should be fed to maximize food utilisation is a question often asked by aquarists. This study, using growth as the marker, investigated the number of times per day a goldfish (Carassius auratus) should be fed to make the most efficient use of a daily food ration.

A total of 120 goldfish were offered a food allowance of 2.0% bodyweight (g/g) at a feed frequency of 1, 2, 4 or 6 times per day. Three replicates, with 10 fish per tank, were used per treatment. The fish were individually weighed at the study start and every 2 weeks thereafter; the following equations were used to calculate growth:

Specific growth rate (SGR) = \(100 \times \frac{\ln w_2 - \ln w_1}{\text{trial days}}\),
where \(\ln w_1\) and \(\ln w_2\) is the log of weight of the fish on day 0 and end day, respectively.

Food conversion (FC) = \(\frac{\text{Bodyweight gain (g)}}{\text{Food fed (g)}}\).

Fish fed 4 times/day displayed the greatest growth for each parameter measured, showing significantly greater SGR, FC and % growth/fish compared with feeding 1 time/day. All the other frequencies were parity with each other.

Values in rows for each measurement that do not share a common superscript are significantly different (\(P<0.05\)).

This study shows that to achieve maximum growth for a set daily food allocation a goldfish should be fed four times per day, assuming good quality water conditions.
Dietary manipulations that could have a subtle effect on adiposity and/or energy expenditure would be useful to face the increasing prevalence of obesity in cats. Although CLA appeared to attenuate increases in body weight and body fat in several animal models, trials in humans found little evidence to support that CLA reduces body weight or promotes repartitioning of body fat and fat-free mass in man. Similarly, conflicting results have been reported about the ability of Garcinia cambogia extracts containing hydroxycitric acid to lower body fat synthesis and accumulation. The current study aimed to determine the effect of these two supplements on the body composition and least observed metabolism (LOM) of adult neutered cats.

Twenty-four normal-weight cats (12 males, 12 females, all neutered) participated in this study. They were allotted to three groups on the basis of sex, body weight, and body composition. Each group was given for 6 months one of the three experimental extruded diets (60kcal ME/kg BW, diet 3960 kcal ME/kg as fed) (1: control, 2: control + 0.4% CLA (mixture 50/50 of c9, t11, t10, c12), 3: control + 0.3% Garcinia cambogia extract). The amounts of active compounds had been chosen according to previous studies in dogs and humans, respectively. Body composition was assessed using deuterium oxide dilution at the beginning and the end of the study. The least observed metabolism (LOM) was assessed at the end of the test period using gas exchange monitoring.

Group 2 cats consumed 60mg/kg body weight CLA mixture, and Group 3 cats 45mg/kg body weight Garcinia cambogia. Body weight and body composition did not (significantly) change in groups 1 and 2. While the increase in body weight, and the decrease in fat-free mass were not significant, the fat mass increased (p<0.05) as well as the percentage of body fat (p<0.05) in group 3. Mean daily energy expenditure was 214±8 kJ/kg BW, and LOM 159kJ/kg BW (300 kJ/kg fat-free mass), without significant difference between the groups.

Our results did not support, at the dosages we tested, any beneficial effect of CLA or Garcinia cambogia on body composition and energy expenditure in normal weight cats. These data should be confirmed in obese animals as well as in the prevention of adiposity increase following neutering.
In human diabetes type II, overweight as well as lack of exercise is documented as significant risk factors. So far no published data have proven obesity or exercise to be risk factors in canine diabetes of type I or II.

To indicate risk factors for canine diabetes mellitus (CDM), an epidemiological pilot study was conducted on 20 consecutive dogs diagnosed with CDM at the Small Animal Clinic at the veterinary faculty of the Swedish University of Agricultural Sciences in Uppsala between 2000 and 2003.

A control group of 40 dogs, matched for age, sex and breed, was recruited from the register at the Swedish Kennel Club. The owners of cases and controls received a questionnaire on feeding patterns, diet, activity, weight and owner perceived body condition score prior to a diagnosis of CDM in cases and at a similar period for controls. A telephone interview explored the variables in more detail two weeks after the owners having received the questionnaire.

The results showed no difference between groups in the proportion fed home-made food or table food versus commercial diets, but owners of cases gave treats more regularly. There was a significant difference between the groups in the amount and intensity of exercise received before the diagnosis of CDM. Fewer dogs that developed CDM received high intensity training and they received training less often. A difference was also found in weight as evaluated by the owner. Dogs that developed CDM were significantly more often overweight at the age of diagnoses and more often had a history of lifelong overweight.

The findings indicate that also in dogs, overweight and lack of exercise should be considered as risk factors and taken into account in preventive measures as well as design of research to elucidate on the multi-factorial background of diabetes in dogs.
Many theories exist as to why dogs eat grass. Dogs may consume grass to induce vomiting in response to illness, to control intestinal parasites, to supplement a deficient diet, or due to behavioral problems such as pica. Plant eating in domestic dogs may also reflect an instinctive predisposition acquired from wild wolf ancestors among whom plant eating has been observed. The goal of this study was to acquire data about the nature of plant eating in domestic dogs and to gather information that might relate to causes of plant eating.

Using strict exclusion and inclusion criteria, an Internet survey was utilized to obtain information from owners of dogs that consumed plants. Owners were questioned regarding how frequently their dog consumed plants, what types of plants were consumed, behavior patterns before and after eating plants, diet, medical history and behavioral history. Statistical comparisons were made using Chi-squared goodness of fit tests.

Data from 1,694 viable surveys were analyzed. Grass was eaten most frequently. The frequency of plant eating was not related to sex or gonadal status, breed, diet, presence of intestinal parasites, receipt of antihelmintic medication, or other behavior problems. Most dogs did not exhibit illness behavior prior to consuming plants nor vomit after ingestion. Dogs reported to typically exhibit signs of illness prior to consuming plants (P<0.001) or with known gastrointestinal disease (P<0.01) were significantly more likely to vomit after consuming plants.

The occurrence of vomiting after eating grass may be correlated with, but not causally related to, gastrointestinal illness and altering a dog’s diet is unlikely to change plant eating behavior. As a proximate cause could not be discerned, plant eating behavior in dogs may have initially evolved in wild canid ancestors and have been conserved through the domestication process.
The aim of this work was to evaluate effect of dietary cellulose on food intake in cats.

Six healthy adult cats were fed a complete moist cat food as a basal diet. Cellulose was added stepwise (2, 4 and 6% of cellulose on wet weight basis, increase every two days, final crude fibre content 18% dry matter). Throughout the experiment dry matter intake remained at about 16g/kg body weight. Consequently energy intake decreased with increasing cellulose content. When the food contained 6% of cellulose the cats ate only 74% of their maintenance energy requirement.

With this type of experiment the question remains open whether the decrease of energy intake with increasing fibre content is mostly related to decreased palatability of the food or whether it is due to increased gastric filling.

Immediately following the previously described experiment (i.e. after a period of decreased energy intake) the cats were offered moist food without cellulose after they had eaten from their 6% cellulose diet. The cats increased total dry matter intake to 24g/kg body weight and energy intake to 124% of maintenance requirements. To elucidate the role of low energy intake before the experiment the design was repeated after a period of normal energy intake. The cats were first offered the moist food with 6% cellulose and then offered moist food without cellulose.

In this case they did not increase dry matter intake and their energy intake remained even below maintenance energy requirements.

These results are markedly different from a similar study in dogs (Dobenecker and Kienzle, 1998; J. Nutr.; 128: 2674S-2675S), and they strongly suggest that gastric filling plays a more important role in food intake of cats than of dogs.
Entire male cats have been known to excrete 3 to 6 times more felinine in their urine compared to castrated males and females. This study investigated the rate of change in felinine excretion after administration of estradiol and/or testosterone to castrated male and testosterone alone to intact female cats.

Three groups of five castrated adult male cats were allocated to three i.m. treatments: control (carrier), testosterone, testosterone+estradiol, while five entire adult female cats received testosterone i.m. Testosterone (Durateston, Intervet Ltd, 50mg/mL which provides androgenic activity for up to 6 weeks) was administered once (day 0) at a rate of 25mg/kg bodyweight. Estradiol (Mesalin, Intervet Ltd, 200mg/mL) was administered at days 0, 2, 4, and 6 at a rate of 14mg/kg bodyweight. Urine was quantitatively collected during a 5-day pre-period and daily up to 12 days after the start of the injections. Blood samples were taken from each cat at set intervals. The cats were housed in metabolism cages and had free access to an AAFCO-approved moist maintenance diet and fresh water. Urine samples were analyzed for felinine and creatinine by HPLC and a Roche diagnostic kit, respectively, while blood plasma was analyzed for testosterone and estradiol using Diasorin RIA-kits.

Blood testosterone and estradiol concentrations were low during the pre-period but increased sharply in the cats receiving these respective hormones. Felinine: creatinine ratio was similar (0.12, P>0.05) for the four groups during the pre-period and increased in the three groups receiving testosterone to 0.43, 9-12 days after injection. The felinine: creatinine ratio remained low in the control group throughout the study. Estradiol injections did not change felinine: creatinine ratios.

Testosterone increases felinine excretion in castrated adult male and intact female cats, whereas estradiol does not increase or decrease felinine in male cats.
INFLUENCE OF GENISTEIN, POLYUNSATURATED FATTY ACIDS, AND VITAMINS D3 AND K1 ON BONE METABOLISM IN BEAGLE DOGS

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Although many female dogs are neutered no osteoporotic problems (comparable to vertebral fractures in humans) are seen in these dogs. Anyhow, the decrease in sex hormone levels (estrogen) as induced by ovariectomy in dogs is accompanied by a high bone turnover, which subsequently could lead to an increased risk of rapid bone loss. The aim of this study was to evaluate the effect of a combination of genistein, PUFA, and vitamins D3 and K1 on preventing the subsequent loss of bone mass induced by ovariohysterectomy (OHX).

A total of 12 female Beagle dogs (18 months of age) were randomized into a treated (15mg Genistein/kg BW, 5% PUFA, 1500 IU Vitamin D3/kg BW, 2000mg Vitamin K1/kg BW) and a control group (1mg Vitamin K1 and 10 IU Vitamin D3/kg BW) and fed for nine months. After five months all dogs were ovariohysterectomized to investigate a possible protective effect of the substances on bone. Blood and urine were collected at intervals to determine marker of bone formation (bALP) and bone resorption (collagen crosslinks deoxypyridinoline and pyridinoline).

Bone resorption (Figure: urinary pyridinoline excretion) in the control group doubled during the weeks after OHX. This strong increase was clearly prevented by administration of the combination of the substances. Bone formation in the control group turned to be numerically higher when compared to the treatment group (Serum bone specific alkaline phosphatase in IU/l at week 8 post OHX: 19.67 + 7.93 vs 12.44 + 2.09, respectively).

*Statistically significant difference (p<0.05) between the two groups at one time point

The results of this study show that the combination of the substances tested reduces a high bone turnover and therefore can have a protective effect on bone in intact as well as in the ovario-hysterectomized dogs.
MATERNAL PLASMA PHOSPHOLIPID FATTY ACID ALTERATIONS AND IMPROVEMENT OF RETINAL FUNCTION OF YOUNG DOGS FROM DAMS FED LONG CHAIN N-3 POLYUNSATURATED FATTY ACIDS DURING DEVELOPMENT AND THE PERINATAL PERIOD.

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2Nestlé Purina PetCare, St. Louis, MO. USA

Long-chain polyunsaturated fatty acids (LCPUFA) are essential for neural and retinal development. This study investigated electroretinograms (ERGs) of puppies born to mothers fed diets varying in marine n-3LCPUFA content. Maternal plasma-phospholipid (PL) fatty acids were also evaluated during gestation/lactation. One of four diets varying only in type/amount of PUFA were fed to 12 bitches (n=3 per group) as sole nutrition source beginning with estrus and breeding, throughout gestation/lactation. Puppies were weaned to the same diets which varied only in their amounts of linoleic and linolenic acid (ALA) and n-3LCPUFA (i.e. 20/22 carbon n-3 fatty acids). Milk supplied exclusive nutrition during suckling diets were designated Lo/Lo, Lo/Mod, Lo/Hi, and Hi/Lo, based on ALA/n-3LCPUFA content.

EDTA plasma samples were collected at selected times during gestation and lactation after withholding feed. Lipids were extracted, total phospholipids (PL) fractionated, and PL-fatty acids determined. No time effects or time*diet interactions were found during gestation or lactation (repeated measures ANOVA). Data from the sample periods were therefore pooled and diet-differences analyzed. Plasma-PL fatty acids during both gestation and lactation significantly reflected the diets (p<0.05) and n-3LCPUFA dose responses were noted. By contrast, dogs fed the high ALA diet (Hi/Lo) showed no accumulation of DHA although EPA and DPA were increased (p<0.05). We have previously reported this phenomenon in non-parous adult dogs fed ALA.

ERGs of puppies (12 weeks) revealed significantly improved visual performance in the Lo/Hi group with superior rod response (improved a-wave amplitude and implicit times, p<0.05). Puppies in the lowest dietary n-3LCPUFA group exhibited poorest ERG response. Initial intensity at which the a-wave was detectable (threshold intensity) demonstrated that retinal response of the n-3LCPUFA puppies occurred at lower light intensity, exhibiting greater rod sensitivity, than other groups. Preformed dietary n-3LCPUFA vs ALA is a more effective means of enriching maternal plasma-DHA and results in improved visual performance in their puppies.
EFFECTS OF DIETARY MODIFICATION ON LIPIDS IN EARLY CANINE CHRONIC VALVULAR DISEASE.
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The purpose of this study was to assess the effects of a moderately reduced sodium diet enriched with n-3 fatty acids, antioxidants, B vitamins, and certain amino acids (Cardiac Diet) on lipids in dogs with asymptomatic chronic valvular disease (CVD). After a baseline cardiac evaluation, blood was collected for a biochemistry profile and for measurement of serum lipids by automated enzymatic method [total cholesterol, triglycerides, high-density lipoproteins (HDL), and non-HDL]. After baseline measurements in the dogs with CVD were compared to those of healthy control dogs, all dogs with CVD were changed from their usual diets to a Run-In diet. After 4 weeks of exclusive feeding of the Run-In diet, dogs were re-evaluated and then randomized to receive either the Cardiac or Placebo Diet (Table) exclusively in a double-blind fashion. A final evaluation was performed after dogs had eaten the Cardiac or Placebo Diet for 4 weeks. All measurements obtained at baseline were repeated at the 4- and 8-week visits.

### Total fat and selected polyunsaturated fatty acid contents of Cardiac and Placebo Diets

<table>
<thead>
<tr>
<th>Nutrient content (mg/100 kcal)</th>
<th>Cardiac Diet</th>
<th>Placebo Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>4.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (mg)</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>Docosahexaenoic acid (mg)</td>
<td>18</td>
<td>1</td>
</tr>
</tbody>
</table>

At baseline, there were no significant differences between the CVD (n=29) and healthy control groups (n=12) in median age or body weight. Triglycerides, total cholesterol, HDL, and non-HDL were not different between dogs with CVD and healthy controls at baseline, nor were there significant changes in these lipid parameters during the Run-In phase. However, dogs eating the Cardiac Diet had significantly bigger increases in triglycerides (P=0.03), total cholesterol (P<0.001), and HDL (P=0.004). Dogs in the Cardiac Diet group also had a significant reduction in cardiac size compared to dogs in the Placebo Diet group. The increase in cholesterol in the Cardiac Diet group appears to be primarily due to an increase in HDL. No detrimental effects of the increase in lipid levels were seen in this group of dogs, but further study of this effect is needed.
The WALTHAM International Nutritional Sciences Symposium

Innovations in Companion Animal Nutrition

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The study was carried out at the Department of Medicine, Veterinary College, Bangalore, India for a period of one year and included three groups of dogs. Group I fed on traditional homemade vegetarian food made up of rice, bread, lentil, milk and vegetables, Group II on homemade food with meat, egg, beef, chicken in addition to rice, bread and milk and Group III on imported commercial feed. The dogs had the same diet throughout the course of the study.

A total of 1229 dogs of different breeds were used in this study and were randomly allocated to different groups. Occurrence of diseases of digestive system, urinary system, skin disorders and metabolic diseases were recorded. The dogs were monitored throughout the course of the study by this case history sheet maintained in the Department of Medicine.

It was observed that the number of dogs affected and the percentage incidence of parasitic enteritis, bacterial enteritis, non-specific anorexia, indigestion and ascites was 19(5.82), 13(3.98), 12(3.68), 9(2.76) and 2(0.61) in dogs receiving commercial food whereas those in Groups I and II it was 43(8.86), 28(5.77), 24(4.94), 22(4.53) & 7(1.44) and 39(9.33), 29(6.93), 19(4.54), 18(4.30) & 5(1.19) respectively.

Results on percentage incidence of dermatological disorders like ticks, fleas, pyoderma, dermatomycoses, atopic dermatitis, hormonal disorders, dry coat, sarcoptes, demodicosis, pruritus and dandruff in Group III animals was 10(3.06), 7(2.14), 8(2.45), 7(2.14), 5(1.53), 3(0.92), 5(1.53), 4(1.22), 2(0.61), 6(1.84) & 6(1.84) as against 20(4.12), 16(3.29), 18(3.71), 18(3.71), 15(3.09), 9(1.85), 15(3.09), 11(2.26), 5(1.03), 12(2.47) & 8(1.64) and 18(4.30), 17(4.06), 15(3.58), 15(3.58), 13(3.11), 7(1.67), 14(3.34), 9(2.15), 4(0.95), 13(3.11) & 9(2.15) in Groups II and III.

The percentage incidence of renal diseases which included renal failure, nephritis, cystitis and urinary calculi in Group III was 48(14.72) as compared to Group I 86(17.73) and Group II 88 (21.05).

Results of occurrence of metabolic diseases indicated that the percentage of rickets and zinc responsive dermatosis in animals receiving commercial food was 3(0.92) and 2(0.61) which was much less as compared to 23(4.74) and 12(2.47) and 27(6.45) and 9(2.15) in Group I and Group II.

The results of overall incidence of disease in dogs indicated that percentage occurrence of disease in Group III animals was less as compared to Group I and II.

The mortality pattern of pups till weaning indicated that the percentage survival of pups in Group III fed on commercial food was higher 867(94.44) as compared to Group I 681(86.75) and Group II 626(87.92).

This study concludes that commercial food is balanced and meets the requirements of the animals better than homemade food.
Osteoarthritis is a degenerative and inflammatory painful condition of dog joints. Over 90% of dogs are affected after the age of five. Previous studies have illustrated the benefits of a green-lipped mussel (GLM) supplementation in the management of this syndrome. The aim of this study was to validate, in field conditions, the efficacy of a GLM-supplemented dry diet* in osteoarthritic dogs.

Dogs were fed the GLM diet for 50 days. Evaluations of osteoarthritic signs were carried out at day 0 (baseline) and at day 50 by practising veterinarians. Each dog was visually scored for mobility reduction (scale 0 to 3). Individual joints of each limb were clinically scored for degree of pain, swelling, crepitus and reduction in range of movement. Summation of the previous scores provided a Total Arthritic Score (T.A.S.) for each dog. Statistical comparisons between initial and final arthritic states were made using a repeated-measure ANOVA Test.

85 clinically-confirmed arthritic and privately owned adult dogs, seen at 25 veterinary clinics, completed the multicentric trial. 7 giant (>45kg), 46 large (26-45kg), 20 medium (11-25kg) and 12 small (<10kg) breeds were represented. All dogs consumed the feed properly. The mean TAS, visual and clinical mobility reduction scores and pain were significantly (p<0.05) decreased compared to baseline (Figure). 60% of the dogs had their TAS decreased by more than 30%.

Veterinarians and dog-owners reported improvements in clinical signs in 94% of the cases over the 50-days study. The GLM Diet appears an option to alleviate osteo-arthritic signs in dogs.

*Veterinary Diet™ Mobility Support™ Royal Canin, Aimargues, France.

**Keywords**: dog, osteo-arthritis, dry-feed, Mobility Impairment
DETECTION OF REACTIVE OXYGEN SPECIES (ROS) IN FIBROBLASTS EXPOSED TO UVA RADIATION

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Ultra-violet (UVA and UVB) irradiation is considered a major environmental problem and has been linked to DNA damage, cell ageing and cancer in mammals. Cellular damage is thought to occur by the action of reactive oxygen species (ROS) within the cellular environment.

This investigation evaluated the reagents 2',7'-dichlorofluorescin-diacetate (DCFH-DA) and hydroethidine (HE), which measure hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) respectively, for their fluorescent response to UVA induced ROS damage in a primary canine fibroblast cell culture.

DCFH-DA or HE were added to fibroblast cell cultures before or after UVA irradiation, subsequent changes in mean fluorescent intensity (MFI) levels of DCFH-DA and HE were monitored over a 12 hour period by flow cytometry. In brief, fibroblast cells were exposed to a UVA (1J/cm²) source for 30 minutes in an ice bath, to prevent overheating. Care was taken to protect non-irradiated control cells from the UVA source. Non-viable and apoptotic cells were detected using propidium iodide (PI), and the mitochondrial marker tetramethyl rhodamine ester (TMRE), respectively.

The MFI of DCFH-DA was only seen to increase over time when added after UVA irradiation. In these experiments a 140-fold increase was observed in DCFH-DA MFI when compared to non-irradiated control cells (Figure 1). The addition of DCFH-DA prior to UVA irradiation gave an initial twelve fold increase in MFI although, following this increase, little change was subsequently seen in MFI. This initial increase in DCFH-DA when added prior to irradiation is potentially due to photoreactivity of DCFH-DA to UVA, not as a result of cellular ROS levels. HE showed little change in MFI regardless of addition prior or following UVA irradiation. In all experiments a second distinct population of cells was identified, these cells stained positively with PI but not with TMRE, suggesting they were non-viable (necrotic) cells.

Dual staining of the cells with PI and DCFH-DA, added after irradiation, appears to offer the most sensitive method for detecting UVA induced cellular damage in primary canine fibroblasts. This method offers a potential in vitro model system to measure environmental stress at the cellular level.

**Figure 1.** Increase in DCFH-DA MFI, relative to control cells at time zero, in irradiated (filled squares) and non-irradiated cells (open squares).
LYSINE CONTENT IN COMMERCIAL CANINE GROWTH AND MAINTENANCE DIETS CAN BE SEVERELY HEAT DAMAGED

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In foods that are undergoing processing or storage, the free ε-amino group of lysine can react with the carbonyl group of other compounds such as reducing sugars. The formed complex, when digested and absorbed, cannot be utilised by humans or animals. The majority of commercially available dry dog foods are manufactured using extrusion which exposes the food mixture to high pressures and temperatures for a relatively short period of time. The present study investigated the heat damage to lysine in commercially available canine diets.

Fourteen extruded canine growth and 19 maintenance diets were obtained from commercial outlets in Chile and USA. All diets were analyzed for dry matter (DM, by oven drying at 105°C), nitrogen (Kjeldahl method), total and O-methylisourea-reactive lysine (OMIU-reactive, according to the procedure described by Moughan and Rutherfurd, 1996).

Total lysine ranged from 0.68 to 1.83g/100g DM with OMIU-reactive lysine ranging from 0.55 to 1.14g/100g DM in the maintenance diets. The mean total lysine content in the growth diets was 1.37g/100g DM while the OMIU-reactive lysine content was 1.10g/100g DM. The mean crude protein, total and reactive lysine contents of the growth were higher than those in the maintenance diets. The average damage to lysine (an indication of lysine damage can be calculated from the difference between the total lysine and reactive lysine contents of a foodstuff) was higher in the growth diets (20 vs 15%).

<table>
<thead>
<tr>
<th>Component</th>
<th>Maintenance diets (n=19)</th>
<th>Growth diets (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Crude protein (g/100g)</td>
<td>24.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Total lysine (g/100g)</td>
<td>1.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Reactive lysine (g/100g)</td>
<td>0.86</td>
<td>0.18</td>
</tr>
<tr>
<td>Reactive/total lysine</td>
<td>0.85</td>
<td>0.13</td>
</tr>
</tbody>
</table>

¹Values are expressed per unit dry matter

This is the first study reporting damage to lysine in extruded canine diets. Damage to lysine in canine diets, due to the use of ingredients containing damaged lysine (e.g. corn, wheat), extrusion and/or storage, appears to be significant. Although the total lysine content may be sufficient to meet lysine growth requirements (0.51g/100g DM), a large proportion of lysine in diets appears to have been damaged and can be expected to be unavailable to dogs. Studies are underway to determine lysine bioavailability in commercially available canine diets.

Literature cited:
An increased content of fiber in dog food appears to have a clear-cut effect on digestibility of crude nutrients, organic matter and energy. By contrast fiber effect on intestinal mineral metabolism is not unequivocal.

In the present study the effect of 6 cellulose types (microcrystalline versus fibrous: fiber length 60, 300 µm, each from beech as well as from pine and a mixed cellulose) and guar on fecal mineral excretion were investigated in feeding trials with 10 adult beagles (1-6 years, 11-15kg body weight, > 7 dogs per trial, total number of observations n=64). The basal diet consisted of 54% heat dried slaughter by-product, 40.6% isolated maize starch (gelatinized by cooking), and 5.4% sun flower oil. Cellulose was added in an amount of 10% of dry matter, guar was added in an amount of 6% of dry matter. Mineral content did not significantly differ among the diets used, mineral content intake met or exceeded requirements.

The amount of fresh feces was higher when the dogs were given fiber of any type. The extent of the increase of fecal water and fecal dry matter excretion varied between the types of fiber thus generating an opportunity to study the effect of either on fecal mineral excretion. Fecal excretion of sodium was correlated to the amount of feces ($r=0.88$), to the fecal dry matter excretion ($r=0.81$) and to the fecal water excretion ($r=0.85$). In all cases an exponential function gave the best fit. Fecal excretion of potassium was also correlated to the amount of feces ($r=0.75$), to the fecal dry matter excretion ($r=0.59$) and to the fecal water excretion ($r=0.78$). Again an exponential function gave the best fit. Fecal magnesium excretion was exponentially correlated to fecal dry matter excretion ($r=0.58$), but not to fecal water excretion. Fecal phosphorus excretion was exponentially correlated to fecal dry matter excretion ($r=0.74$) and to some extent to the amount of fresh feces and to fecal water excretion. Fecal calcium excretion was correlated to fecal dry matter excretion ($r=0.62$), but not to fecal water excretion.
PRELIMINARY INVESTIGATION INTO GENISTEIN AND DAIDZEIN ABSORPTION IN CATS

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Feline diets contain dietary isoflavones (genistein and daidzein) in concentrations known to exert significant biological activity in mammals1. This pilot study investigated isoflavone absorption and excretion in cats.

After a 12h starvation period, two castrated adult male cats (4.4 and 4.6kg), received an oral dose of 2.7 and 2.1mg/kgBW of genistein and daidzein, respectively, as a tablet with an isoflavone-free meal (providing 35kcal/kgBW). Sequential blood samples were taken through a jugular vein catheter and 12h pooled urine samples were collected up to 120h post-dosing. Samples were analysed by TRFIA2 and HPLC3, respectively.

Approximately 90% of the excreted doses of both isoflavones appeared in the urine within the first 12h with mean (±SEM) total genistein and daidzein excretions of 4.1 (±0.01) and 8.5% (±0.01) of the ingested dose, respectively. Approximately 81% of urinary genistein and daidzein were conjugated to either glucuronic acid or sulfate. Peak plasma genistein concentrations occurred between 0.5 and 1.5h (B). Low plasma genistein concentrations were measurable at 120h. One cat exhibited two distinct peaks in plasma genistein concentration.

Genistein absorption in cats is rapid, although complete elimination from plasma may not occur until after 4 days post-ingestion. Urinary excretion data indicates minimum intestinal genistein and daidzein absorption in cats of 4 and 8%, respectively. This study provides evidence for enteric or hepatic conjugation of genistein and daidzein in cats. The double plasma genistein peak found for one of the cats may represent absorption of free genistein (not requiring deconjugation), or entero-hepatic recycling, as observed in other species4.

References
1Bell et al. The isoflavone content of commercially available feline diets in New Zealand. New Zealand Veterinary Journal (submitted).
2Time Resolved Fluro-Immuno Assay.
3High Performance Liquid Chromatography.
THE EFFECTS OF BRANCHED CHAIN AMINO ACIDS IN CANINE NEOPLASTIC CELL PROLIFERATION

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The use of amino acid supplementation to perturb neoplastic cell growth has been used in many experimental models. Branched chain amino acids (BCAA) are sometimes recommended to ameliorate lean body wasting, and may be of used in the prevention and management of cancer cachexia, and in some cases evidence suggests BCAA may potentiate or inhibit mitogenesis.

Since there is a paucity of information on the effects of BCAA on neoplastic cell growth, we chose to test three canine neoplastic cell lines for their proliferative capacity, soft agar growth, cell survival capabilities, and cell cycling with increasing concentrations of the branched chain amino acids, leucine, isoleucine, and valine. Arginine the most widely studied amino acid for anti-proliferative effects on neoplasia was used as a direct comparison to the BCAA.

Interestingly, leucine (50 mM) exhibited similar anti-proliferative effects when compared to arginine (50 mM) for all three canine neoplastic cell lines. Neither isoleucine (50 mM) or valine (50 mM) exhibited as profound an effect as leucine or arginine. Osteosarcoma cells were the most extensively examined due to their sensitivity to both arginine and leucine. In osteosarcoma cells, arginine and leucine appear to work through different mechanisms leading to caspase activation and cell death at different times, and different points within the cell cycle (See caspase activation below).

Though the amino acid concentrations used to observe apoptotic activity, and profound anti-proliferative capacity, were well above physiologic concentrations, the fact that different mechanisms may be involved in their inhibitory activities is intriguing. Though speculative at this point, our data suggests that amino acids in combination may be able to exert greater inhibitory capacity than supplementation with a single amino acid.
Obesity in dogs is an increasingly prevalent clinical condition, and represents an important challenge to the veterinarian. In humans, obesity is associated with a number of physiological disturbances such as hypertension, diabetes and dyslipemia that increase mortality and decrease quality of life. The relationship between obesity and altered physiology has not been described in dogs. The aim of this study was to assess the relationship between body condition score and blood pressure in a group of 100 dogs living in Spain.

Subjects: A cross-sectional study was carried out during 2004-2005. Data were obtained on 100 client-owned dogs (64 females and 36 males, 30 small, 38 medium, 32 large) recruited at different veterinary clinics during routine visits. All the dogs were urban household pets. Measures included: body condition (BCS, range 1 to 9, Laflamme 1997), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (oscilometric method). Obesity was considered when body condition >6. Hypertension was defined according to the criteria of the Veterinary Blood Pressure Society (SBP >150mmHg and DBP of >95mmHg). Statistical analyses: SPSS (version 12.0) was used throughout. The Kolmogorov-Smirnov test was used to test for normality of distribution. Categorical variables were estimated using the X2 test and the relationship between BCS and blood pressure was assessed by linear regression.

Prevalence of hypertension significantly increased with increased body condition score (Spearman’s correlation 0.43, P=0.01). After controlling for age, there was a small but significant relationship between BCS and DBP (R=0.52, P=0.01) but not between BCS and SBP (P=0.53)

This study shows that obesity in dogs is associated with hypertension. This should be taken into account in veterinary practice, and highlights the importance of weight loss programs to control obesity related health problems.
FOOD SELECTION AND DIGESTIBILITY IN ARATINGA JANDAYA AND ARATINGA AURICAPILLA IN CAPTIVITY

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*Correspondence: Via de Acesso Prof. Paulo Donato Castelane, s/n. Jaboticabal - SP. Brazil ZIP: 14.884-900. e-mail: aulus@fcav.unesp.br

There are very little data on the nutrition and diets for psittacines in captivity. The objective of this study was to evaluate the selectivity and digestibility of a diet based on seeds, fruits and vegetables, commonly given to small psittacines in Brazil.

The study included 10 Aratinga jandaya and 10 Aratinga auricapilla, maintained in individual cages adapted to collection of excreta and food wastage. The diet included sunflower, oat, rapeseed, unhulled rice, birdseed, millet, papaya, beet, banana and kale stem. The food selectivity was determined by the daily average consumption based on dry matter (DM), to estimate this each food was placed in an individual feeder. During 5 days, the amount being offered and the leftovers were weighed. The apparent digestibility coefficients (ADC) were determined by the total collection method, with a 10-day adaptation period and a 5-day fecal collection period. The uric acid was determined in the excreta and was deducted in order to calculate the DM and crude protein (CP) digestibility. Additionally, the nitrogen balance of the birds was determined.

Selectivity was an individual characteristic, but the preference ranking of the foods between the species was different. The average DM consumption was 11.8 grams/bird/day, with no difference between the species. There was no difference (p>0.05) between the species in the average nutritional composition (based on DM) of the diets effectively consumed. The average values were 16% CP, 19.4% ether extract (EE), 50.4% nitrogen-free extracts (NFE) and 11.3% crude fiber. The ADCs of DM, CP, EE and NFE were 89%, 93.4%, 96.6% and 86.5% respectively for A. jandaya and 87.1%, 92.3%, 89.9% and 85.1% for A. auricapilla. Only the ADC of DM was different between the two (p<0.05). The nitrogen balance was 0.14 mg N/bird/day for A. jandaya and 0.098 mg N/bird/day for A. auricapilla.

The diets were well digested by the birds, but the difference in selectivity led to nutritionally heterogeneous diets among the individuals.

Keywords: Aratinga, psittacines, selection, digestion, nutrition
Feeding trials were performed with eight captive black rhinoceroses (Diceros bicornis) from three zoological institutions, quantifying intake by weighing of offered feeds and leftovers, and fecal excretion by total fecal collection. Three to five different zoo rations, consisting of varying proportions of roughage, concentrates, and in some cases browse material, were fed (total n of trials = 32). Representative samples of feeds and feces were analysed for mineral content (Ca, P, Mg, Na, K). Additional data for black rhinoceroses was available from three hitherto unpublished studies (Paros & Dierenfeld, n=2; Woodfine, n=2; Froeschle & Clauss, n=14; not all minerals analysed in all cases). Mineral content was plotted against absorbable (ingested – excreted) mineral content in dry matter, and differences in the resulting regressions to those derived from literature data on domestic horses (Equus caballus) for a comparable range of dietary mineral content were tested by analysis of covariance for macrominerals. In these regressions, the regression slope (a) corresponds to the “true” absorption coefficient, and the negative intercept (b) to the endogenous fecal losses (EFL).

Dietary mineral content ranges, and the regression equations, are summarized in Table 1. Black rhinoceroses had a significantly higher absorption (increased a) of Ca and Mg. EFL of P were significantly lower in black rhinoceroses (difference: 0.07g/100g DM). Regardless of a similar absorption efficiency for Na, black rhinoceroses had significantly higher Na EFL (difference: 0.12g/100g DM). K gave a similar pattern as Na. These data suggest that differences in mineral absorption exist between species. For the management of captive rhinoceroses, the results imply that diets designed according to horse requirements should be adequate, with the exception of Na.

Table 1. Number of observations, dietary mineral content (g/100g DM) range and regression characteristics (absorbable mineral g/100g DM = a * mineral g/100g DM + b) in domestic horses (E. caballus) and black rhinoceroses (D. bicornis). Significant differences to horse data indicated in rhinoceroses by *** (p<0.001)
During The Pet Smile Campaign in Poland, the free dental check-ups were provided for the pets. Those procedures were documented in the standardized questionnaires provided by Dental Working Group of PSAVA.

Each animal was recorded in separate chart in which were included the following information: breed, age, sex of the patient and address of the owner. Apart from this, five other parameters appeared in the chart, and every of them was followed by collecting 0-2 points: 1. Nutrition (home-made and soft food, dry food, mixed) 2. Oral Prophylaxis (daily teeth brushing; other; none) 3. Lymphnodes status (normal, active, enlarged) 4. Dental deposits situation (clear, up to 50%, more than 50%) 5. Periodontal status. (health, gingivitis, periodontitis) In two years’ time over 38,000 valid charts were collected from 730 surgeries involved in PS Campaign. The nutritional aspect of the survey is presented.

### 9074 CATS 23.4 % of surveyed population

<table>
<thead>
<tr>
<th>Oral health</th>
<th>Nutrition profile</th>
<th>Home-made and soft food</th>
<th>Mixed 5208 cats - 57.4%</th>
<th>Dry food 3058 cats - 33.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lymphadenopathy</td>
<td>n</td>
<td>[%]</td>
<td>n</td>
<td>[%]</td>
</tr>
<tr>
<td>No dental deposits</td>
<td>343</td>
<td>42.4</td>
<td>3666</td>
<td>70.4</td>
</tr>
<tr>
<td>No periodontopathy</td>
<td>115</td>
<td>14.2</td>
<td>1474</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>268</td>
<td>33.2</td>
<td>2870</td>
<td>55.1</td>
</tr>
</tbody>
</table>

### 29702 DOGS 76.6 % of surveyed population

<table>
<thead>
<tr>
<th>Oral health</th>
<th>Nutrition profile</th>
<th>Homemade and soft food</th>
<th>Mixed 17138 dogs - 57.7%</th>
<th>Dry food 6683 dogs - 22.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lymphadenopathy</td>
<td>n</td>
<td>[%]</td>
<td>n</td>
<td>[%]</td>
</tr>
<tr>
<td>No dental deposits</td>
<td>3223</td>
<td>54.8</td>
<td>12099</td>
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</tr>
<tr>
<td>No periodontopathy</td>
<td>1012</td>
<td>17.2</td>
<td>4422</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td>2664</td>
<td>45.3</td>
<td>10900</td>
<td>63.6</td>
</tr>
</tbody>
</table>

Conclusion: The number of pets fed with dry food is still not very high in Poland, however this profile of nutrition has significant positive influence on the status of periodontium, lymph nodes and dental deposits.

**Keywords:** dogs and cats oral health, nutrition and oral health
THE EFFECT OF TEXTURIZED VEGETABLE PROTEIN (TVP) ON BLOOD GLUCOSE AND INSULIN CONCENTRATIONS IN DOGS FED HIGH FAT DIETS

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\textsuperscript{2}Texas A\&M University
\textsuperscript{3}Scottsdale, AZ
\textsuperscript{4}University of Minnesota

Traditionally, low fat, high fiber foods are recommended to reduce hyperglycemia in diabetics. Nevertheless, diabetic cats may require less insulin when fed high fat diets and the effect of fiber on glycemic control in animals fed high fat diets is unknown. TVP, an extruded form of soy, contains indigestible carbohydrate that may affect glycemic control. Ten normal hound dogs were fed four canned diets each containing (dry matter) 23\% crude protein, 32\% fat, 31\% nitrogen free extract and 1\% crude fiber in a randomized block design for 1 week/diet. These diets contained reciprocal proportions of protein from TVP (0\% to 57\%) and from beef (100\% to 43\%). Blood glucose and insulin concentrations were measured before and up to 360 min after a daily meal. The power was 80\% to detect changes of 10 mg/dL glucose, 10 U/mL insulin, or 45\% in area under the curve of insulin concentration (AUC\textsubscript{Ins}). Glucose concentrations were unaffected but insulin concentrations increased and the response was different among diets (p<0.05). The AUC\textsubscript{Ins} during the first 120 min was 70\% higher after the 0\% TVP diet than after the 57\% TVP diet but there was no difference over 360 min because substantial irregular insulin secretion occurred from 120-360 min. Insulin concentrations measured in six dogs from 420-720 min after the meal were similar to baseline. Thus, TVP reduced the initial insulin response but this immediate effect was not representative of the whole response. Blood glucose and insulin concentrations should be evaluated for more than 120 min after a complex high fat meal but the insulin response is complete after 7h.
PROTEIN INTAKE DOES NOT AFFECT INSULIN SENSIVITY IN NORMAL WEIGHT CATS

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2Centre de recherche Royal Canin, Aimargues, France
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Diet is known to influence insulin sensitivity. Especially high long term feeding of high carbohydrate diet could be responsible for diabetes in cats. In this species, some authors have then recommended to replace most carbohydrate by protein that would better correspond to the physiology of this carnivorous animal. Moreover, some investigators have also shown that a reduction in plasma insulin and/or IGF1 level would be correlated with increased longevity and retarded ageing. The aim of the study was to compare insulin sensitivity, insulin secretion and plasma leptin and IGF1 in cats fed diets differing in protein and carbohydrate content.

Sixteen normal-weight cats participated in this study that was conducted as a crossover, with two consecutive six-month periods. The extruded diets were isoenergetic either high protein (HP, 528g/kg crude protein) or moderate protein (MP, 297g/kg crude protein). The protein was exchanged isoenergetically for carbohydrate with no other alteration for MP diet. Insulin sensitivity (IVGTT, 0.5g of glucose/kg BW), insulin secretion (arginine stimulation test, 0.1g/kg BW), leptin and IGF1 (radioimmunoassay) were assessed prior to and at the end of the test period.

Plasma leptin level was unchanged after HP or MP chronic diet. The insulin sensitivity and the insulin secretion in response to arginine were the same in cats fed with HP or MP diet. Plasma IGF1 level was not modified after MP diet, whereas it decreased in cats fed during 6 months the HP diet (521±64 ng/ml vs. 369±40 ng/ml, p<0.02).

Similar plasma leptin level in both groups is consistent with their normal weight. Our results suggest that HP would not affect insulin sensitivity nor insulin secretion in normal weight cats. However, the decrease in plasma IGF1 observed in cats fed HP diet level could lead to the hypothesis that high protein diet could protect from diabetes and increase life span in cats.
EFFECT OF DIETARY FERMENTABLE TO NON FERMENTABLE FIBER RATIO ON FECAL PUTREFACTIVE PRODUCTS IN DOGS VARYING IN BODY SIZE

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In dogs, it has been shown that body size affects colonic transit time, and a longer colonic residence time would be related to a poorer fecal quality by promoting fermentation activity. It is also known that dietary fiber can be a major determinant of this activity. The aim of this study was to test the effect of small variations in fermentable to non-fermentable fiber ratio on fecal ammonia and total amines concentrations, particularly in large dogs in which a high fermentation activity was previously hypothesized.

Twenty-four dogs, (six Miniature Poodles (MP): 3.6±0.9 kg, six Standard Schnauzers (SS): 12.8±1.7 kg, six Giant Schnauzers (GS): 23.7±3.2 kg, six Great Dane (GD): 53.6±6.7 kg were used in the study. Four diets differing in fiber amount and fermentable-to-non-fermentable (F:nF) ratio were fed successively for 2-weeks. Diets 1, 2, 3 and 4 contained a F:nF of 0.15, 0.15, 0.36 and 0.48 with 7.7, 9.7, 8.9 and 9.2% TDF, respectively. Ammonia and total amines (putrescine + cadaverine + spermidine + tryptamine + tyramine + histamine) were measured in fresh feces collected on the last day of the feeding period and frozen pending the assay.

We found higher ammonia concentrations in Great Danes (GD) compared to smaller breeds, whatever the diet (from 90.3±16.7 to 169.3±19.3 mmol/gDM with diet 1, respectively for Miniature Poodles and GD). Diets had no effect on ammonia concentration. There was no difference in total amines concentration according to body size. For all breeds, total amine concentrations were significantly lower (50% lower) with diet 4 compared to diet 1.

Fecal ammonia concentrations were higher for large compared to small dogs, whatever the diet. This could confirm a higher fermentation activity in large breeds. We found no difference between breeds in fecal total amines concentrations, but a significant decrease was observed with diet 4. This decrease was higher than with fermentable carbohydrates supplementation observed in other studies. Regarding amine production, dealing with a suitable F:nF ratio could be more efficient than considering only the amount of fermentable fiber.
DOES BUDGERIGAR LYMPHOCYTE DNA CONTAIN A HIGH AMOUNT OF ALKALI-LABILE SITES?

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The Comet Assay is commonly used to assess the levels of DNA damage in a cell population. It has been used to measure the protection offered by diets rich in antioxidants. The most common form of the assay involves electrophoresis at pH13 to reveal the damaged DNA containing both single strand breaks and a variety of alkali sensitive sites.

Using this assay on leukocytes isolated from budgerigar (Melop isittacus undulatus) blood revealed a surprisingly large amount of DNA damage. By repeating the assay at pH12 a lower level of DNA damage was seen, suggesting that the DNA damage seen in budgerigar leukocytes was due to the presence of alkali labile sites rather than single strand breaks. Treating isolated leukocytes with hydrogen peroxide, which induces mainly single strand DNA breaks, increased the level of DNA damage revealed by electrophoresis at either pH equally. Alkali labile sites can be produced by the direct action of reactive oxygen species on DNA, but are also produced in large numbers by the action of repair enzymes that remove damaged nucleotides to produce abasic sites. Despite birds having several methods of protection from oxidative damage, this data suggests that a high level of DNA damage is still being produced in budgerigar blood. Potentially the large numbers of alkali sensitive sites are a consequence of this and are due to oxidatively damaged bases continually being repaired.

Potentially, therefore, when the effect of antioxidant-rich diets for budgerigars are being assessed, the Comet Assay should be carried out at both pH12 and pH13, in order that different types of DNA lesions can be measured.
VALIDATION OF A NEW 7-POINT ALGORITHM-BASED BODY CONDITION SCORE SYSTEM

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Numerous methods exist for quantifying body composition and body fat mass in companion animals. In a clinical setting, the most widely accepted and practical method of body condition evaluation is body condition scoring, using visual assessment and palpation. A 9-point, dual-energy X-ray absorptiometry (DXA)-validated system exists, but training is required and significant between-operator variability can exist. ‘SHAPE’ (Size, Health And Physical Evaluation) is a new 7-point algorithm-based approach, designed to minimise inter-operator variability, and level of expertise required. The current study assessed ability of the SHAPE system to predict body composition in dogs and cats.

32 dogs and 10 cats, referred to the Small Animal Hospital (SAH), University of Liverpool (UOL), participated in the study. All subjects were client-owned and either referred for investigation of obesity to the Royal Canin Weight Management Clinic UOL, or to the SAH for investigation of other problems. All animals were independently condition scored by two operators (A. German and S. Holden) with experience of the SHAPE system, whilst patients were also independently scored by their owners. Subsequently, all animals were sedated, and were scanned in dorsal recumbency with a fan-beam DXA (Lunar Prodigy Advance; GE Lunar; Madison, USA).

Correlation between the experienced operators was excellent for both dogs (Rs=0.959, P<0.0001) and cats (Rs=0.999, P<0.0001). Correlation between the owner-determined SHAPE score, and scores determined by experienced operators was good for dogs (Rs=0.742 [p=0.014] and 0.735 [p=0.015] for client versus A. German and S. Holden, respectively) and cats (Rs=0.777 [p=0.023] for clients versus both A. German and S. Holden). Regression analysis demonstrated a significant association between body condition, as determined by the SHAPE system, and body fat percentage in both dogs (R²=0.746, p<0.0001) and cats (R²=0.723, p=0.001).

The SHAPE system correlates well with body composition, and agreement between experienced operators is excellent. Good agreement exists between measurements by experienced operators and clients, suggesting that the method is reliable when used without prior training.
Obese dogs exhibit lipid metabolism disturbances similar to those observed in humans. Dual inhibition of cholesterol synthesis and absorption is a new strategy for treating hypercholesterolemia. It is especially supported by the enhanced cholesterol absorption that would occur under atorvastatin (an HMG-CoA reductase inhibitor) treatment. However, the cellular mechanisms of the cholesterol transfer from intestinal lumen to the secretion of chylomicron in lymph are poorly described. On one hand, the scavenger receptor class B type I (SR-BI) has been identified in intestine, but its role in cholesterol absorption remains unclear. On the other hand, the microsomal transfer protein (MTP) mediates the assembly of chylomicrons and represents the apolipoprotein B (apoB)-dependent pathway of cholesterol absorption. In order to get further insights into the role of these proteins, we studied the effects of atorvastatin on cholesterol absorption in dog.

Seven beagle dogs were treated with atorvastatin (5mg/kg/day) for 6 weeks. Percent cholesterol absorption was assessed, before and after treatment, by the dual isotope method, using mass spectrometry. Plasma lipids, MTP activity, SR-BI expression and lipid content in duodenum extract were also assessed before and after treatment.

Atorvastatin treatment decreased plasma total cholesterol (4.82±0.18 to 3.33±0.23mmol/l, p<0.05), phospholipids (4.52±0.16 to 3.53±0.08 mmol/l, p<0.05) and triglycerides (1.03±0.09 to 0.82±0.07 mmol/l, p<0.05). After atorvastatin treatment, percent cholesterol absorption was higher (74.9±2.9 to 91.5±2.8 %, p<0.05) and total cholesterol in duodenum was lower (0.78±0.18 to 0.64±0.14mg/g, p<0.05), while triglycerides and phospholipids did not change. Neither MTP activity nor SR-BI protein expression assessed by Western blot did change.

Our data suggest that in dog atorvastatin would upregulate cholesterol absorption by an apoB-independent pathway. Moreover, the present study shows that SR-BI would play a minor role in cholesterol absorption by intestinal cells.

**Keywords**: cholesterol absorption, SR-BI, MTP, dog
As in human beings, it has been shown that life span can be shortened in overweight and obese dogs. It has been established that obesity is closely associated with insulin resistance, type 2 diabetes, and other chronic diseases such as hypertension and dyslipidemia. Fenofibrate is a member of the fibrate class that mainly exert hypolipidemic actions via the activation of peroxisome proliferator-activated receptors alpha. Fenofibrate markedly reversed obesity and corrected insulin resistance and lipid profile in several species. The aim of this study was to examine whether fenofibrate was effective on the lipid lowering in obese dogs.

In order to investigate this question, six adult obese beagle dogs were administered fenofibrate, 10mg/kg daily just before their meal, for 15 days. Jugular vein blood samples were collected on day 0 (D0), day 8 (D8) and day 15 (D15) in 24h-unfed state and 6 hours after feeding (the time when the maximum plasma concentration in fenofibrate is reached). Plasma triglycerides, total and free cholesterol, free fatty acids and phospholipids were measured in blood samples.

Fasting triglycerides, total and free cholesterol, free fatty acids and phospholipids concentrations significantly decreased by 29%, 30%, 34%, 30% and 25%, respectively. In postprandial state, they were also reduced by 33%, 25%, 20%, 30% and 15% respectively. Body weight slightly but significantly decreased by 3%.

These results support the efficiency of fenofibrate as a hypolipidemic agent in hyperlipidemic obese dogs.
Energy digestibility in dog and cat food can be predicted by linear regression of the fiber content on dry matter basis. This has been demonstrated for crude fiber (AOAC method) in both species and for Total Dietary Fiber (Englyst-method) in dogs. However, this last study, were based on a small number of observations for total dietary fiber (TDF; by AOAC-method) and energy digestibility. Consequently there was not enough data points (independent variable TDF, dependant variable experimentally determined energy digestibility) to calculate a reliable regression equation.

In the present study 610 digestion trials with dog food and 261 digestion trials with cat foods were evaluated in retrospect. Digestion trials were carried out according to AAFCO-protocols. Energy in food and feces was determined by bomb calorimetry. In 495 of the 610 trials with dog food crude fiber (CF) content was also analyzed. The following regression equations between energy digestibility (%) and TDF in % dry matter (DM) were calculated from the resulting data: dog food: energy digestibility = 96.6 - 0.96 x TDF (% DM); r=0.94** (** p<0.01). Cat food: energy digestibility = 95.6 - 0.89 x TDF (% DM); r=0.88**. For CF in dog foods the regression equation was: energy digestibility = 92.9 - 1.6 x CF (% DM); r=0.87**. Confirming earlier results CF proved useful to predict energy digestibility in dog food. TDF may give even better results than CF.
Riders often presume that problems with their horses such as unresponsiveness or stiff back are caused by problems of muscle metabolism, and may be alleviated by supplementation of extra vitamin E and/or selenium. These popular theories were used to create a model for the potential of placebo effects.

Ninety sports horses with a history of mild muscle problems were given either placebo or extra selenium or vitamin E (2 times requirements) for 120 days. One horse was withdrawn because of behavioural problems, five others for reasons unrelated to the study. All horses ate typical rations (hay, grain, supplements), there were no indications of selenium or vitamin E deficiency. Vitamin E and selenium plasma levels were determined before and after supplementation and the riders were interviewed using a standardized questionnaire. Questions aimed at the above mentioned problems as subjectively perceived by the riders.

Wherever possible responses were scaled from 1 meaning that this particular issue was not a problem for this horse to 7 identifying it as a major problem. In the selenium group plasma selenium tended to higher values after supplementation (87±33 vs. 96.7±31 µg/100 ml). Vitamin E plasma levels were within normal range in all horses (4.2±1.6 µg/ml). They decreased significantly during the study, independent of supplementation. Several subjectively perceived parameters such as unresponsiveness, fretful mouth, too hot improved significantly. Unresponsiveness improved in 6 horses from 7 to 1.

The results demonstrate that statements on the success of treatments for comparable problems are entirely valueless without a double blind placebo control. The study does not exclude that the supplementation of vitamin E or selenium above the requirements has any effects on the muscles of sport horses because the placebo effects may have masked smaller differences between groups.
ROUGHA GE / CONCENTR AT E RATIO MODIFIES THE EFFECT OF AMMONIUM CHLORIDE ON ACID BASE BALANCE IN HORSES

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Acid-base balance is an important consideration in the management of hypokalemic periodic paralysis, urolithiasis, and exercise induced acidosis. Reports on the effects of acidifying diets in horses are contradictory. It was hypothesized that the roughage to concentrate ratio might have considerable influence on acid base balance. In the present investigation the effect of ammonium chloride on acid base balance was investigated in two isoenergetic rations with a roughage to concentrate ratio of 7:1 and 2.3:1 respectively.

Four ponies (250-350kg BW) were available for the investigation. The basal rations consisted of either 1.1kg grass hay and 150g oats per 100 kg of BW (diet HAY) or 30g grass hay and 700 g oats per 100kg of BW (diet OATS). Ammonium chloride was added at two levels 4.7 and 9.4g per 100 kg BW (cross over experimental design). Cation-anion-balance (CAB) in the food was calculated as follows: CAB mmol/kg DM = 49.9Ca+ 82.3Mg+ 43.5Na+ 25.6K- 59.0P- 13.0Meth/Cys- 28.2Cl (concentrations in g/kg DM). The ponies were fed twice (8.00am, 4pm). They were adapted to the diets for seven days (after a washout-period of seven days between experiments), then urine and feces were collected for three days. The ponies were kept on a paddock with plastic sheeting for several hours and urinated when they were allowed to return to their stalls. Urine was then manually collected with a bucket. pH was measured immediately after collection. In order to get an estimate of potential large intestinal acidosis the fecal pH was measured in the OATS diet (mean 6.6, no values below 6.2). Blood pH was measured from arterial blood before and after feeding.

In the HAY diet ammonium chloride only had a mildly acidifying effect (Table 1). But the same amount of ammonium chloride had a strong acidifying effect when added to the OATS diet. Because of the basal nature of the two diets the CAB differed between them even when the same amount of ammonium chloride was added. The basal OATS diet and the HAY diet with the smaller amount of ammonium chloride however, had a comparable CAB (131 versus 111 mmol/kg DM), which could still be considered to be slightly alkalizing. Urine pH was higher in the OATS diet. The OATS diet with the smaller dose of ammonium chloride and the HAY diet with the higher dose were also comparable with a CAB usually considered to have an acidifying effect (CAB 53 versus 43 mmol/kg DM). However, such an effect was only found for the OATS diet. Blood pH showed a similar pattern. The results indicate a difference in acid-base-metabolism between horses fed predominantly with grain and horses fed predominantly with hay.

| Table 1: Average urinary pH (mean ± SD; rations with comparable CAB in the same column) |
|---------------------------------|---------|---------|------|
| **Ration**                      | Hay-based diet: HAY | Grain-based diet: OATS |      |
| CAB mmol/kg dry matter          | 179     | 131     | 53   |
| NH₄Cl g/100 kg body weight      | 0       | 4.7     | 4.7  |
| Urine pH                        | 7.48±0.25a | 7.36±0.19b | 7.29±0.18b |
| Blood pH before feeding         | 7.45±0.01a | 7.45±0.01a | 7.40±0.02bc |
| **CAB mmol/kg dry matter**      | 43      | 53      | 26   |
| NH₄Cl g/100 kg body weight      | 9.4     | 4.7     | 9.4  |
| Urine pH                        | 7.29±0.18b | 6.78±0.53d | 5.30±0.17e |
| Blood pH before feeding         | 7.40±0.02bc | 7.39±0.03bc | 7.36±0.03c |

Means not sharing a superscript letter are significantly different.

The study was funded by the H. Wilhelm Schaumann Foundation.
DIET, EXERCISE AND WEIGHT AS RISK FACTORS IN HIP DYSPLASIA AND ELBOW ARTHROSI S IN LABRADOR RETRIEVERS

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Secondary arthritis from hip and elbow dysplasias is a significant cause of lameness and euthanasia, especially in large sizes of dogs. Among the environmental factors involved, overfeeding have proven (experimental studies) to be detrimental to both hip and elbow dysplasia. Over-supplementation of calcium (experimental/epidemiological studies) has been shown to be a risk factor for osteochondrosis, one of the main constituents of elbow dysplasia.

An epidemiological retrospective case-control design was applied within a birth-cohort of Labradors screened at an age of 12-24 months and surveyed shortly after that. The dogs were matched by sex and age. From the registries at the Swedish Kennel Club, 325 Labradors born in the year 2000 with known status on both hip and elbow conformity were allocated as cases or controls. The dog owners were sent a previously validated questionnaire containing 39 questions about demographics, appetite, feeding patterns, diet, exercise, living patterns, weight and owner perceived body condition score. After two weeks they were interviewed by telephone. Most dogs were also born from both sires and dams unaffected by both conditions.

The response rate was 90% (292/325) and equal for cases and controls. Owner perceived appetite as well as body condition score, proportion fed home-made versus commercial diets and dogs given treats were evenly distributed between cases and controls for both diseases.

Indicated risk factors for both hip dysplasia and elbow arthrosis were when feeding the dogs ad lib, and exercise by repeatedly throwing balls and sticks. For elbow arthrosis, body weight as well as high intake and proportion of energy from fat was also shown to be significant risk factors.

The conclusion is that in Swedish Labradors bred for hip and elbow conformity feeding patterns, diet, weight and exercise have to be taken into account as proven and significant risk factors.
CHEMICAL COMPOSITION OF TURTLES AND TORTOISES
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Carcasses of 91 turtles and tortoises (six species) of different ages were analysed. The carcasses originated from commercial animal traders and private persons. Shell, femur bone, liver and the remaining body (without gut and bladder content) were separated if possible. Proximates, major minerals and trace elements were analysed. In adults the shell weight amounted to about 30% of the body weight.

In hatchlings shell weight amounted only to 20% of body weight. There was no obvious difference between species. The shells of tortoises contain more water than those of turtles (51.6% versus 29.9%). In hatchling tortoises, water content of the shell amounted to an average of 70%, whereas the shell of adult tortoises contained only about 34% water.

In average, the calcium content in dry matter in shell was higher in turtles (247mg Ca/kg dm) than in tortoises (163mg Ca/kg dm). In tortoises calcium content in shell dry matter increased from hatchling to semi-adult. Iron content in shell decreased in tortoises from about 150mg/kg dm in hatchlings to 65mg/kg in adults. By contrast in turtles it increased from 250mg/kg dm in juveniles to 670mg/kg in adults.

These values are still lower than iron content of body without shell and liver. Zinc content in shell showed small species and age effects. It ranged from 93-236mg/kg dm. The zinc content of body without liver was in a similar range. Copper content of shell did not show any effects of species or stage of life. It amounted to 4.5mg/kg dm compared to an average body content of 5.3mg/kg dm. Liver copper content showed a greater variation and a higher mean (27mg/kg dm) in tortoises than in turtles (mean 12mg/kg dm). It was considerably lower than in most domestic animals.
A NOVEL NON-INVASIVE TECHNOLOGY (ELECTROSONOPHORESIS) TO MEASURE PLASMA GLUCOSE LEVELS IN CATS

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A new, sophisticated, non-invasive blood sampling technique (electrosonophoresis, ESOP) has been developed in our group1. It utilises ultrasound and low voltage to create a transdermal flux between underlying blood vessels, extracellular fluid and the skin and an osmotic gradient to collect the exudate, which is concentrated and processed similar to normal blood serum. This study aimed to investigate the potential of the ESOP technique in cats.

Twenty adult cats sedated with an injection of Domitor® (80mg/kg)/ Ketamine (5mg/kg) i.m. for routine 6-month dental examination were blood sampled by jugular venepuncture from one side of the neck. A commercial ultrasound conductive gel was applied to the contra-lateral site and exudate samples were obtained using the ESOP apparatus. Samples were collected during application of continuous ultrasound (1MHz frequency and 0.35 W/cm² power) for 1 minute using a specialised collection head fitted over the end of the ultrasound wand. Collection occurred through a semi-permeable dialysis membrane, and was augmented with a 9-volt driven electric field. The exudate was collected in 70% alcohol which was pumped through a collection chamber beneath the membrane. Serum samples were analysed for glucose on a Cobas Fara Analyser. Exudate samples were concentrated by blowing over nitrogen gas and by applying vacuum. Samples underwent a pre-derivitisation step and were then eluted into a HPLC microinjection system and analysed for glucose, against standards.

The study established that glucose was present and could be measured from exudate samples when blood glucose was at the high end of the normal physiological range. A strong linear correlation ($R^2>0.99$) to serum levels was observed across this range (Figure). However, glucose levels in the exudate samples were 0.01% of blood serum levels.

An improvement of exudate collection parameters should enable measurement of serum glucose across the full physiological range.

References

EX VIVO PLATELET FUNCTION IN TAURINE DEFICIENT DOGS
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Taurine (Tau) deficiency is clinically characterized by low blood taurine concentrations in cats. Upon taurine depletion in cats, platelet taurine concentration decreases, and as a result, the ex vivo aggregation potential increases1. This study was designed to determine whether taurine depletion in dogs affects platelet taurine concentration and function.

Fifteen milliliters of blood were collected from seven male adult taurine-sufficient dogs (Tau+) and six male adult taurine-deficient dogs (Tau-). CBC, platelet isolation and taurine analysis were performed in all samples using standard procedures. Ex vivo platelet function was measured by optical aggregometry (OA) and whole blood electric impedance aggregometry (WBEI).

Blood taurine concentrations were 201±18 and 89±15nmol/mL (mean±SEM; P=0.001), for Tau+ and Tau- respectively. CBC counts were not different between groups. Platelet taurine concentrations were greater (P=0.01) in Tau+ than in Tau- (464±47 and 212±64 nmol/106 platelets respectively). Blood taurine was better correlated to platelet taurine concentrations in the Tau+ group (R=0.94) than in the Tau- group (R=0.63). Nonetheless, blood and platelet taurine concentrations were well correlated (R=0.87) when the data was analyzed as a whole [Blood taurine (nmol/mL) = 0.3671*platelet taurine(nmol/mL) + 19.1; R² = 0.76]. Ex vivo collagen-stimulated aggregation (OA) did not differ between groups, 63±8 and 51±12% for Tau+ and Tau- respectively (P=0.63). Lag time (WBEI) was not different between the groups (00:03:54±00:01:45 Tau+ vs. 00:01:30±00:01:28 Tau-; P=0.16). Once aggregation was detected, platelets from Tau+ dogs tended to aggregate faster than of Tau- dogs (slope of 2.6±0.6 vs. 1.3± 0.2 respectively; P=0.06).

These results show that platelet taurine concentrations are directly correlated with blood taurine concentrations. Taurine deficient platelets do not seem to be hyper-reactive in dogs with mild taurine deficiency. Further studies with a larger number of dogs with more severe taurine deficiency are needed to determine if there is a difference in aggregation function in taurine deficient dogs, ex vivo and clinically.

Keywords: taurine, platelet, dog

References
EFFECTS OF BOVINE COLOSTRUM IN CANINE SKIN FIBROBLAST PROLIFERATION

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Bovine Colostrum (BC) is a rich source of bioactive components such as immunoglobulins, IGFs, epidermal growth factor, tumor necrosis factor, basic fibroblast growth factor, vaso-endothelial growth factor and telomerase. Moreover, BC has shown several therapeutic functions as gastroenteritis and intestinal damage prevention, improvement of tissue repair and athletic performance, and reduction of the incidence of upper respiratory tract infections. The aim of this assay was to evaluate the effects of a freeze dried BC source (Coplexan, Ultra Grade, Colostrum Technologies GmbH, Germany) as an in vitro stimulator of canine skin fibroblasts proliferation and activity. This in vitro model is used as a model for wound repair efficiency, which is commonly impaired in senior dogs and stressed animals as dogs after a surgery.

Fresh abdominal skin samples (n=4) for this assay were obtained from dogs during routine surgery. Cultures of normal canine dermal fibroblasts were established by enzymatic digestion of canine skin, and fibroblasts were used at passages 2-5. Fibroblasts proliferation was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells were plated at a density of 10,000 cells/100 ml culture medium with different BC concentrations (0, 0.1, 0.3, and 1mg/ml) and incubated at 37 ºC in 5% CO2 humidified atmosphere for 24 and 48 h. MTT (10 ml) was added to 100 ml of medium per well and incubated for 4h. Finally, well absorbance was read using a multiwell scanning spectrophotometer (ELISA reader) at 570nm. Relative activity (RA, %) of each BC dose was estimated as the cell growth ratio to the control, and was:

In conclusion, Bovine Colostrum significantly (p<0.05) stimulated dog fibroblasts growth (20 to 32%) at any dose used (0.1 to 1mg/ml) at 24h culture incubation, and the effect remained significant at 48h for the dose of 0.3 and 1mg/ml (12-30%).

![Fibroblast activity stimulated by colostrum](image)
PROTEIN TURNOVER IN LACTATING MINK DAMS
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The mink is a strict carnivore, and may therefore serve as a model for the cat. Rapid kit growth is supported by a high milk yield and we have previously shown superior milk yield in dams fed low protein diets (Fink et al., 2004, 2005). The objectives of this study were to develop a 15N-glycine end-point method for measurement of protein turnover in lactating mink dams and to evaluate if different levels of dietary protein supply did affect protein synthesis or breakdown.

Twelve mink dams, six fed a medium (M) protein diet (41:43:16% of ME from protein, fat and CHO) and six fed a low (L) protein diet (28:44:28% of ME from protein, fat and CHO) were used. Measurements in lactation weeks 2, 3 and 4 included N-balance, respiration experiments and 15N-glycine end-point measurements (oral dose 5mg/kg BW; urine collected and weighed 0, 6, 12, 24, 36 and 48h after dose). Flux, synthesis, breakdown and net protein synthesis were calculated. Milk N excretion was estimated from milk intake per g kit gain and chemical composition of milk (Tauson et al., 2004).

Kit growth was not affected by protein supply, indicating similar milk yield in both groups. This was probably caused by poorer dietary amino acid composition than in previous investigations (Fink et al., 2004, 2005). There was a tendency for higher flux among M dams and for flux to increase with period, but there were no diet effects on synthesis and breakdown. Net synthesis increased with period. There was a fair agreement between net synthesis and estimated N excretion in milk.

Keywords: protein supply, milk yield, protein turnover

References
energy expenditure and water turnover in hunting dogs

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Hunting dogs display an extraordinary working capacity. They can work for several hours a day, for a week or more and cover considerable running distances. Knowledge of their energy expenditure is therefore important to allow for precise feeding recommendations. This study aimed at measuring energy expenditure (EE) in relation to running distance during three different types of exercise: hunting, running on roads beside a bicycle, and running on a treadmill (not presented). Body water turnover was also examined, since water intake is considered crucial for high endurance. Two dogs (Brittany spaniels) with different running willingness were used in the experiments. In the hunting session the dogs ran for three hours a day over seven successive days (high activity), followed by a seven-day period of low activity. Similar procedures were followed in the road running session. Running distance in the high activity period was recorded by GPS. The doubly labelled water technique (H2O18) was used to measure energy expenditure, and H2 turnover was calculated to determine water turnover.

During the hunting period, the difference in running willingness between the two dogs was demonstrated by the different distances recorded by the GPS. The EE for the two dogs were different, and EE was highest for the dog that ran the longest distance (Dog 2). If the EE during the low activity period is used as estimate for maintenance energy consumption, the EE associated with activity amounted to between 45 and 201kJ/km during hunting and road running. The large variation may be due to different temperatures and the fact that the GPS only recorded horizontal movements. There was a discrepancy in body water content for Dog 2 between the two sessions, which may have affected the EE measurements. Body water turnover was highest during the heavy exercise periods.

Hunting dogs have high EE and water turnover during high activity performance. Extra attention should therefore be given to provision of a high energy diet and abundant water supply during these periods.

<table>
<thead>
<tr>
<th>Body weight, body water, running distance, energy expenditure and water turnover in hunting dogs.</th>
<th>Body weight (kg)</th>
<th>Body water (%)</th>
<th>Distance run per day, km</th>
<th>Energy expenditure kJ/day</th>
<th>Body water turnover g/day</th>
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<tr>
<td><strong>Hunting period</strong></td>
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<tr>
<td>Average temp: -9º C</td>
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Hunting dogs have high EE and water turnover during high activity performance. Extra attention should therefore be given to provision of a high energy diet and abundant water supply during these periods.
EFFECT OF EXERCISE ON NUTRIENT DIGESTIBILITY IN HUNTING DOGS
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Dogs used for bird hunting have a high energy requirement during working seasons due to prolonged heavy exercise. Common practice is to feed the dog after the daily hunt and to avoid large meals immediately before and during physical activity as this may inflict with digestive processes. This study aimed at studying nutrient digestibility in six hunting dogs during a 4-day period of exercise (30 km per day on a treadmill) compared with corresponding digestibility in a resting period without exercise. The experimental food was an extruded dog food added 0.01% yttrium oxide (Y2O3) as inert marker. Exercise periods were divided into four 7.5 km runs at 10km/h. Total length of stay in the laboratory was six hours per day including breaks of 30 minutes each. The GLM procedure of SAS was applied to test differences in digestibility.

The dogs (two Brittany spaniels, two Gordon setters, one English setter, and one German pointer) were offered water ad libitum and consumed 20-25% of the daily food ration between the daily runs. The rest of the food was consumed before and after the daily exercise period.

Digestibility values were not significantly affected by the exercise (P<0.05). The digestibility values of two dogs were surprisingly low during resting and were therefore excluded.

Average digestibility (%) of main nutrients during resting and exercise. SEM = standard error of the mean.

<table>
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<th>Activity</th>
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<th>SEM</th>
<th>P-values</th>
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</table>

The experiment showed that exercise on a rather moderate level had no impact on nutrient digestibility in dogs.
THE EFFECT OF SUPPLEMENTATION WITH BRANCHED-CHAIN AMINO ACIDS* ON COGNITIVE FUNCTION IN ACTIVE DOGS
Fretwell, L.K.*, McCune, S., Fone, J.V., Yates, D.J.
WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK
*Patent granted
**Correspondence: laura.fretwell@eu.effem.com

Branched chain amino acids (BCAA) ingested prior to, and during, a cross-country race improved the cognitive performance of human athletes. The aim of this study was to determine if BCAA had a similar effect on cognition in exercising dogs.

The dogs (n=24) represented two age groups; young (aged 1.5-3.0 years) and senior (>8 years). All dogs underwent 7 weeks training over a standard, 7-obstacle agility course. The test comprised 3 consecutive circuits of this standard (familiar) course, followed immediately by a fourth (novel) course, which was sited in a different area, using a different configuration of the apparatus. BCAA were provided approximately 2.5 hours before the start of the test to the supplemented group (n=12) in the form of a low protein, high carbohydrate rice cake coated with a baste of valine (40%), leucine (35%) and isoleucine (25%) in a carbohydrate solution (7g/100g water). Unsupplemented dogs received the rice cake with no BCAA.

There was no effect of supplementation or sex on baseline performance (counting the number of errors made over course 2 (familiar)), although young dogs tended to make more errors compared to senior dogs. This age-effect was significant over course 4 (novel), with young dogs making almost double the number of errors compared to senior dogs, but there was no effect of supplementation, breed or sex. However, following correction for baseline performance, errors over course 4 (novel) tended to be less in supplemented compared to unsupplemented dogs irrespective of age.

These findings suggest that BCAA may improve the ability of dogs to sustain cognitive performance during exercise which may impact on overall performance.
THE EFFECT OF ALTERING ENERGY DENSITY ON THE REGULATION OF INTAKE IN THE CAT
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WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

Historically it was believed that the domestic cat had a unique ability to regulate its intake. Approximately 40% of cats however are overweight\(^1\). Some species, (locust and rat) regulate intake in the face of a variety of dietary challenges. No long-term large-scale studies however have been conducted in cats.

Forty-eight cats participated in a randomised, 70-day, three-way cross over study with 10 weeks in each arm. Cats were matched for age, sex, weight and body condition score. Each cat was presented with one of three trial diets, twice daily, for a period of one hour. Diets varied in energy density by manipulation with water (Diet A 59kcal/100g, Diet B 89kcal/100g, Diet C 120kcal/100g). 400g were presented in the morning and any uneaten food plus an additional 200g presented in the afternoon. Cats were weighed twice weekly and body composition was determined using DEXA at the beginning and end of each trial phase.

Due to problems associated with refusal to eat and excessive weight loss with diet A, 22 cats were withdrawn from the study (two days refusal of food, 5% weight loss). Data from 26 cats on Diets B and C only are available for analysis. There was no significant effect of diet order on any of the measured parameters. Overall, there was no significant difference in caloric intake over time with either diet B or C. Individual cats however demonstrated a variety of intake behaviours with 9 cats demonstrating some ability to adapt volume eaten to maintain caloric intake. There was also no significant difference in body mass with either diet B or C. Only four cats maintained a body mass within 5% of baseline and 12 cats within 10% of baseline on both diets.

There is no evidence from this study of ability to regulate caloric intake from diets with varying energy density.
Owners of five canine breeds (Beagle, Cocker spaniel, Labrador, Dachshund and Rottweiler) were recruited to answer to a questionnaire on ‘feeding habits’ of their pet. The questionnaire was available during five weeks on internet and was composed of 65 questions (multiple choice or other) dealing with dog’s description (including a Body Condition Score – BCS – fixed by the owner on a 5-points scale), exercise and housing, human-dog relationships, dietary habits, medical records, perception and prevention of the risk of obesity by the owner and profile of the owner. The goal of the study was to compare normal (BCS=3) and overweight or obese dogs (BCS>3) for all parameters. Differences between the two groups were examined using independent chi square tests.

The total number of answers was 517, in which 501 were considered as valid. 470 questionnaires were related to dogs with a BCS of at least 3 according to the owner, and BCS was >3 for 22.8% of the dogs. There were significant differences between the 2 groups for parameters as spontaneous activity, gender, age, evolution of adult body weight, the number of- and time spent to exercise, the appetite, the response to the dog’s begging for food, the type of diet, the number of treats and snacks, the fact of knowing or not precisely the daily quantity of food, the medical record, the perception of obesity as a serious risk for the health of the dog and age of the owner.

However, there was no difference according to BCS for housing, sleeping habits, place and time of feeding, the fact of feeding snacks, feeding costs, means used by the owner for the prevention of obesity. BCS was not related to characteristics of the owner as gender, control of body weight, job, diploma and body mass index.
Obesity is the most common nutritional disease in dogs. In human subjects or in dogs, obesity is frequently associated with modifications in blood lipid concentrations. Short-chain fructooligosaccharides (sc-FOS) are synthetic indigestible oligosaccharides of small length (2 to 4 units) which have prebiotic and dietary fibre properties. Sc-FOS supplementation is sometimes used in hyperlipidemic obese patients according to their hypothesized hypolipidemic properties. The present study in the dog investigated the long term effects of weight loss (WL) induced by a low-energy high-protein diet, offered alone or supplemented with sc-FOS, on blood lipids concentrations.

Twelve chronically obese Beagle dogs of both genders and aged between three and nine years were used. Mean body weight (BW) was 22±0.8kg. The obese dogs were allotted in two subgroups and submitted to a WL protocol with a commercial low-energy high-protein diet supplemented or not with 2% sc-FOS until they reached their optimal BW. Finally, the dogs were maintained at their optimal BW for 6 months; during this period, dogs were fed a maintenance diet alone or supplemented with 3% sc-FOS.

Weight loss with low-energy high-protein diet, supplemented or not with sc-FOS, resulted in a significant decrease in blood lipid concentrations and this decrease was maintained during the 6 months of observation at constant BW. Although the hypolipidemic effects of sc-FOS during the weight loss protocol were not always significant, straight decreases in blood lipid concentrations were observed, mainly for total plasma cholesterol, HDL cholesterol, total plasma triacylglycerol and VLDL triacylglycerol concentrations.

Footnotes
134% protein, 10% fat, 15% carbohydrate, 15% crude fiber, 8% water, 2900 kcal/kg, as is
224% protein, 16% fat, 38% carbohydrate, 2.5% crude fiber, 8% water, 4100 kcal/kg, as is
INVESTIGATION OF FELINE URINARY \( \gamma \)-GLUTAMYLFE LINYLGLYCYNE METABOLITES
Rutherfurd-Markwick, K.J.\(^1\), McGrath, M.C.\(^1\), Hendriks, W.H.\(^2\)
\(^1\)Institute of Food, Nutrition & Human Health, Massey University, Palmerston North, New Zealand
\(^2\)Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University and Research Centre, Wageningen, The Netherlands

Felinine is a unique amino acid excreted by certain Felidae species, and thought to be a precursor to odorous urinary volatiles. We recently demonstrated that felinine is transported in cat blood as part of a tripeptide: \( \gamma \)-glutamylfelinylglycine, which is synthesised through a condensation reaction of isopentenylpyrophosphate and glutathione. We also showed that the renal metabolism of the tripeptide occurs in a similar manner to that of glutathione conjugates in other mammalian species by showing that N-Acetyl-felinine (NAc-felinine) is present in the urine of cats.

The aim of this study was to investigate the ratio of felinine to NAc-felinine in urine from cats of different gender. Twenty-four hour urine samples were collected from 30 cats (10 entire males, 10 castrates, 10 females), and analysed for felinine (HPLC), NAc-felinine (HPLC) and creatinine (Roche diagnostic kit). The cats were housed in metabolism cages during the collection period, with free access to an AAFCO approved moist maintenance diet and fresh water.

Results showed that while felinine excretion was gender dependant, with intact males excreting at least four times the concentration (mean±SEM, 0.40±0.05mol/mol creatinine) of that of castrates (0.09±0.01) and females (0.13±0.01), NAc-felinine excretion was not gender dependant. There was no significant difference (mol/mol creatinine) in the level of excretion of NAc-felinine between intact males (0.12±0.02), castrates (0.14±0.03) or females (0.17±0.03). Total urinary felinine metabolite excretion was significantly higher (P<0.001) for intact males than castrates and females, while the level excreted by females was significantly higher than that of castrates (P<0.01).

These findings indicate that N-Acetyl transferase, which catalyzes the acetylation of felinine, appears to be rate limiting in the synthesis of NAc-felinine in cats. It also indicates that the sulphur amino acid requirements, for the synthesis of felinine and related metabolites are substantially higher for castrates and females, as well as intact males, than previously reported.
Taurine is a unique amino acid with antioxidant properties. Studies have shown that plasma taurine decreases in response to oxidative stress. The objective of this study is to compare taurine status between normal cats and cats with inflammatory bowel disease (IBD).

A group of 11 healthy and 11 IBD cats were fed an adequate taurine food (1000 ppm) for four weeks. All foods met minimum AAFCO requirements for taurine and were nutritionally adequate, and indicated for GI distress. The cats were housed individually but interacted with other cats and the caretakers daily. Blood samples were taken for measurement of taurine and white blood cells glutathione status and urine samples for taurine and creatinine excretion.

The results showed that although the taurine level was not deficient, it significantly decreased the urinary excretion of taurine in both groups of cats (p<0.05), from 3076 to 272 µM in normal cats and 2644 to 440 µM in IBD cats from baseline values. There was no difference in whole blood taurine levels. The minimum level of taurine also significantly reduced the level of reduced, oxidized and total glutathione. However, the level of GSH:GSSG ratio in both groups of cats were maintained and even slightly increased. There was no difference in the glutathione status between the normal and IBD cats.

In conclusion, the data suggests that taurine requirement and usage for the maintaining the glutathione system at the systemic level are similar in normal and IBD cats. The minimum AAFCO level was sufficient for maintaining taurine status as reflected by the whole blood taurine. However, it is not clear whether this level is sufficient to maintain the integrity of the glutathione system, particularly at the cellular level in the GI tract.
The aim of genomics is to decipher how genes function and provide an understanding of the link between genotype and phenotype. Therefore accurate characterization of phenotype is integral to genomic advancements. The diagnosis of canine hip dysplasia (CHD) has for decades been based on an empirically accepted phenotype derived from the ventrodorsal radiograph of the canine hips and pelvis. This radiograph is scored subjectively based on the presence of subluxation of the coxofemoral joint, or secondary osteoarthritis (OA). It has been generally accepted that the subjective scoring of hip phenotype at one or two years of age accurately reflects the true hip phenotype of the dog. This investigation compared popular scoring systems including the BVA/KC hip score at one year of age and OFA and PennHIP scores at two years of age, with end-of-life radiographic and histopathologic hip phenotypes.

48, 8-week-old Labrador Retrievers from seven litters were followed for life. Hips were radiographed at 30, 42, and 54 weeks of age, then yearly till end of life. Pelvic radiographs were evaluated using the BVA/KC scoring criteria at one year of age followed at two years of age by OFA and PennHIP methods. Radiographic OA scores were also recorded. Histopathology of the hips was performed on 45/48 dogs after succumbing to natural causes.

Radiographic OA increased linearly from 15% at two years of age to 67% at end-of-life. OFA-type scoring at two years of age scored 29 of 48 dogs to be ‘normal’. Of the 29 ‘normal’ dogs 16 (55%) were radiographically dysplastic by end-of-life and 92% had histopath evidence of OA. Of dogs approved for breeding by BVA/KC scoring at one year of age, 57% developed radiographic OA by end of life and 94% showed histopath OA. At two years of age PennHIP scores predicted all 48 dogs to be susceptible to OA (DI range 0.36 – 0.92): 67% showed radiographic OA at end of life: 98% showed Histopath OA. Median disease free interval was three years for dogs with DI>0.6 and 12 years for dogs with DI<0.4.

The linear increase in OA over life refutes the common belief that hip OA occurs either early in life or much later in the geriatric years. In fact, idiopathic old-age OA of the hip is likely a misnomer. The high rate of ‘false negative’ diagnoses for the BVA/KC and OFA methods explains the slow observed progress in making genetic improvements in hip phenotypes via selective breeding, and for the future this understanding confounds advancements in genomic research. Though not tested, other scoring systems based on hip-extended radiography would likely fare similarly. The PennHIP DI predicted with 97% accuracy the dogs that would go on to develop the OA of CHD later in life. Results suggest that rapid genetic improvement in hip phenotype will occur using the PennHIP method as a selection criterion. Similarly, more rapid progress in genomic research can be expected by keying on this newer hip phenotype.
A COMBINATION OF ARGININE, ZINC, VITAMIN C, VITAMIN E, CURCUMIN AND GLUCOSAMINE INCREASES CANINE DERMAL FIBROBLAST MIGRATION IN VITRO

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WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

Two-dimensional fibroblast culture has long been used as a model to mimic the activity of skin during the healing process. The aim of this study was to use such a model to measure the effect of a number of supplements on the ability of canine dermal fibroblasts to re-populate a denuded area.

Fibroblasts were isolated and cultured in six well plates (4x10^5 cells/well, DMEM containing 10% Fetal Calf Serum (FCS) and 1g/l glucose). After 48 hours the media was replenished with media supplemented with 0.5% FCS containing either, arginine, zinc, vitamin C, vitamin E, curcumin or glucosamine at a range of concentrations. Half of the confluent sheets of the cell monolayers were then removed using a cell scraper as described previously. The area into which fibroblasts re-populated was recorded using an automated inverted microscope and measured by image analysis (termed area of re-population, \( \mu m^2 \)).

Both stimulatory and inhibitory effects were observed when fibroblasts were cultured with the individual supplements (Table 1). The effect observed when the supplement mixture was combined (full) was greater than the individual supplements added together (Table 1, Figure 1). When curcumin and vitamin E were removed from this supplement mixture, this enhancement of activity was markedly reduced (Figure 1).

These data point to some degree of synergy between some or all of the components of the supplement mixture. Although it is not possible to deduce any key participating activity of vitamin E and curcumin, their removal markedly reduces the overall effect. In general the data support the addition of the full supplement in vivo to help promote wound healing.

References

THE EFFECTS OF ANTIOXIDANTS ON COGNITIVE FUNCTION IN DOGS
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Cognitive function often declines during natural ageing. One potential mechanism may be oxidative stress. This study aimed to assess the effect of an antioxidant cocktail on cognitive ability in young (aged 1-3, n=8) and senior (aged >8 years, n=13) dogs (6 beagles, 15 Labrador retrievers), using an adaptation of methodology published previously. For at least one month prior to, and throughout, all dogs were fed a commercially available complete diet to maintain bodyweight; the supplemented group (n=10; 4 young & 6 senior) received a daily dose of antioxidant cocktail sprinkled on their food, and the non-supplemented group (n=11; 4 young & 7 senior) received no cocktail. An oddity discrimination test was conducted in which the dog had to move a rewarded object which was a different colour/shape from the other two (identical) objects and reach a predefined criterion within 40 days.

There was no significant effect of age or supplementation on the number of errors or number of sessions to reach criterion. Beagles tended (ANOVA P=0.08) to commit more errors (45.1±8.5) compared to Labradors (26.5±4.6), and took significantly (P=0.04) more sessions to reach criterion (11.0±1.2) compared to Labradors (7.8±0.7). When the test was made more difficult by making the rewarded object more similar to the other two, the dogs (n=19; 2 Labradors not tested) all reached criterion.

Supplemented dogs tended to require fewer sessions compared with unsupplemented dogs (43.6±15.5 errors, 10.7±2.3 sessions for supplemented; 73.9±14.6 errors, 14.6±2.2 sessions for unsupplemented) (P=0.1). Senior dogs tended to commit more errors (P=0.1) and require more sessions (P=0.1) to reach criterion, compared with young dogs (78.9±12.1 errors, 15.6±1.8 sessions for senior; 38.6±19.7 errors, 9.7±2.9 sessions for young).

This study suggests this antioxidant cocktail could improve cognitive function, particularly in aged dogs, supporting work with other antioxidants in dogs.
THE RAT GROWTH ASSAY IS A SUITABLE MODEL FOR ESTIMATING LYSINE BIOAVAILABILITY IN THE CAT

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¹Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, California
²Department of Animal Science, University of California, Davis, California

Lysine has chemical characteristics that increase its sensitivity to processing damage, leading to a potentially decreased bioavailability relative to other amino acids.

The purpose of this study was to use a rat growth assay to determine bioavailable lysine in damaged casein and to compare the results with those previously obtained using kittens¹.

Eighty male 26-28-day-old Sprague Dawley rats were assigned to one of eight dietary groups. Five kilograms of powdered casein, containing 7.85% lysine, was mixed with 250g of dextrose and 5kg of water and autoclaved for 2 hours. Three control diets provided crystalline amino acids in excess of requirements; one contained the amino acids as the sole source of nitrogen, while two diets contained an additional 45g/kg of diet heated or untreated casein. Three additional diets supplied lysine at 4.5, 3.0 and 1.5g/kg of diet. Processed or unheated casein was also added to two lysine-free formulations at 45g/kg of diet.

Addition of heated casein to diets as the sole source of lysine resulted in lower average daily gains compared to untreated casein (0.32g/d and 1.78g/d, respectively, p<0.01). Linear regression analysis of growth data from rats fed graded concentrations of lysine (R²=0.998) allowed estimation of the amount of bioavailable lysine supplied by casein in lysine-free diets. The ratio of the amount of lysine available to support growth to the amount of total lysine in the casein was taken as the bioavailability of lysine in casein for growing rats. Lysine bioavailability was calculated as 48% and 96% in the processed and untreated casein, respectively. These values are similar to those previously determined for kittens (56% and 96% bioavailable lysine).

Growth assays for lysine bioavailability yield similar values for weanling rats and kittens, thus rats may be a good model for determining amino acid bioavailability for the cat.

Keywords: rat, cat, growth, bioavailability, lysine

References
The health and welfare of a fish can be influenced by a variety of physical properties ranging from water quality to territorial difference, unnatural habitats, stocking density and available nutrition. Stocking density is well publicised as a factor that can influence fish health and growth rate since overstocking a tank can negatively impact on water quality and food availability. However low stocking density is a factor often overlooked by the hobbyist that may also compromise welfare preventing fish from reaching their full potential. This may be particularly relevant for shoaling fish such as the Black Widow Tetra.

Using growth, as an indicator of health, the aim of these studies was to monitor the growth of 87 Black Widow Tetras (Glymnocorymbus ternetzi) and 75 Goldfish (Carassius auratus) when housed either singularly or in shoals of 4, 8, 12 or 16. The fish were offered AQUARIAN tropical or goldfish flake at 2% bodyweight as appropriate for the species.

Fish were individually weighed at the study start and every two weeks thereafter; and the following equation was used to calculate growth (specific growth rate):

Specific growth rate (SGR) = 100 x (ln(w2-1n.w1)/ trial days)

where w1 and w2 is the weight of the fish on day 0 and end day, respectively.

There was no significant difference in growth rate between the group sizes in either species [Black Widow Tetras (f=0.93, p=0.47); Goldfish (f=0.51, p=0.68)], although singularly housed fish tended to have greater growth (figure).

Lowering the stocking density of the shoaling Black Widow Tetra or Common Goldfish did not adversely affect growth rate with singularly housed fish tending to produce the highest growth rate.
MODELS FOR PREDICTING BODYWEIGHT FROM BODY LENGTH AND FOOD ALLOWANCE IN TROPICAL AND COLDWATER ORNAMENTAL FISH SPECIES

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Aquarists often overlook the importance of feeding a balanced diet at an appropriate allowance for fish in an aquarium. Although most commercial food manufactures do offer an on pack-feeding guide, these guides are very general and vague, and may result in overfeeding and poor water quality. The aim of this research was to establish simple mathematical models that predict bodyweight from body length and to link these with energy requirement data to allow the formulation of accurate feeding allowances.

Power models were developed using over 9000 individual fish lengths from 18 ornamental fish species. The power models were developed for individual species in the form, weight = a(length)b or weight = Y0 + a(length)b where Y0, a and b are the model parameters.

To develop a feeding allowance it was necessary to establish the energy required to achieve an appropriate specific growth rate (SGR) of around 0.1%/day, for aquarium fish. SGR was measured using the following formula:

\[
SGR = 100 \times \frac{\ln(w2) - \ln(w1)}{\text{trial days}},
\]

where \(\ln(w1)\) and \(\ln(w2)\) is the log of weight of the fish on day 0 and end day, respectively.

Energy requirements were determined for Fancy Goldfish (90 J/g BWT/day), Common Goldfish (94 J/g BWT/day) and 13 tropical species, ranging from 208 J/g BWT/day for a Peppered Corydoras to 57 J/g BWT/day for an Angelfish. Predicted weight and energy requirement were then translated into a food allowance for individual fish according to the energy content of the food offered.
ASSESSMENT OF EX VIVO RESPONSES TO T-CELL MITOGENS AND OXIDATIVE STRESS IN LYMPHOCYTES FROM HEALTHY ADULT AND SENIOR CATS

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1Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, Northern Ireland
2WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK
3Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham, UK
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Immune senescence is the functional decline in immune response in the ageing mammal. In humans it has been associated with increased susceptibility to infectious disease and poor vaccine efficacy. We have previously demonstrated that ageing cats (>10 years) have lower circulating T-cell numbers and attenuated vaccine responses when compared with young adult cats.

In order to assess age-related differences in functional status we examined both the proliferative responses of peripheral blood lymphocytes (PBL) to T-cell mitogens Con A and PHA, and the susceptibility of these cells both to spontaneous and oxidatively induced apoptosis. Thirty seven domestic shorthaired cats were assigned to one of two age groups, adult (2-5 years, n = 15) and senior (10-14 years, n=22). Methyl-[3H]-thymidine incorporation was used to assess cell mitogenesis in both whole blood and peripheral blood lymphocyte cultures from each group of cats. T-cell counts were measured in whole blood samples using a combination of flow cytometry and automated hematology analysis.

Consistent with previous findings, mean T-cell counts were significantly lower in the older group of animals. Significant age-associated differences in proliferative response to mitogen were observed in both lymphocyte and whole blood assay cultures. However, when data from whole blood assays were adjusted for T-cell concentration, there was no significant effect of age on proliferative response nor was there an effect of age on spontaneous or induced early apoptosis.

Our findings suggest that T-cells from cats aged 10-14 years do not demonstrate impaired proliferative response to mitogens nor increased sensitivity to apoptosis. We suggest that lower circulating numbers of reactive T-cells rather than cellular dysfunction can contribute to attenuation of the acquired immune response in vivo in this age group of animals.
USE OF GASTROINTESTINAL LUBRICANT AND DIETARY FIBRES IN A CAT TREAT DESIGNED TO ENHANCE FECAL ELIMINATION OF INGESTED HAIR

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Accumulation of groomed hair in the stomach of a cat predisposes the animal to coughing up trichobezoars (‘hairballs’) and in severe cases may lead to blockage of the gastrointestinal (GI) tract. Problems with products intended for hairball control include poor efficacy, low acceptance by cats and difficulties of administration. The use of GI lubricant (petrolatum) together with dietary fibres formulated into a commercial cat treat1 to enhance fecal elimination of groomed hair was evaluated in this study.

The feeding trial involved 20 cats, split into two groups of 10 cats in a crossover study lasting 28 days (4 days pretrial, 10 days Trial I, 4 days ‘washout’ and another 10 days Trial II). Fecal hair weight was analysed using ANOVA for linear model crossover design (Kuehl, 2000) including the effect of sequence, treatment, cat and period. Treat acceptance during the trial (10 pieces/cat/day) was 100%.

There was a significantly greater (23%) amount of hair in the feces of cats receiving the test treats than in the same cats without them (P<0.05). There was no significant effect of sequencing (i.e. the order in which the treats were offered) but results between individual cats and between the two periods were different (P<0.05). The data from this study indicated a statistically significant increase in hair output in response to administering 10 pieces of the test treats. The high palatability of the treat makes it very acceptable to cats and easy to administer.

References
1Whiskas®/MD Temptations®/MD HAIRBALL CONTROL Treat for Cats
DOCOSAHEXAENOIC ACID ACCUMULATES IN PLASMA OF CANINE PUPPIES SUCKLED ON MILK FROM DAMS FED ALPHA-LINOLENIC ACID-RICH DIET.

Bauer, J.E.1, Heinemann, K.M.1, Waldron, M.K.2

1Companion Animal Nutrition Lab and Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX
2Nestlé Purina PetCare, St. Louis, MO, USA

Milk of dogs fed linseed oil diets is enriched in linolenic acid (ALA) but not eicosapentaenoic acid (EPA) nor docosahexaenoic acid (DHA). DHA is important in neurological development. Its presence in the diet during suckling or its synthesis from shorter chain precursors may thus be significant. This study investigated whether puppies suckling ALA-rich milk results in DHA synthesis. Bitches were fed a high-ALA diet (11.6g/kg linoleic acid (LA) and 68.2g/kg ALA) vs a control-diet (17.5g/kg LA and 1.4g/kg ALA) (all DM basis) from estrus, breeding, and throughout gestation/lactation (n=3 per group). Diets were isocaloric, similar in nutrients, and formulated for all life stages.

Puppies were suckled then weaned to the same diets as their mothers. Milk was collected at d4, d10, d16, and d28 during lactation; puppy plasma was obtained at these same times and at d70 and d84 after weaning. For sampling, puppies were separated from their mothers for 2-3 hours during suckling or fasted overnight after weaning. Total milk-lipids were extracted and plasma-phospholipids (PL) were extracted/fractionated and fatty acids analyzed using gas chromatography. A statistically significant milk-ALA enrichment in the high-ALA group was found at all times (p < 0.05). Also, statistically significant enrichment of both EPA and DHA was observed in the suckling PL samples vs controls (p < 0.05). After weaning, this difference was not seen. We have previously found no accumulation of PL-DHA in adult dogs fed ALA-enriched diets. Thus, puppies are capable of synthesizing/transporting DHA when diets are rich in ALA yet contain no appreciable long-chain n-3 fatty acids. It is not known what minimal amount of dietary ALA supports this conversion nor to what extent relative amounts of LA may impact it. Nonetheless, neonatal canines appear to synthesize DHA at a time of life where demand for it is high.
The ability of an organism to reduce damage caused by free radicals is linked to a number of health benefits, including increased immune function and reduced incidence of cancer. Free radicals can be derived from external sources but are also produced during respiration, and organisms with high basal metabolic rates suffer from high levels of oxidative damage. Many of these free radicals are formed by the reduction of oxygen. It could be expected that fish, with a lower basal metabolic rate than mammals and living in a low oxygen environment, would have fewer requirements for a high level of antioxidants in their blood. It was, therefore, decided to measure both FRAP/FRASC (Ferric reducing-antioxidant power/ Ferric Reducing antioxidant power and ascorbic acid) values of plasma from a tropical ornamental fish species, the Midas cichlid (Amphilophus citrinellus) and to investigate the protection offered to piscine lymphocyte DNA by an exogenously added free radical producer. FRAP/FRASC values were measured using an Olympus AU400 auto analyser. DNA damage was measured by the single cell gel electrophoresis (Comet) assay.

Cichlid FRAP and FRASC values were higher than those found in cats and dogs or those reported for humans. In addition, treating lymphocytes isolated from fish blood with hydrogen peroxide produced similar amounts of DNA damage to a similar treatment of mammalian lymphocytes. This suggests that fish maintain a high level of protection from oxidative damage despite themselves living in low oxygen conditions. These factors may have to be considered when diets are designed for fish.
The measurement of various hematological parameters is often used for both health assessment and to assess the effect of dietary intervention in companion animals. Many of these measurements are complicated in fish species by the presence of nucleated erythrocytes, which cannot be clearly distinguished from leukocytes by automated blood cell counters. This study describes the use of the mitochondrial dye DIOC6 along with a fixed number of fluorescent beads to determine the numbers of erythrocytes and leukocytes in the blood of three ornamental fish species, goldfish (Carassius auratus), the midas cichlid (Amphilophus citrinellus) and koi carp (Cyprinus carpio). Further subdivision of the leukocyte population into lymphocyte and thrombocytes, granulocytes and monocytes was carried out. Hematocrit measurements were made using a hematocrit centrifuge and hemoglobin was measured using a Hemocue Hemoglobin 201+ meter.

No significant differences between the three species were seen in the red blood cell concentrations, although Koi had an increased hemoglobin content (101 SEM ± 4.5 g/l) when compared to cichlids (76±2.2 g/l) and goldfish (69±4.4 g/l). The hematocrit was also greater in koi (34.6±1.4%) when compared to the other two species (27.9±1.3 and 23.9±1.5 %). Both Koi and cichlids had a higher leukocyte count (46x10⁶ and 57x10⁶ cells/ml respectively) than the goldfish (19x10⁶ cells/ml). In all three species, the predominant leukocyte populations were lymphocytes and/or thrombocytes, which are indistinguishable in this assay. Goldfish and Koi had approximately equal numbers of monocytes and granulocytes (between 8.5x10⁵ and 1.3x10⁶ cells/ml), but virtually no granulocytes were apparent in the cichlid blood. These measurements can be used to measure the benefits of any particular diet or could potentially provide some stress measurements for these three common species.
There has been some interest in determining whether the distribution of MHC alleles and haplotypes is identical within the same breeds of American and European dogs. This information would be highly relevant to disease susceptibility and other clinical studies.

We have characterised the DLA-DRB1, DQA1 and DQB1 alleles and haplotypes in 3000 dogs from around the world. We have been able to establish a database of DLA haplotypes, and can predict which haplotypes will be found in particular breeds.

Previous data have suggested that pure bred dogs from America may be less variable within breeds than their European counterparts. However, these data were generated based on small groups of American dogs (n=6 to 22) being compared to much larger groups of European dogs (n=14 to 66).

New analyses of four breeds (Doberman, Labrador retriever, Shih Tzu and Yorkshire Terrier), based on much larger groups of American dogs (n=86 to 129), indicate that there are no major differences in the MHC allele and haplotype frequencies between American and European dogs of the same breed.

These data suggests that it would be valid to pool all data within each breed regardless of the origin of the dogs. This will allow better comparison between breeds, and will facilitate clinical analysis of MHC data in this species.
The WALTHAM International Nutritional Sciences Symposium

Innovations in Companion Animal Nutrition

BEYOND THE CANINE GENOME
SATURDAY, 17 September

13:30  Introduction and welcome: Graeme Blackwood.

13:30 - 14:15  OVERVIEW OF THE CANINE GENOME, Dr. Elaine Ostrander, National Institutes of Health, USA
General introduction to the rapidly developing field of canine genetics from the historical perspective of genome project, the outcomes of this piece of work and the implications for advancing our understanding of the canine species

14:15 - 15:00  SINGLE GENE DEFECTS AND TESTS, Dr. Paula Henthorn, University of Pennsylvania, USA
The state of play in our understanding of single gene conditions for canine. What are the main diseases, what are the genetic polymorphisms underlying them and how can we best manage the tests for the good of the dog world?

15:00 - 15:15  Coffee break

15:15 - 16:00  HEALTH, WELFARE AND BREED DIVERSITY, Dr. Jeff Sampson, The Kennel Club, UK
The genetics of breed diversity; what has breed structure meant for the dog genetics and how might our understanding of the canine genome be beneficial for the world of the pure bred?

16:00 - 16:45  OVERVIEW OF COMPLEX DISEASES, Professor Bill Ollier, University of Manchester, UK
General review of the area - the mechanisms by which a number of polymorphic genes can interact to increase susceptibility to certain diseases.

16:45 - 17:30  COMPLEX DISEASE, RISK AND THE ROLE OF ENVIRONMENT, Professor Jose Ordovas, Tufts University, USA
The role of the environmental component in the risk and manifestation of complex diseases - lessons from cardiovascular diseases in the human

17:30pm  Session closes.

SUNDAY, 18 September

08:30 - 09:15  COMPLEX DISEASE STUDY - CANINE HIP DYSPLASIA, Professor Rory Todhunter, Cornell University, USA.
A study of the genetic and environmental factors which contribute to malformation and subsequent abnormal laxity of the canine hip joint.

09:15 - 10:00  COMPLEX DISEASE STUDY - PERIODONTITIS, Dr. Neale Fretwell, WALTHAM, UK.
Identification of a number of polymorphisms that appear to contribute to increased risk of periodontitis in dogs. How these genes might be operating within the pathological mechanism?

10:00 - 10:15  Coffee break

10:15 - 10:45  NUTRITIONAL PREVENTIVE THERAPY FOR CANINE PERIODONTITIS, Dr. Adrian Watson, WALTHAM, UK
The potential to compensate for predisposition to inflammatory gum disease by nutritional means.

10:45 - 11:30  NUTRITIONAL GENOMICS, Dr. Kelly Swanson, University of Illinois, USA
How might the environmental component, in the form of nutrition, be manipulated to influence the genetic component of complex canine conditions? What we know so far

11:30 - 12:00  Discussion and close
Elaine Ostrander PhD
Senior Investigator and Branch Chief of Cancer Genetics at NH/NHGRI

Dr. Elaine Ostrander remains an Affiliate Member of the Divisions of Clinical Research and Human Biology at the Fred Hutchinson Cancer Research Center where she was from 1993 until recently. Dr. Ostrander received her PhD from the Oregon Health Sciences University, and did her postdoc training at Harvard. She then went to UC Berkeley and Lawrence Berkeley National Labs where she began her work on the genome project. Dr. Ostrander's lab works on the genetics of cancer, as well as development of the canine system for genetic analysis of complex traits. She has co-authored over 130 papers on these subjects and will talk about her work in canine genetics.

Paula Henthorn PhD
Associate Professor of Medical Genetics, University of Pennsylvania, Philadelphia

Dr. Henthorn received her PhD in genetics from the University of Wisconsin and has studied the molecular basis of mammalian genetic diseases for more than 20 years, beginning with human genetic diseases. She expanded her interests to domestic animal genetic diseases upon joining the Section of Medical Genetics at the University of Pennsylvania School of Veterinary Medicine in 1990. Her current research focuses on the determination of the specific gene defects in canine and feline genetic diseases, comparative genetics, and treatment, and control of genetic diseases of man and domestic animals. Dr. Henthorn is actively involved in genetic education for veterinarians.

Jeff Sampson PhD
Canine Genetics Coordinator, The Kennel Club, UK

With a first degree and PhD in Biochemistry from the University of Sussex, Dr. Sampson then worked for approximately five years as a Scientific Officer for the Imperial Cancer Research Fund (now incorporated into Cancer Research UK) studying various aspects of gene expression, particularly with relevance to cell differentiation. He was appointed to a Lectureship in Biochemistry in the Medical School of the University of Leicester, UK, in 1978, being promoted to Senior Lecturer in 1988. In the late 80s he changed the research emphasis of his group to studying methods to identify the genes involved in inherited diseases in the dog. He quickly established a major collaboration with the Animal Health Trust in Newmarket, UK, and together they were founder members of DogMap, a European consortium of laboratories established to develop a canine genome map. He left Leicester University in 1998 to take up his present position as Canine Genetics Coordinator with the Kennel Club in the UK. This new initiative by the KC was designed to facilitate the use of new DNA technology to identify genes involved in inherited diseases and develop breed-specific DNA tests for inherited disease that can be used by breeders to select against these mutant genes and reduce their frequency within a breed’s gene pool.

Bill Ollier PhD
Professor of Immunogenetics, University of Manchester and Director of the Centre for Integrated Genomic Medical Research

He also currently holds honorary positions with Salford Royal Hospitals NHS Trust, and Veterinary Medicine, University of Liverpool. Professor Ollier's research has now spanned more than 30 years and he has published more than 360 reviewed publications. Over the last 10 years, Professor Ollier's research interests have focussed largely on investigating the genetic basis of common complex disorders, including rheumatic diseases, inflammatory and autoimmune conditions, neurocognitive disorders, and complex traits in companion animals.

Jose M. Ordovas PhD
Professor of Nutrition and Senior Scientist (USDA-HNRC), Tufts University, Boston, Mass and Director of the Nutrition and Genomics Laboratory, HNRCA.

Dr. Ordovas’ major research interests focus on the genetic factors predisposing to cardiovascular disease and their interaction with the environment and behavioural factors with special emphasis on diet. Dr. Ordovas has published more than 400 scientific articles in peer review journals, including The Lancet and the New England Journal of Medicine and Science. He is editor for Current Opinion in Lipidology (Genetics Section), consulting editor for the American Journal of Clinical Nutrition and Associate Editor for the Journal of Nutrition, Metabolism and Cardiovascular Disease.

Rory J. Todhunter BVSc, MS, PhD
Diplomate American College of Veterinary Surgeons, Associate Professor of Surgery, Department of Veterinary Medicine, Cornell University, Ithaca NY 14853

Dr. Todhunter earned his bachelor of veterinary science (DVM equivalent in the USA) from the University of Sydney in 1975, Australia. He was an equine surgery resident at Michigan State University from 1980–1983 where he also gained an MS degree. He was Instructor in Surgery at Cornell University from 1983-1986 and entered the PhD program in 1987, graduating in 1992. He has been on the Cornell faculty since 1993 where
he is an Associate Professor and a small animal orthopedic surgeon in the Companion Animal Hospital. He is the recipient of the Pfizer Award for Excellence in Research. Dr. Todhunter’s research interest is in hip dysplasia pathogenesis and secondary osteoarthritis with an emphasis on genetics and quantitative trait locus mapping of the underlying genes. He is also developing a clinical research program in bone healing using bone marrow-derived stem cells.

Neale Fretwell PhD
Technology Development Manager, WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

Dr. Neale Fretwell completed his PhD in Human Genetics at the University of Leicester. Following postdoctoral experience on the molecular investigation of inherited human cardiovascular disease, he obtained a Research position at the Waltham Center for Pet Nutrition in 1999. His primary interests are the molecular investigation of canine inherited traits of an immune-related predisposition.

Adrian Watson PhD
Solutions Development Manager, WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

Dr. Watson gained his first and second degrees in Biochemistry and Genetics from the University of Nottingham, UK. Subsequently he undertook five years of post-doctoral studies in the Anatomy Department at Cambridge University looking at early development of the human feto-placental unit. Joined Waltham in 1999, spending four years on the skin and coat research programme. More recently joined a team looking into the interaction between genes and nutrition.

Kelly S. Swanson MSc, PhD
Assistant Professor, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, USA

Dr. Kelly Swanson completed his PhD in Nutritional Sciences at the University of Illinois in 2002. Following post-doctoral experience focused on canine nutritional genomics, he obtained an Assistant Professor position in the Department of Animal Sciences at the University of Illinois in 2004. His primary interests include intestinal health, obesity and diabetes, and the nutritional effects on gene expression and protein profiles, much of which is focused on dogs and cats.
GENOME ARCHITECTURE AND MAPPING OF COMPLEX TRAITS IN THE DOMESTIC DOG
Ostrander, E.A., PhD
Senior Investigator and Branch Chief of Cancer Genetics at NH/NHGRI Cancer Genetics Branch, Bethesda, MD 20892-8000
University of Utah, Department of Biology, Salt Lake City, UT 84112-0840

As a result of differential selection for phenotypic traits, dog breeds are, by definition, closed breeding pools. That is, because registration to a particular breed requires that one's parents were also registered, genetic diversity can always decrease, but not increase. Small numbers of founders, population bottlenecks, and popular sire effects have thus conspired to make the domestic dog an ideal resource for mapping genes associated with both simple and complex traits, and for understanding the dynamics of population evolution. We have evaluated this in several ways.

First, using data from five unrelated dogs of each of 85 breeds, we demonstrated previously that breed barriers result in strong genetic isolation, with breed membership accounting for ~30% of total genetic variation among dogs. In addition, genetic cluster analysis based on microsatellite data groups modern breeds into four populations reflecting similarities in morphology, behavior and geographic origin. We now expand on that analysis by including data from 40 additional breeds to more precisely describe the historical relationship between founding populations.

In addition to the above, we have evaluated the extent of linkage disequilibrium (LD) in five well-selected breeds of unique ancestry. We found that LD in dogs is 20-100 times more extensive than in human populations and varies by as much as 10-fold between breeds. This suggested that a correspondingly smaller number of markers would be required for association studies in dogs than humans. We also evaluated haplotypes and found that dog breeds, in general, are characterized by low haplotype diversity and high haplotype sharing. Thus, we predict that a single SNP map of 10,000-15,000 SNPs will be useful for mapping traits of interest in most breeds. Overall these findings set the stage for mapping genes associated with disease susceptibility.

Lastly, we and others have increasingly turned our attention to the identification of loci associated with morphologic variation. Towards that end, collaborators Gordon Lark, Kevin Chase and colleagues have used principal component analysis of skeletal variation in a population of Portuguese Water Dogs (PWDs) to reveal systems of traits defining skeletal structures. A total of 21% of variation in skeletal size of PWDs results from differences between females and males. More than half of this sexual dimorphism results from an interaction between a QTL associated with CHM on the X-chromosome and a QTL associated with FH2017 on CFA15. Positional cloning of the underlying genes and identification of relevant variants is underway using SNP-based haplotype approaches and progress to date will be discussed.
Reports of approximately 450 different canine genetic diseases appear in the biomedical and veterinary literature. For more than 50 of these diseases, all of which are inherited as single gene traits, the mutations that gave rise to disease-causing alleles are known, thus providing the basis for as many as 80 DNA-based genetic tests. Nearly 40 different tests are available commercially, and this number is rapidly increasing.

The vast majority of diseases for which there are DNA-based genetic tests are not those diseases that appear in the ‘top ten’ lists of diseases of concern to breeders, as compiled from health surveys\(^1\), at least in part because they do not show simple modes of inheritance. Scientists are only just beginning to understand the pathogenesis of inherited diseases that do not show single gene patterns. Generally, the assumption is that more than one gene is involved in a particular disease, and that any single allele occurring individually is not responsible for disease. Disease or increased risk of disease results when particular alleles at several loci occur together, and with environmental appropriate conditions. Hence the term disease susceptibility allele is perhaps more appropriate than disease-causing allele. The organization of dogs into breeds that are reproductively isolated should make the study of complex disease more amenable than in humans\(^1\).

While genetic testing is currently used primarily by breeders to choose breeding pairs that will not produce offspring affected with a particular genetic, DNA-based genetic tests will be applied more broadly in the future. More comprehensive programs have the possibility of using genetics tests to eliminate disease-causing or disease susceptibility alleles from a population, for diagnosis of symptomatic individuals, and for predictive testing of animals at risk for late onset diseases. While many new genetics tests will appear in the near future, the challenges of using these tests for making breeding choices remain the same. Are the monetary and societal incentives strong enough for genetic testing to become widespread? Will selective breeding to eliminate disease-causing or disease-susceptibility alleles result in overall reduction of genetic diversity? Are educational and counselling programs adequate to provide veterinarians and breeders with the knowledge to make appropriate decisions that will affect the future health of a breed? An already substantial array of genetic tests will undoubtedly expand in the near future, and play a much larger role in companion animal health maintenance.

References
HEALTH, WELFARE AND BREED DIVERSITY
Sampson, J., PhD
Canine Genetics Coordinator, The Kennel Club, UK

By their very nature, purebred dog breeds represent very unusual genetic populations with restricted genetic variation. Most, if not all, will be founded on a relatively small number of dogs and the early years of breeders' selection will have been concentrated on fixing the new desired breed type using a small number of the early progeny. Inevitably, once established, the breed will have been recognised by one or more of the national Kennel Clubs, imposing new restrictions to breeding by insisting that no dog may become a registered member of a breed unless both its parents are also registered members of the breed. Genetic variation within a breed will have been further reduced over the years by breeders' insistence on using so-called popular sires, or matadors. Thus, purebred dog breeds start out with very shallow gene pools that quickly become closed by the establishment of breed registers.

Although these very restrictive breeding patterns have been highly successful at producing breeds of dog with essentially homogenous phenotype, they have not been without collateral damage to the breeds, particularly in the area of inherited disease. Around 400 inherited diseases have been described in the purebred dog population, with many being restricted to a particular breed or group of related breeds. The vast majority of these diseases result from recessive mutations, often in single genes. The very restrictive breeding patterns have not been the cause of these mutations, but they have been responsible for a rapid increase in the frequency of a mutant allele following either a de novo mutation or the introduction of a mutant allele from elsewhere. Increasingly, inherited diseases are imposing a serious disease burden on most, if not all, breeds of dog.

One of the initial aims of the Canine Genome Project was to provide easier and more reliable tools to identify the genetic mutations that underlie inherited diseases in the dog. Once a disease causing mutation has been identified it becomes a relatively simple task to develop a DNA test for the mutation, which can be used on an individual dog to reveal its genotype with respect to the disease. In excess of 40 different breeds now have a DNA test for at least one inherited disease that occurs in their breed. All of the available DNA tests are for diseases that result from single gene mutations and the vast majority are for recessive mutations.

Recent progress in our understanding of the canine genome will certainly make the detection of gene mutations easier and gives us the very real prospect of identifying mutations involved in polygenic diseases like hip dysplasia, epilepsy, heart disease and auto-immune diseases, and give breeders the tools needed to select against these diseases as well.
OVERVIEW OF COMPLEX DISEASES
Ollier, W.E.R., PhD
Professor of Immunogenetics, University of Manchester, UK and Director of the Centre for Integrated Genomic Medical Research

Most biological variation is a consequence of complex interactions between genes and how they are modulated by external environmental factors. As significant genetic variation exists between individuals, any particular observed characteristic (phenotype) represents the complex interactions of multiple gene variants (alleles) and environment. In the case of diseases, these are referred to complex genetic conditions.

Although defects (mutations) in single genes can have dramatic clinical consequences (e.g. Duchenne’s Muscular Dystrophy) these are usually rare. In contrast, the majority of diseases seem to have a complex etiology (polygenes and environment) and are common (e.g. diabetes, obesity, cardiovascular disease). Thus, these conditions have a significant impact on the global health burden.

If inherited risk factors can be identified, this information can predict those at risk of developing disease. Such information may be useful in offering earlier or more intensive screening. It may also provide important insights into identifying novel therapeutic targets. If environmental triggers can also be identified, it could lead to suggestions of lifestyle choices or pre-disease onset prophylaxis for those at risk.

Although polygenic phenotypes have been recognised since Mendel, our ability to unravel both genetic and environmental components has been hampered by analytical complexity and lack of information. This is now changing rapidly as genomes of different species are sequenced and we gain access to breakthrough technological advances in molecular genetics, informatics and computing.

In parallel with the international effort to identify genetic and environmental risk factors for human conditions such as asthma and schizophrenia, the veterinary community now appreciates the clinical potential of investigating complex diseases. This will lead to identifying disease genes in companion animals and also provide a powerful comparative genomics approach for investigating homologous conditions in humans.

Veterinary complex genetic analysis has significantly more potential than human studies to identify gene-environment interactions. This is particularly the case for gene-nutrition interactions. Diet is a major environmental modulator of gene function and may have both protective and deleterious inputs into disease development. Diet histories, analysis and compliance is notoriously difficult in human studies due to lifestyle, access and freedom of choice. Such issues can be more rigorously controlled and documented in companion animal studies. This will lead to groundbreaking studies being pioneered in companion animals.

The basis of complex disease in companion animals and how they are being analysed will be discussed along with how such developments can impact on animal health and welfare.
GENETICS, DIET AND HEALTHY AGING: THE IMPORTANCE OF DISEASE PREVENTION

Ordovas, J.M., PhD
Professor of Nutrition and Senior Scientist (USDA-HNRC), Tufts University, Boston, Mass and Director of the Nutrition and Genomics Laboratory, HNRCA

The most economical and successful approach to achieve healthy aging comes from disease prevention. It has been estimated that removal of the major disease risk factors will increase global healthy life expectancy by 9.3 years. Specifically, when it comes to ischaemic heart disease, this should result in a reduction in population disease of 80-87% and on mortality of 77-84%. Most of those risk factors are driven by a combination of genetic and environmental factors interacting with each other to produce the biochemical or clinical phenotypes and the disease risk. Therefore, we need to highlight that most gene markers for chronic diseases and specifically for cardiovascular disease (CVD) are not determinant of disease, but predisposing factors in combination with unhealthy environmental exposures.

Thus, the relevance of understanding the cross-talk between genes and environment and more specifically with dietary factors. At this regard, nutrigenetics is emerging as a multidisciplinary field focusing on studying the interactions between nutritional, genetic factors, and health outcomes. The ultimate goal is to elaborate efficient individual dietary intervention strategies aimed to preventing disease and thus achieving healthy aging.

Our studies, using a wide array of population and intervention studies have found already significant evidence for associations between CVD risk factors and specific alleles at candidate genes. More important, we have found significant interactions between dietary factors, genetic variants and biochemical markers of CVD. The traditional approach of recommending low fat, low cholesterol diets for the entire population has been the subject of heated discussion and controversy. Now, based on the accumulating knowledge, we can begin to characterize individuals that may respond better to one type of recommendation or another. Therefore, a low fat, low cholesterol strategy may be especially beneficial for lowering plasma cholesterol levels for subjects carrying the apoE4 allele at the APOE gene.

Moreover, HDL concentrations are determined also by dietary, behavioral and genetic factors. We have demonstrated significant interactions at the APOA1, CETP and LIPC genes modulating the effects of dietary fats on HDL-C concentrations. Similar models are being used to identify genetic interactions between other chronic disorders, such as cancer and osteoporosis and food components; however, those efforts are hampered in part by the added complexity of the phenotypes involved in those diseases as compared to those used in the CVD field.

This knowledge could pave the way for most successful dietary recommendations based on genetic factors that may help to reduce disease risk more efficiently than the current universal recommendations.
COMPLEX DISEASE STUDY: CANINE HIP DYSPLASIA
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Cornell University 1College of Veterinary Medicine and 3Animal Science, 4Biotechnology
2College of Veterinary Medicine, Texas A&M University, College Station, 77843 TX

Canine hip dysplasia is a common developmental complex trait in dogs characterized by hip joint laxity and subluxation and secondary hip osteoarthritis (OA). Trait-free greyhounds and dysplastic Labrador retrievers were crossed to produce a 3-generation pedigree. Dysplastic traits recorded at 8 months of age included the distraction index, the dorsolateral subluxation score, the Norberg angle, and the extended-hip radiograph score. 152 dogs in 3 generations were genotyped with up to 250 microsatellites at the NHLBI Mammalian Genotyping Service, Marshfield WI.

We invoked a web-based software (QTL Express™) which regresses identical-by-descent marker probabilities on the phenotypes. We reported (Todhunter et al, 2005 Mammalian Genome) the results of this genome-wide screen in which putative QTL for canine hip dysplasia (p<0.05, chromosome-wide) were found on CFA04, 09, 10, 11 (p<0.01), 16, 20, 22, 25, 29 (p<0.01, LOD score 2.8), 30, 35, and 37.

Genotyping these dogs and 130 Labrador retrievers for the 323 markers in Minimal Screening Set 1 continues. Association mapping based in linear mixed models that account for pedigree structure will be used to combine genotypes and phenotypes from all these dogs to confirm and extend these results. Fine mapping will be based on single nucleotide polymorphism (SNP) haplotypes that span the chromosomes with the highest LOD score peaks on these dogs and on unrelated Labrador retrievers and other breeds susceptible to canine hip dysplasia.

Complementary microarray analysis and results of published work on hip dysplasia in dog and human will help guide selection of candidate genes for mutation screening in affected and unaffected dogs. Chase et al. mapped 2 QTL on CFA01 for the Norberg angle in Portuguese Water Dogs (2003) and a single QTL for acetabular osteophytes on CFA03 (2005). These studies suggest that the genes that contribute to normal and abnormal hip conformation in dogs will be found based on localization through initial coarse genome-wide screens.
In dogs, dental problems represent one of the most common reasons for veterinary consultation and most dogs will suffer from some degree of periodontitis during their lifetime. Many pet food manufacturers now develop specific products that are designed to help owners improve their pet’s oral health care. Most of these are complimentary oral care products, which are designed to reduce tartar and plaque accumulation through mechanical abrasion. Although these products have been shown to be efficacious, owners often only recognise a problem once irreversible changes to the periodontium have occurred and adoption of lifelong pet oral care regimes remain rare. Periodontitis may affect any breed of dog, however specific breeds have been observed to be more susceptible, particularly toy breeds which often show signs of severe disease within the first few years of life. It is thought that this biased occurrence in the small breeds is probably not solely an anatomical issue, since some breeds with evidently crowded dentition appear relatively resistant to early onset periodontal problems.

Recent advances in the human genetics field have highlighted that there is a substantial genetic basis for susceptibility to periodontitis. Variants in several immune-related genes, including the Interleukins (IL)-1α and IL-1β, IgG Fc receptor (CD16), N-formyl peptide receptor and Cathepsin C, have been implicated as potential risk factors for the development of human periodontal disease. Although susceptibility to periodontitis is clearly a complex genetic disease, with multiple genetic and environmental components, it is becoming clear that certain genes are important in determining both resistance to pathogenic bacterial species, and which individuals will suffer early onset of disease.

Some genetic factors protect the host against pathogenic bacteria of the oral cavity, but others may also control the metabolic pathways that promote the sporadic connective tissue loss and bone destruction characteristic of the condition. In order to try to advance knowledge of the factors that pre-dispose dogs to developing periodontitis, we have conducted a survey of four canine breeds looking at clinical and genetic factors that may play a role in disease susceptibility. The research presented will allow us to assess if there are inter- and intra-breed differences in genetic susceptibility to periodontal disease, and should lead to the development of more efficacious oral-care solutions in the future, with potential to target nutrigenomic modulation of specific host pathways.
NUTRITIONAL PREVENTATIVE THERAPY FOR CANINE PERIODONTITIS.

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Periodontal disease is probably the single most common disease seen in small animal veterinary practice with the great majority of dogs over the age of three years already affected by an extent of disease that warrants professional intervention. What is more there is growing evidence that infection in the oral cavity may cause disease of distant organs such as the liver and the kidneys.

The inflammatory component of the immune system provides essential protection for the body against microbial attack. However, the conditions of gingivitis and, in particular, periodontitis are examples of what can happen to the host when concomitant with the inflammatory response comes damage to surrounding tissues. Periodontal disease is a direct consequence of the inflammatory response to the oral bacteria of dental plaque. Gingivitis is inflammation of the gingiva and is the earliest sign of disease. Individuals with untreated gingivitis may then develop periodontitis. The inflammatory reactions in periodontitis result in destruction of the periodontal ligament and alveolar bone. The result of untreated periodontitis is ultimately exfoliation of the affected tooth.

Based on what is known of the disease mechanism there are two main targets for the prevention of both gingivitis and periodontitis. These are the invading pathogenic bacteria and the host immune response. There is now clear evidence that certain breeds, or even specific dogs have a predisposition to the development of periodontitis. In these dogs the host immune system appears to respond particularly aggressively to plaque bacteria resulting in the activation of a cascade of degradative enzymatic activity. As we are beginning to understand some of the molecular factors that lead to this predisposition it is possible to focus treatment and prevention regimes on the key events in the etiological process. The aim of such regimes would be to negate the influence of predisposing factors and thereby return the chance of an otherwise high-risk individual getting the disease to that of a non-predisposed individual. The potential for achieving this re-balancing of risk through the use of nutritionally delivered active ingredients will be discussed.
Phenotype is the product of genotype, environment, and the interaction between these two factors. Diet is the primary environmental factor influencing phenotype. The importance of nutrient-gene interactions, and their impact on health and disease, is becoming increasingly evident. While genotype affects nutrient absorption, metabolism, and transport, diet, in turn, impacts epigenetic, genomic, and proteomic events. Human genes are thought to contain ~10 deviations (polymorphisms) in its code from the ‘standard gene.’ Most canine genes are also likely to have numerous polymorphisms. While many polymorphisms do not impact gene function, a considerable number are thought to have mild effects on functionality. Identifying polymorphisms affecting nutrient or drug metabolism is important for studying canine health and disease. Diet may regulate gene expression through epigenetic mechanisms or by acting as transcriptional factors. Various dietary manipulations have been reported to impact epigenetic inheritance in rodents and humans. Metabolic programming, often used to describe epigenetic inheritance impacting life-long metabolism, continues to gain interest due to its link to chronic diseases. Evidence also suggests that bioactive food components, including vitamins, minerals, carotenoids, and flavonoids, act as transcriptional factors. Finally, nutrient intake may also impact downstream events such as post-translational modifications of newly synthesized proteins.

Canine genome sequence data and advances in molecular biology, nanotechnology, and computer science have supplied scientists with an extensive set of experimental tools. Researchers must take advantage of these tools and apply them to companion animal research. Although genetic diseases are present in many purebred populations, the majority of diseases affecting canine health and longevity are complex in nature. Experiments focused on diseases such as obesity, hip dysplasia, diabetes, intestinal disorders, and cancers are high priority and may be improved by incorporating genomic technologies. While a ‘reductionist’ strategy is important for understanding individual gene and protein function, integrative approaches are required to fully understand the behavior of complex systems. Thus, a “systems biology” approach that simultaneously measures phenotypic, genetic, and genomic parameters may be implemented to increase our understanding of these diseases and identify nutrition’s role in prevention or treatment strategies.

Nutrigenomics research will soon change the paradigm by which pet food professionals and veterinarians understand and apply companion animal biology. Although the tools are currently available for this paradigm shift to occur, significant science-based research initiatives are required before these concepts become reality. My presentation will provide a brief overview of nutrigenomics and its application to canine nutrition, health, and disease.
The mission of the ACVIM Foundation is to enhance the diagnosis, treatment, and prevention of disease in all animals by supporting the acquisition and distribution of new knowledge with direct clinical implications in veterinary medicine.