The WALTHAM International Symposium

Pet Nutrition Coming of Age

Abstracts

Vancouver
Canada

7-8 August 2001
Foreword

PET NUTRITION COMING OF AGE

WALTHAM has been host to International Symposia for almost 25 years. These events bring together academics, nutritionists, veterinarians and practitioners alike to share their interest and expertise in the fields of nutrition, behaviour and care of pets. This year, we focus on understanding the ageing process in companion animals and how nutritional intervention can be used to maximise health and longevity both in the healthy ageing pet and in those suffering from age-related disease.

Ageing, or senescence of organisms is inevitable, and despite the best efforts of science, still poorly understood. Different species are known to age at different rates; but the rate of ageing, or at least many of the outward signs of ageing, can vary between individuals of the same chronological age. And, whereas the duration of life may be genetically determined, many physiological aspects of ageing are under the control of a range of environmental and lifestyle factors not the least of which is nutrition. Through WALTHAM research we have learned that we can decrease the effect of damaging free radicals at the cellular level. Validating the Comet Assay for use in the dog and cat has allowed us to directly measure and then nutritionally ameliorate the difference in damage to perhaps the most important cellular constituent; the genetic code, or DNA. This could have potential long-ranging effects on ageing, and the development of age-related diseases such as cataracts, diabetes and cancer.

As we live longer and our 'aged' population increases the major health problems encountered shift. The same is true with our pets. Our increased understanding of nutrition and health of pets means that the life expectancy of domestic cats and dogs is higher than ever before.

With over 90 research abstracts and 200+ delegates from 23 countries, this year’s symposium will be one of our biggest ever. It promises to be a stimulating and varied symposium and is a tremendous opportunity to share current understanding of nutrition, health and longevity in companion animals. As our scientific methodologies and insights become more sophisticated we can truly begin to unlock some of the mysteries of the ageing process and make a difference to the health and well-being of pets. It is our responsibility as scientists to share our knowledge and WALTHAM will continue to publish and provide the opportunity for sharing of expertise for years to come.

Pauline Devlin, BSc, PhD
Scientific Communications Executive, WALTHAM

Karyl J. Hurley, DVM, DACVIM (Internal Medicine)
Global Scientific Communications, WALTHAM

E. Jean Harper, BSc, MSc
Head of Innovation, WALTHAM
# The WALTHAM International Symposium
**August 6-7 2001, Vancouver, BC**

## SUNDAY, 5 August

**WELCOME RECEPTION** – Wine and Cheese, Registrations

## MONDAY, 6 August

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<td>7:30-8:30</td>
<td>Breakfast: Buffet in the Seawall Bistro</td>
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<td>8:45</td>
<td>WELCOME: Dr. Jean Harper, Head of Innovation, WALTHAM</td>
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<tr>
<td>9:00 – 10:00</td>
<td>PLENARY SESSION: Health and Ageing, Professor Carl Keen</td>
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<td>10:00 – 10:15</td>
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<td>10:15 - 10:35</td>
<td><strong>ABSTRACT SESSION: HEALTH AND AGEING</strong></td>
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<tr>
<td>10:55 - 11:15</td>
<td>Age-related changes in dogs' flavour perception. McCune, S.</td>
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<td>12:00 - 1:00</td>
<td>LUNCH</td>
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<td>1:00 - 1:45</td>
<td>PLENARY SESSION: Living fast, dying when? The metabolic basis of ageing Professor John Speakman</td>
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<td>2:00 - 2:20</td>
<td><strong>ABSTRACT SESSION: ANTIOXIDANTS</strong></td>
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<td>Effect of a large single dose of b-carotene on plasma levels of b-carotene and Vitamin A in cats. Schweigert, F.L., Raila, J., Wichert, B., and Kienze, E.</td>
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<td>3:00 - 3:15</td>
<td>Coffee break</td>
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<td>3:15 - 3:35</td>
<td>Changes in circulatory antioxidants and markers of oxidative damage during endurance competition. Marlin, D., Fenn, K., Smith, N., Deaton, C., Roberts, C., Harris, P., Dunster, C., and Kelly, F.</td>
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<td>4:00 - 5:00</td>
<td>ROUND TABLE DISCUSSION: Ageing and Immune Function</td>
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<td>Moderator: Prof. Roger Batt</td>
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<tr>
<td>6:00 – 8:00</td>
<td>POSTER SESSION – Wine and hors d’oeuvres</td>
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<tr>
<td>8:00 – 10:30</td>
<td>Dinner: Canadian Barbeque by the pool with Steel Band accompaniment</td>
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TUESDAY, 7 August

7:30 - 8:45  Breakfast: Buffet in the Seawall Bistro

9:00 – 10:00  PLENARY SESSION: Diseases of the older pet amenable to nutritional intervention  
Professor Sharon Center  

ABSTRACT SESSION: DISEASES AMENABLE TO NUTRITIONAL INTERVENTION

10:00 - 10:15  Coffee break  

10:15 - 10:35  Dietary flavanols: Their potential for preventing age-associated vascular pathologies. Schmitz, H.  


12:00 - 1:00  LUNCH  

1:00 – 1:45  PLENARY SESSION: Comparative Neurosenscence; Prof John Mayer  

ABSTRACT SESSION: EFFECTS OF DIETARY MANIPULATION

2:00 - 2:20  Predictive equations for the quantitation of polyunsaturated fats in canine plasma and neutrophils from dietary fatty acid profiles. Bauer, J.E., Waldron, M.K., Spencer, A.L., Hannah, S.S.  

2:20 - 2:40  ‘Red hair’ in black cats is reversed by addition of tyrosine to the diet. Morris, I.G., Yu, S., and Rogers, Q.R.  

2:40 - 3:00  The influence of chronological age on barrier function properties of the canine epidermis. Watson, A., Fray, T., Clarke, S., Yates, D., and Markwell, P.  

3:00 - 3:15  Coffee break  


4:00 - 5:00  ROUND TABLE DISCUSSION: Ageing and Clinical Nutrition  
Moderator: Peter Markwell  

8:00 – 11:00  VANCOUVER AQUARIUM EXTRAVAGANZA!  
Buses will leave the Westin at 7:30 and return at 11:00
POSTER PRESENTATIONS – Posters will be available for review throughout the meeting, and the authors are asked to stand with their presentations for Q&A during the Monday Evening Poster Session, 6-8pm.

**Canine**

1) Validation of single-cell gel electrophoresis assay (comet assay) for assessing levels of DNA damage in canine and feline leukocytes. Heaton, P.R., Ransley, R., Charlton, C.J., Mann, S.J., Smith, B.H.E., and Harper, E.J.


6) The use of the servo med ep-2 evaporimeter to measure the transepidermal water loss (tewl) in canine skin. Watson, A., Fray, T., Clarke, S., Gunn, N., Yates, D., and Markwell, P.

7) Influence of calcium- and phosphorus supply on the apparent digestibility of these minerals in growing dogs. Dobenecker, B., and Kienzle, E.

8) Bioprotein – a new potential feed ingredient in dogs diets. Skrede, A. and Ahlstrøm, Ø.


11) Influence of body size on intestinal permeability in growing dogs. Weber, M., Martin, L., Dumon, H., Biourge V., and Nguyen, P.


13) Compatibility and digestibility of mixed diets with various hydrocolloid and water contents in 3 different breeds of dogs. Zentek, J., Kaufmann, D., and Pietrzak, T.


18) Nutritional lens opacities in two litters of Newfoundland dogs. Ranz, D., Gutbrod, F., and Kienzle, E.

19) A placebo controlled double blind study on the effect of nutraceuticals (chondroitin sulfate, mussel extract) in dogs with joint diseases as perceived by their owners. Dobenecker, B., Beetz, Y., and Kienzle, E.

20) Large intestinal metabolism of dietary nutrients in dogs. Hendriks, W.H. and Sritharan, C.

21) Age, breed, sex and period effects on skin biophysical parameters for dogs fed canned dog food. Young, I.A., Dodge, J., Guest, K., Cline, J.L., and Kerr, W.W.


23) Conversion of essential fatty acids by delta-6 desaturase in dog liver microsomes. Dunbar, B.L., and Bauer, J.E.

24) The use of sorghum and corn as alternatives to rice in dog foods. Twomey, L.N., Pethick, D.W., Rowe, J.B., Choc, M., Pluske, J.R., Brown, W.


28) Simultaneous determination of total body water and plasma volume in conscious dogs using the indicator dilution principle. Wamberg, S., Sandgaard, N.C.F., and Bie, P.


CANINE AND FELINE:


34) Calculation of gross energy in pet foods: do we have the right figures on heat combustion? Kienzle, E., Schrag, I., Butterwick, R., and Opitz, B.

35) Further developments in the prediction of metabolizable energy (me) in pet food. Kienzle, E.


FELINE:


40) Are changes in apparent digestibility in ageing cats linked to changes in feeding behaviour? Peachey, S.E. and Harper, E.J.

41) The effect of toothbrushing on periodontal disease in cats. Ingham, K.E., Gorrel, C., Blackburn, J.M., and Farnsworth, W.


43) Prevalence of feline odontoclastic resorptive lesions in an ageing cat population. Ingham, K.E., Gorrel, C., Blackburn, J., and Farnsworth, W.

44) Rice bran decreases plasma and whole blood taurine in cats. Stratton Phelps, M., Backus, R.C., Rogers, Q.R., and Fascetti, A.J.

45) Effect of processing of dietary protein on lysine bioavailability in growing kittens. Larsen, J.A and Rogers, Q.R.


50) Breath hydrogen responses of cats given commercial dry and canned diets indicate intestinal microfloral activity varies with diet type. Backus, R.C., Puryear, L.M., Crouse, B.A., Biourge, V.C., and Rogers, Q.R.

51) In vitro fermentation characteristics of mannan oligosaccharides by dogs and cats. Hussein, H.S. and Healy, H.P.

**EQUINE:**

52) How does age influence play in domestic horses? Hughes, C.F., Goodwin, D., Harris, P.A. and Davidson, H.P.B.

53) Iodine balance in relation to iodine intake in ponies. Wehr, U., Engelschalk, B., Kienzle, E., Rambeck, WA.

54) Serum response after oral application of different zinc compounds in horses. Wichert, B., Kreyenberg, K., and Kienzle, E.


56) Serum response of ß-carotene in ponies after feeding ß-carotene either by grass meal or a synthetic beadlet preparation with and without added dietary fat. Kienzle, E., Kaden, C., Hoppe, P.P., and Opitz, B.

57) Supply with the trace elements zinc, copper and selenium in horses in bavaria. Wichert, B., Frank, T., and Kienzle, E.

58) Interactions between mixed feed and roughage on apparent energy and nutrient digestibility in horses. Kienzle, E., Fehrle, S., and Opitz, B.

59) Systemic and pulmonary bioavailability of two different forms of ascorbic acid in equids. Deaton, C.D., Marlin, D.J., Smith, N.C., Roberts, C.A., Kelly, F., Harris, P., and Schroter, R.C.


64) A clinical trial to assess the efficacy of two nutraceuticals for the amelioriation of lameness in horses. Dyson, S.J., Park, N.R., Harris, P and Preston, S.

**EXOTIC & OTHER:**


66) The ferret as an animal model to study vitamin A metabolism in carnivores. Raila, J., Gomez, C., and Schweigert, F.J.

67) Plasma concentrations of leptin mirror changes in body weight but do not influence the pattern of the pre-ovulatory luteinizing hormone (LH) surge in mink (mustela vison). Tauson, A-H. and Forsberg, M.

68) Substrate oxidation and energy expenditure in male blue foxes (alopex lagopus) during feeding, fasting and realimentation. Tauson, A-H., Chwalibog, A. and Ahlstrom, Ø.

69) Gut loading to enhance the nutritional composition of insects as food for reptiles: a mathematical approach. Finke, M.D.
SPEAKERS AT THE PLENARY SESSIONS:

Prof. Carl L. Keen
Carl received his BS and PhD in nutrition with a minor in physiological biochemistry from the University of California, Davis. He has been Chairman of the Department of Nutrition at the University of California, Davis since 1993 and a Professor of Nutrition since 1981. Prior to that he was a National Institute of Dental Research postdoctoral fellow and a Proctor and Gamble postdoctoral fellow again at the University of California, Davis. As well as being a member of several nutritional and scientific associations (including the American Society for Nutritional Sciences and the Society for Experimental Biology and Medicine), Carl serves on the editorial board of a number of scientific journals. Carl was given the Borden Award from the American Institute of Nutritional Research in 1995.

Prof. John Speakman
Professor John Speakman has been in working in Aberdeen since 1984 as a member of Aberdeen University Department of Zoology and was appointed to Head the Appetite and Energy balance group in July 2000, as a joint Rowett/University appointee. In 1998 Professor Speakman was the Royal Society of Edinburgh Caledonian Foundation Research Fellow and in 2000 he was a Royal Society of London Leverhulme Senior Research Fellow. He is currently chairman of the Aberdeen Centre for Energy Regulation and Obesity (ACERO). Professor Speakman’s research interests principally concern the quantification of energy expenditure and body composition using isotope based methodologies. These include the doubly-labelled water method which was the subject of a book written by him in 1997. He has also edited a book on comparative non-invasive body composition methods, to be published in 2001. His work uses these approaches to provide novel insights into the functioning of free-living animals. His work in zoology has yielded several publications in Nature, and in 1996 he was awarded the Zoological Society of London’s Scientific medal. His future work will bring to bear these isotope methodologies and his insights from the zoological evolutionary perspective on the causes of the international obesity epidemic.

Prof. Sharon Center
Prof. Sharon Center completed her veterinary training at the University of California at Davis in 1975. Following an internship at Cornell University in 1976, she worked in private practice for three years, then completed a residency in Small Animal Internal Medicine at the New York State College of Veterinary Medicine at Cornell University (1980-1982). Dr. Center then joined the faculty of the college and currently holds the position of Professor in Small Animal Internal Medicine. Dr. Center has written 27 book chapters and published more than 150 papers including peer-reviewed journals and proceedings with 90% of this work focused in the area of hepatobilliary disease in the dog and cat.

Prof. R. John Mayer
Professor R John Mayer graduated in Biochemistry with a first class honours degree from the University of Birmingham in 1965. Subsequently, he obtained a PhD which included the seminal discovery of the invertebrate equivalent of glucagon. He has studied intracellular proteolysis for thirty years, including extensive studies on the ubiquitin proteasome pathway for the last fourteen years. He was made a Fellow of the Royal College of Pathologists on the basis of the application of ubiquitin immunocytochemistry to discover dementia with Lewy bodies. This disorder is second only to Alzheimer’s disease as a cause of cognitive decline in the elderly. As in Alzheimer’s disease, the neuropathology includes extracellular amyloid deposits but, in the case of dementia with Lewy bodies, cortical neurones contain large ubiquitilated inclusions which are Lewy bodies. The distribution in the brain of Lewy bodies, as well as their intensity, correlates neuropathologically with disease progression. The Nottingham Group for the Study of Neurodegenerative Diseases not only established the neuropathological basis of dementia with Lewy bodies, but also established the clinical criteria for the diagnosis of the disease. These criteria were adopted in 1996 as the global standards for dementia with Lewy bodies. The Nottingham Group also showed by the application of ubiquitin immunocytochemistry that inclusions are present in the anterior horn cells in the spine and in the motor cortex of patients succumbing to motor neurone disease (amyotrophic lateral sclerosis). Others have demonstrated intraneuronal inclusions in a variety of other diseases including polyglutamine expansion diseases, eg Huntington’s disease. He is devoted to further understanding of the role of the ubiquitin/26S proteasome pathway in health and disease.
ABSTRACTS

Abstracts of oral presentations are arranged in chronological order, followed by poster abstracts.
The Comet Assay: A measure of DNA damage validated in the cat and dog for nutritional studies.

WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire, UK

A variety of defence mechanisms exist to quench potentially damaging free radicals. Despite these defence systems damage still occurs within the cell and it is thought accumulation of unrepaired, oxidatively damaged DNA may contribute to a variety of disorders associated with the ageing process. Previous WALTHAM research has shown that it is possible to sustainably increase some of the key dietary antioxidants1,2, and it is clear that maintenance of a balanced antioxidant status and reduction in levels of oxidative stress is becoming a major focus of nutritional research. One of the requirements to achieve these objectives is the ability to accurately measure levels of free radical damage and assess how dietary intervention may be able to reduce such damage in cats and dogs. At WALTHAM, we have developed and validated the comet assay (modified from Singh et al., (1988)3), for measuring levels of DNA damage in cat and dog blood samples for use in nutritional and oxidative stress-related studies.

In recent WALTHAM studies endogenous and exogenous (i.e. after in vitro exposure to varying concentrations of H2O2), DNA damage was measured in cats and dogs fed either control diets or control diets supplemented with antioxidants. Figure 1 shows measured endogenous damage in cats which had been fed an antioxidant supplemented diet for a period of two years. All of the results prove significant (p<0.05) reductions in levels of DNA damage in the antioxidant supplemented groups when compared to the non-supplemented controls.

Whilst it is not yet clear whether this observed reduction in DNA damage is due to a reduction in free radical damage or increased levels of DNA repair, the overall effects prove that WALTHAM has shown reduction of DNA damage through dietary antioxidant supplementation in cats and dogs. This reduction may reduce susceptibility to degenerative disorders (including the ageing process in general).

References:
Telomeres are specialised DNA-protein complexes that cap the ends of linear chromosomes. In vertebrates, telomeres consist of tandem repeats of the sequence TTAGGG and a number of telomere associated proteins. Telomeres maintain genomic integrity by protecting chromosome ends from recombination, fusion and from being recognised as DNA damage. In normal human somatic cells, telomeres shorten with each cell division. This telomeric attrition has been attributed to the inability of the DNA replication machinery to efficiently replicate the 5' ends of linear chromosomes (end replication problem). Thus telomeres shorten progressively with cell division and telomere shortening to a critical length has been proposed to limit the life span of somatic cells in humans and play an important role in the process of cellular senescence. Consistent with this model, telomere shortening has been observed in vivo during aging of normal somatic tissues as well as in in vitro cultured human fibroblasts. In addition, cells from patients with premature aging syndromes show evidence of premature telomeric erosion.

The aim of this study was to investigate telomere lengths and telomerase activity in a range of canine tissues. Telomere repeat fragment (TRF) analyses were employed to study telomeric lengths in 60 canine Peripheral Blood Mononuclear Cells (PBMC) samples from dogs of varying breeds and ages. TRF analysis in canine PBMCs and tissues demonstrated mean TRF lengths to range between 12 kbp to 23 kbp with heterogeneity in telomere lengths being observed in a range of normal somatic tissues. These data did not demonstrate any statistically significant correlation between mean or peak TRF with donor age. However, within the Retrievers a trend indicating telomeric attrition with increasing donor age was observed. TRF patterns generated from young and old suggest that age-specific differences in telomere lengths may exist. We conclude that the dog has similar telomere biology to humans and that telomeric attrition may contribute to the aging process.
AGE RELATED CHANGES IN DOGS’ FLAVOUR PERCEPTION
McCune, S.
Waltham Centre for Petcare & Nutrition, Melton Mowbray, Leicestershire, U.K.

Age-related changes in dog behaviour have become the focus of many studies recently. These studies have focussed on disease, motor function and cognitive ability. This study looks at flavour perception in the dog as a means of assessing age-related changes in sensory function. In a series of preference tests, dogs were assessed for their tolerance of increasing levels of a flavour (chilli powder) expected to be unpalatable, at least at high levels of addition. The flavour was added to a standard complete prepared dog food. Flavour addition started at very low levels (0.0005%) to avoid strong aversion and gradually increased to a maximum inclusion of 1.0%. Flavour was added at concentrations of 0.0005%, 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.0075%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.075%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% & 1.0%. Flavour was dissolved in water and made up to a fixed volume. This was added to standard product and tested against a control of standard product with an equal volume of water added. Each day of testing, dogs were offered one test product against control product. Fifteen dogs were used: 5 in each of 3 age categories: i) less than 3 years of age; ii) 3-8 years; and iii) over 8 years of age. All dogs failed to discriminate between control and test product until flavour was added at a 0.05% level, above which the flavoured product was increasingly rejected. At concentrations above this a significant effect of age was found (ANOVA F=-3.3, p<0.005). Older dogs were significantly less likely than younger dogs to reject the product with flavour added until flavour was added at a level greater than 0.075%. Beyond this, virtually all dogs increasingly rejected test product as flavour concentration increased. Within this limited population, breed differences were also found; Yorkshire Terriers and Miniature Poodles were less likely to reject flavoured product than West Highland Terriers and Dachshunds (ANOVA F=5.46, p<0.0005). Age-related differences in feeding behaviour were also found. Older dogs were more likely than younger dogs to exclusively eat just one product within a single test. These results show that older dogs respond differently towards flavour cues than younger dogs. Older dogs were more tolerant than younger dogs of the chemical cues contained in chilli peppers suggesting their ability to detect flavour deteriorates with age. Older dogs may be unable to fully appreciate palatability cues in standard dog food which is usually developed to maximise acceptance in the average dog. The addition of stronger flavours to food may provide a means of maintaining appetite for older animals suffering from inappetence due to sensory loss.
The past decade has seen a major shift in equine demographics: it is estimated geriatric horses now comprise 10 – 20% of the equine population. Despite the increasing number of aged horses, minimal research has been done to investigate the physiological process of ageing in the horse. There are several recognized quantitative and functional immunological changes associated with ageing in other species. Total lymphocytes, T-cells, B-cells, CD4+ cells, and CD8+ cells decrease in humans and rodents with advancing age, likely a result of thymic involution. T-cell mediated immune responses including interleukin-2 (IL2) production, IL2 receptor production, delayed hypersensitivity, and mitogen stimulation are decreased in elderly humans and rodents. Immunoglobulin serum concentration of IgG and IgA increase with age in healthy old people, IgM does not. Proinflammatory cytokines interleukin 6 (IL6) and tumor necrosis factor-a (TNF-a) as well as acute phase proteins increase in aged humans and laboratory animals. The exact mechanism of these changes remains speculative.

The purpose of this study was to characterize age-associated changes in lymphocyte population subsets and immunoglobulin isotypes by comparing young and aged horses. Thirty healthy light breed horses 20 years or older (mean age 23.3 years) were selected from the NCSU field service population. All horses were clinically normal with no history of illness or trauma in the previous 6 months. All horses had a normal hair coat and showed no clinical evidence of pituitary dysfunction (Cushing's disease.) Thirty healthy horses age 5-12 (mean age 9.1 years) were selected from the same environment to serve as controls. All sixty horses had a normal complete blood count and serum chemistry panel.

Whole blood was collected in EDTA tubes and white cells isolated by whole blood lysis. Cells were labelled with monoclonal antibodies against equine cell surface markers CD5 (a pan-T cell marker), CD4, CD8 or IgG and IgM heavy and light chain (B-cell marker) then secondarily labelled with fluorescent antibody. Surface fluorescence was quantitated by flow cytometric analysis. Total lymphocyte count from the concurrent complete blood count was used to calculate absolute lymphocyte subset counts for each horse.

Determination of serum immunoglobulin concentration was performed using a commercial single radial immunodiffusion kit. Immunoglobulin types IgM, IgG, IgG(T), and IgA were determined using 3 ml of serum. Reference standards provided in the kit were used to calculate a linear regression line used to determine the concentration of the samples.

Values of aged horses were compared with values of control horses using the Student's t-test, or when data was not normally distributed, using the Mann-Whitney rank sum test. A p value <0.05 was considered significant.

Absolute cell counts of total lymphocytes, CD5+, CD4+, CD8+ and B cells of aged horses were significantly less than those of younger horses (p<0.0001). There was a significant decrease in the percentage of CD8+ cells in the aged population (p=0.039). There was no significant difference in immunoglobulin isotypes in the aged horse when compared to the control horses, however there was a tendency towards a higher concentration of IgG and IgA (p=0.07) in the aged group. Results of this study suggest an age-related decline of total lymphocyte count, as well as lymphocyte subset cell counts in horses, similar to other species. These changes may contribute to an age-associated decline in immunocompetency.
Studies to assess age-related differences in feline immune phenotype in 51 Domestic Shorthaired cats (Adults; n=26 and Seniors; n=25) at the WALTHAM Centre for Pet Nutrition revealed significant differences between the two groups. The senior group had lower total leukocyte (p<0.05) and lymphocyte (p<0.001) counts. Analysis within the lymphocyte population, revealed that the overall percentage of cells staining for pan T-cell marker was similar in both groups. Within the T-cell population, the percentage of CD4+ cells was lower (p<0.001) and CD8+ cells higher (p<0.05) in the senior group, resulting in a lower CD4+:CD8+ ratio (p<0.001).

Further studies utilising 288 Domestic Shorthaired cats at the WALTHAM Centre for Pet Nutrition, ranging from 0.2 to 15.9 years confirmed these significant changes in immune parameters with age. Linear regression analysis identified a significant decrease in CD4 (R2 = 0.12, p<0.001), a significant increase in CD8 (R2 = 0.15, p<0.001), with a corresponding decrease in the CD4:CD8 ratio (R2 = 0.23, p<0.001) with increasing age. Using discriminant analysis on the CD4:CD8 ratio data we were able to define two statistically distinct groups, kittens (2 to 8 months) and adults (8 months to 15.9 years), with an overall correct classification of 77% (cross-validated). These findings are in agreement with age-related changes observed in other mammals, including humans, suggesting that these species share similar characteristics of immunosenescence. The data also show the potential for using markers of immune status for defining life-stage classifications.
Interest in feeding carotenoids to cats has increased because of potential beneficial antioxidative effects. With the exception of a lack of intestinal carotenase activity (Gershoff et al. 1957), little is known with regard to the metabolism of β-carotene in cats. In the present investigation the plasma response for β-carotene and vitamin A (retinol and retinyl esters) including their content in the chylomicrons after a single oral bolus of β-carotene (100 mg/kg body weight, BW) as well as the urinary excretion of β-carotene and vitamin A was examined in six adult domestic cats (2-5 y; 3.8-6.0 kg BW). The cats were fed a vitamin A deficient UV-irradiated diet for a period of 28 days. Control samples were taken at day 9 and the bolus was given after day 23. Blood samples were taken before the bolus was eaten and after 2, 4, 6, and 12 hours. Urine was collected for 96 hours. β-Carotene, retinol and retinyl esters from plasma and urine were determined by rp-HPLC. Chylomicrons were separated by ultracentrifugation (d<1.006 g/ml). Lipids were measured by enzymatic methods.

β-Carotene levels continuously increased for 12 hours after the bolus was eaten (Table 1). Neither plasma retinol nor retinyl palmitate or stearate were affected and no differences were observed between the control and experimental period. β-Carotene, but not retinol was excreted in the urine with maximum levels at 48 hours. Although there was a postprandial increase of chylomicron associated triglycerides, the β-carotene and retinyl ester content in this fraction was not systematically affected.

The results can be interpreted as a confirmation that β-carotene is absorbed but not transformed into vitamin A at least as long as cats are not vitamin A deficient. The kinetic of the plasma response is different to known kinetics for humans (v.d. Berg and v. Vliet, 1998). The increase occurred slower and the elevated plasma levels persisted longer in the cats. This invites speculations with regard to species specific differences in the site and mechanism of β-carotene absorption.

References:

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<td>β-Carotene</td>
<td>n.d.</td>
<td>74 ± 63</td>
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<td>Retinol</td>
<td>134 ± 14</td>
<td>67 ± 32</td>
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<tr>
<td>Retinyl palmitate</td>
<td>97 ± 49</td>
<td>112 ± 67</td>
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<tr>
<td>Retinyl stearate</td>
<td>161 ± 67</td>
<td>183 ± 103</td>
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Fish oil is a rich source of polyunsaturated fatty acids (PUFAs), which are susceptible to oxidation. There have been numerous reports in the literature on vitamin E deficiency in cats due to the feeding of diets high in PUFAs. Vitamin E is a natural scavenger of free radicals and as such protects PUFAs from oxidation in vivo. NRC (1986) recommends increasing the vitamin E content of diets for cats by 3-4 fold if the diet contains a high level of PUFAs. AAFCO (2000) recommends that dietary vitamin E levels should be increased by 10 IU for every g of fish added per kg diet dry matter. The aim of the present study was to determine the vitamin E requirements of adult cats fed a diet with a high PUFA content.

Thirty-two (16 male, 16 female) adult domestic cats (Felis catus) were randomly allocated to four groups according to sex and fed one of four fish oil (30% of dry matter) containing experimental diets (0, 1.2, 2.8, and 4.3 IU DL-a-tocopheryl acetate/g dry matter) for 26 wks. The cats had previously been fed a complete and balanced commercial cat food (AAFCO 1998) for 6 months prior to the start of the study. Blood samples were obtained at the start, biweekly until 18 wks and at 26 wks of the study. Blood samples were subjected to plasma a-tocopherol, red blood cell haemolysis, the ferric reducing ability of plasma, lymphocyte proliferation (to concanavalin A, pokeweed and phytohemagglutinin), plasma alkaline phosphatase and plasma triglyceride analysis. Data were analysed using repeated measures ANOVA with diet and gender as variables and time as the repeated factor.

All cats remained healthy throughout the study and no clinical signs of vitamin E deficiency were observed. Plasma a-tocopherol levels responded to dietary inclusion of a-tocopherol, with the cats fed the unsupplemented diet maintaining a low plasma a-tocopherol concentration throughout the study. There was an effect (P<0.05) of diet on plasma a-tocopherol levels, the ferric reducing ability of plasma and red blood cell haemolysis. Plasma triglycerides, plasma alkaline phosphatase and whole blood lymphocyte proliferation to concavalin A, pokeweed and phytohemagglutinin were similar (P>0.05) between groups. No effects of gender, diet x gender, gender x time or diet x gender x time were found for any of the response parameters.

Red blood cell haemolysis and the ferric reducing ability of plasma of the cats fed the unsupplemented diet were significantly compromised in comparison to the cats fed the supplemented diets as a result of the low a-tocopherol content of the diet. The failure to induce vitamin E deficiency symptoms in the cats was believed to be the result of the quality fish oil used and the high selenium content of the diets in the study. The fish oil used was of a high quality as lipid peroxides were not detected in the diets at the start of the study. In vivo oxidation rate may have been relatively low thereby “sparing” vitamin E. Selenium levels in the diet were on average 1.0 ppm dry matter. Addition of fish oil to diets for adult cats increases vitamin E requirements by approximately 5 IU per g of added fish oil per kg diet dry matter.

References
Racing greyhounds have a comparatively short life-span and most dogs are withdrawn from racing at five years of age. During exercise, increased production of reactive oxygen species may overwhelm the body’s natural antioxidant defenses. It is possible that cellular damage resulting from this oxidative stress may shorten a greyhound’s active career. Vitamin C is an antioxidant that is not considered an essential nutrient for dogs because it is synthesized in adequate amounts. Nevertheless, the requirement for vitamin C may increase beyond the synthetic capacity of the liver during strenuous exercise. Previous experiments have shown that oxidative stress as measured by the ratio of thiobarbituric acid reducing substances (TBARS; a marker of oxidation) to trolox equivalent antioxidant capacity (TEAC; a measure of total antioxidant capacity) increases after exercise. To date, the effects of chronic vitamin C supplementation on oxidative stress in the racing greyhound have not been studied. The objective of this experiment was to determine if supplementation with pharmacological doses of ascorbic acid influences plasma ascorbate levels, antioxidant capacity, or markers of oxidation in greyhounds after a 500m sprint. Preliminary experiments showed that circulating vitamin C concentrations return to baseline within 6 hours after administration of 1000 mg ascorbate. Five racing greyhounds (all female, weighing 27.6±0.6 kg (mean ± SD) and aged 2.9±0.4 years) in training were assigned to receive each of 3 treatments for 4 weeks, in a Latin square design. Dogs were randomly assigned to 3 treatment groups: (1) no supplemental ascorbate; (2) 1000 mg ascorbate daily, administered orally with food (after exercise or racing); (3) 1000 mg ascorbate daily, administered orally 1 hour prior to racing on race days, and administered orally with food after exercise on non-race days. At the end of each treatment period, blood was collected before racing, and 5, 60 and 1440 minutes after racing. Blood was analyzed for ascorbic acid, TBARS and TEAC. Vitamin C increased in dogs receiving ascorbate prior to racing (p<0.05) compared to unsupplemented dogs at 5 and 60 min after racing. The mean ratio of TBARS/TEAC did change after racing (p<0.05) but the difference was small and there was no evidence of an effect of vitamin C administration either before or after racing on TBARS, TEAC or the ratio of TBARS/TEAC compared to unsupplemented dogs. This experiment failed to show any benefit of chronic vitamin C administration or increased circulating vitamin C concentrations on oxidative stress in trained racing greyhounds though the number of dogs may have been too small to show a difference. Further experiments are necessary to ascertain whether vitamin C may be of benefit in untrained dogs undertaking strenuous exercise or whether supplementary vitamin C may be of benefit in combination with other antioxidants.
CHANGES IN CIRCULATORY ANTIOXIDANTS AND MARKERS OF OXIDATIVE DAMAGE DURING ENDURANCE COMPETITION
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Prolonged exercise in man, and more recently in sled dogs, has been shown to increase systemic oxidative stress and markers of oxidative damage. Such changes may be associated with membrane and tissue damage, particularly in muscle. We therefore expected that oxidative stress would be apparent in horses competing in endurance rides at intensities around 50% of maximal oxygen uptake for 10-12 hours.

Three venous blood samples were collected from 40 horses before (Pre), at the end (End) and after 16 hours recovery (+16h) following a 140 km competitive race ride. Plasma was analysed for CK, AST, vitamin E, ascorbic acid, TBARS, uric acid and iron. The concentrations of GSSG (oxidised glutathione) and TGSH (total glutathione) in red blood cells and the glutathione redox ratio (GRR; GSSG/TGSH) and GSH (TGSH-GSSG; reduced glutathione) were calculated.

The mean speed of the horses sampled over the total 140 km was 16.5 ± 1.6 km/h and ranged from 13.9 to 19.7 km/h. GSH, GSSG and TGSH all decreased significantly from Pre to End exercise. GSH and TGSH showed no further change from End to +16h, but GSSG was further decreased at +16h (P<0.001). However, GRR did not change significantly with exercise or following recovery. Plasma ascorbic acid concentration in individual horses either increased, decreased or remained unchanged from Pre to End exercise and overall was not significantly different. However, plasma ascorbic acid concentration was significantly decreased at +16h compared to both Pre (P<0.00001) and End (P<0.0001). Plasma ascorbic acid concentration at End was significantly correlated with mean speed (r=0.622; P=0.0004). Plasma vitamin E concentrations were not significantly altered by exercise or at 16hrs recovery. The concentration of TBARS in plasma before exercise showed a large variation (66 to 1048 nmol/l) and there was an overall increase with exercise (P<0.001), but no further change between End and +16h (P>0.05). There was a relatively narrow range in plasma uric acid concentrations before exercise (7.3 to 17.4 umol/l) and a significant increase in uric acid at the end of exercise (P<0.00001). By +16h the median uric acid concentration (9.8 umol/l) had fallen to below that at Pre (11.8 umol/l).

Prolonged endurance exercise causes changes in blood and plasma antioxidants and there is evidence of oxidative stress in some horses but the response is highly variable between individuals. This may be related to factors such as diet and warrants further investigation.
LIPOIC ACID AS AN ANTIOXIDANT IN MATURE GELDINGS: A PRELIMINARY STUDY

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Oxidative stress is evident in horses undergoing intense and endurance exercise; this warrants the testing of antioxidant supplements. Alpha-lipoic acid (LA) has illustrated antioxidant effects in recent studies (Packer et al., 1995). Lipoic acid helps scavenge free radicals and recycle other antioxidants, like vitamin E, C and glutathione. It is effective in the rat at a dose of 10 to 100 mg/kg (about 40 to 200 mg/kg0.75) and in humans at a dose of 600 to 1200 mg (about 8.5 mg/kg or about 25 mg/kg0.75 and about 17 mg/kg or 50 mg/kg0.75, respectively); however, it caused gastrointestinal distress at the high dosage. It may be a useful antioxidant supplement in horses with oxidative stress during exercise. Our hypothesis was that a dose of 10 mg/kg (about 50 mg/kg0.75) diminishes oxidative stress in horses without causing any clinical signs of toxicity.

Ten mature Thoroughbred geldings were supplemented with LA for 2 weeks. The geldings were maintained on pastures of winter bluegrass/fescue with free access to alfalfa/orchard grass hay. The supplement consisted of 10 mg/kg body weight dL-a-lipoic acid (Sigma Chemical) once a day. The LA was mixed into a supplement containing molasses (30 g) and sweet feed (40 g) and offered by hand to 5 of the geldings; the other 5 geldings received a control supplement (CON, molasses and sweet feed only). Blood samples were collected in tubes containing sodium heparin at 1300 h just prior to supplementation. They were obtained at baseline (before start of supplementation period), after 1 and 2 wk of supplementation, then 48 hours without supplementation. Samples were centrifuged and processed into red blood cell, white blood cell, and plasma aliquots, then frozen at -80°C until analysis. Blood fractions were analyzed for glutathione (GSH), glutathione peroxidase (GPx), and lipid hydroperoxides (LPO).

An experienced veterinarian observed no adverse clinical signs of toxicity, including gastrointestinal distress. Plasma LPO was 0.32 ± 0.16 mM at baseline with no difference between groups. The LPO in the LA group was 0.12 ± 0.05 and 0.19 ± 0.08 mM at 1- and 2-wk, respectively, and lower (P = 0.064 and 0.034, respectively) than corresponding CON values (0.25 ± 0.05 and 0.41 ± 0.06 mM, respectively). Plasma LPO returned to baseline after 48 hr. For GSH and GPx no differences were found between groups at any stage. At baseline, GSH concentration was 69 ± 7 and 115 ± 13 mmol/mg protein in the white and red blood cells, respectively. GPx activity was 47 ± 4 and 26 ± 5 U/g protein in the white and red blood cells, respectively. The GPx activity decreased with time (R² = 0.91, P = 0.042) in CON, but not LA.

The results show that a dose of 10 mg/kg of LA was absorbed in the horses gut, had no evident adverse effect, and reduced the oxidative stress of horses allowed light voluntary activity. Plasma LPO may be regarded as a cumulative and persistent response, whereas both GSH concentration and GPx activity may have changed transiently in response to LA then reverted to normal values after 24 h when the next blood sample was taken. The present findings encourage testing of LA supplementation in horses subjected to strenuous exercise and suggest that the timing of supplementation may be critical.

References
Cardiovascular disease is one of the leading causes of death in humans in the developed world, and rapidly achieving similar status in the developing world. Though less well understood, it is known that cardiovascular disease can be a significant issue in some companion animal species as well. The development of vascular pathologies over time clearly plays a central role in cardiovascular disease progression, and can also play a key role in the progression of other disease processes, eg, renal disease. Flavonoids are a large and diverse class of naturally occurring antioxidants ubiquitous in plant foods, and an increasing body of literature suggests that certain members of this class may inhibit the progression of age-associated vascular pathologies. In particular, the flavanols and their related oligomers, the procyanidins, are the subject of intensive investigation. In this context, our group in collaboration with others have shown significant biological activity related to modulation of eicosanoid synthesis, platelet function and inhibition of lipoprotein oxidation has been. Taken together, these results provide strong evidence that dietary flavanols and procyanidins can potentially play a role in preventing age-associated vascular pathologies in both humans and companion animals.
Nutrition can play an important role in the management of patients with cardiac disease. Dogs with cardiac disease often have nutritional alterations (e.g., anorexia, cardiac cachexia) and a variety of nutritional modifications have been proposed (e.g., sodium restriction, nutritional supplementation). Nutritional modulation requires knowledge of the usual dietary patterns of dogs with cardiac disease. Dietary patterns may change when dogs are initially diagnosed with cardiac disease or may vary with increasing severity of disease. In addition, dogs with cardiac disease are typically older and may have other diseases that could also require dietary modifications. The purpose of this study was to determine the dietary patterns of dogs with cardiac disease. Dogs with stable (>1 month on the same medications) cardiac disease were included in the study. Canine patients with cardiac disease presented to Tufts University School of Veterinary Medicine were identified from the cardiology database. Owners of these dogs were contacted by telephone and were given a standardized questionnaire regarding their dogs' diet history and food intake over the last 24 hours.

Twenty-four owners of dogs with cardiac disease were contacted. Two dogs had died and one owner declined to participate; thus, 21 dogs with cardiac disease were included in the study. All 21 dogs had chronic mitral valve disease (endocardiosis). The mean murmur grade was IV/VI. The mean age of these 21 dogs was 12.1 ± 3.2 years. The dogs were predominantly male (14; 11 neutered) with only 7 female dogs (7 neutered). The most common breed represented was the poodle (5 miniature, 2 toy). There were also multiple Cavalier King Charles spaniels (3) and Chihuahuas (2). Dogs were predominantly small breed dogs, with a median body weight of 8.1 kg (range, 2.4-22.7 kg). Body condition scores were available for 17 of 21 dogs. The mean body score was 5 ± 1. Most dogs had a body condition score between 4 and 6 (14). Only 2 dogs had a body condition score of 3 and 1 dogs had a body condition score of 7.

The majority of dogs included in the study were asymptomatic for the cardiac disease (12) but 9 dogs had a prior episode of congestive heart failure. The median length of time since initial diagnosis of the cardiac disease was 21 months (range, 5-108 months). Seventeen dogs were receiving cardiac medications. Many dogs (13) also had other diseases including hyperadrenocorticism, osteoarthritis, atopy, systemic hypertension, intervertebral disk disease, urolithiasis, collapsing trachea, seizure disorder, chronic renal failure, and cancer. Some dogs had multiple disease processes.

Only 5 owners changed their dogs' diets when the cardiac disease was first diagnosed. Another 13 owners changed the diets later during the course of disease. Anorexia was present, either currently or at some point in the past, in 7 dogs (33%). Fourteen dogs had never experienced anorexia. Anorexia was significantly more likely in symptomatic dogs (6/9) than in asymptomatic dogs (1/11; p=0.005). Diets eaten were varied. Dogs ate commercial dry dog food only (4), commercial canned food only (5), or both (9). Three dogs ate no commercial dog food (these dogs consumed homemade diets exclusively). Five dogs were eating therapeutic diets (3 for cardiac disease, 1 for obesity, and 1 for calcium oxalate urolithiasis). Nineteen of 21 dogs (91%) ate commercial dog treats and 16 (76%) ate human food as treats. Nutritional supplements were given to 7 dogs. These included n-3 fatty acids (2), coenzyme Q10 (2), multivitamin supplements (2), vitamin E (2), and glucosamine (2). Twelve of the owners used foods, either dog food or human foods, to administer medications. Many of these foods were high sodium foods such as lunch meats. This information can assist nutritionists in determining the optimal diet for dogs with cardiac disease and where nutritional modifications could be beneficial.
Telomeres are specialised DNA-protein complexes that cap the ends of linear chromosomes. In vertebrates, telomeres consist of tandem repeats of the sequence TTAGGG and a number of telomere associated proteins. Telomeres maintain genomic integrity by protecting chromosome ends from recombination, fusion and from being recognised as DNA damage. In cells that proliferate indefinitely such as germline cells and transformed cells, the enzyme telomerase provides a mechanism for the maintenance of telomere length. Telomerase is a ribonucleoprotein reverse transcriptase capable of synthesizing terminal TTAGGG telomeric repeats thereby extending telomere length and compensating for telomeric attrition during replication.

The aim of this study was to evaluate telomerase activity in normal and cancerous tissues of the dog. Using a combined PCR/ELISA method, telomerase activity was analysed in multiple adult canine tissues derived from both tumour bearing and non-tumour bearing dogs. Telomerase activity was detected in tumour and gonadal tissues with little or no activity present in normal somatic tissues. Telomerase activity was also assessed in a panel of canine immortalised cell lines. High levels of telomerase activity were detected in several canine cell lines. Two cell lines (MDCK, A72) were negative for enzyme activity, suggesting that an alternative pathway for telomere maintenance may operate in these cell types. Based on our finding we propose that telomerase may contribute to the maintenance of cancer in dogs and may represent a target for therapeutic intervention.
Traditionally, green-lipped mussel has been shown to reduce arthritic symptoms in humans. We evaluated the efficacy of GLM powder of alleviating arthritic symptoms in dogs when it is added onto a dry diet or processed into a semi-moist treat by a patent-pending method designed to retain activity. Freeze-dried stabilized GLM powder was supplied by McFarlane Laboratories, Australia. The design of both studies were double blind, randomised, controlled, using mixed breed/sex dogs (4-13 yrs old) exhibiting varying degrees of arthritic symptoms at least 4 months to several years. For evaluation of arthritic symptoms, each dog was scored for mobility (lameness in walking, trotting and climbing stairs) and all joints were individually scored for degree of pain, swelling, crepitus and reduction in range of movement (0 = no symptoms; 1 = mild; 2 = moderate; 3 = marked; 4 = severe). Summation of all scores for an individual dog comprised their total arthritic score. To evaluate the effect of GLM powder, 32 dogs were randomly assigned to two groups (Control (n = 15) and GLM (n = 17) at baseline. GLM powder was added on top of the standard food before feeding (>34 kg = 1000 mg GLM powder/day; 34-25 kg = 750 mg/day; <25 kg = 450 mg/day). To investigate the efficacy of a GLM-containing treat, 33 dogs were randomly assigned to two groups, Control (n = 16) and GLM Treat (n = 17) at baseline. Treats were fed based on body weight to deliver the following GLM dosage levels: >34 kg = 1000 mg GLM/day (2 pieces); 34-25 kg = 750 mg GLM/day (1.5 pieces); <25 kg = 450 mg GLM/day (1 piece). Dogs in both studies were fed the same dry base diet at a level to maintain body weight. In both studies, the changes in total arthritic scores, joint pain, swelling and crepitus scores at the end of 6 weeks showed significant improvement in the GLM powder-supplemented (Group 1) and GLM Treat (Group 2) groups when compared to their respective Control groups (p < 0.05). In Group 1, 82.4% (14/17 dogs) of the dogs in the GLM supplemented group demonstrated a 30% or greater reduction in total arthritic scores (indicating an improvement), however, only 6.67% (1/15 dogs) of the dogs in the Control group showed a 30% or greater improvement. In Group 2, 53% (9/17 dogs) of the dogs in the GLM Treat group exhibited a 30% or greater reduction in total arthritic scores and 0% (0/16 dogs) of the dogs in the Control group demonstrated a 30% or greater improvement after 6 weeks of treatment. The above data provide evidence that GLM powder supplemented onto a diet or as a processed treat helped alleviated arthritic symptoms in dogs.
The formation of urinary tract stones composed of either calcium oxalate (CaOx) or magnesium ammonium phosphate (MAP) is an important clinical problem in dogs and cats. In order to assess the probability of stone formation in a particular animal, or to evaluate a dietary or other intervention, it would be useful to have a biochemical measure of the risk of MAP or CaOx precipitation in urine. Computer programmes are widely used in humans to calculate relative supersaturation (RSS) as a predictor of mineral crystallisation. The aim of this study was to compare and validate two computer programmes, SUPERSAT and EQUIL, for use in dogs and cats.

10 healthy adult cats and 9 dogs were fed standard diets for 3 weeks. Urine was collected over 24 (dogs) and 48 h (cats), filtered and the pH measured. 20 ml was acidified to pH 2 and frozen for analysis. Additional aliquots were incubated with 1g of seed crystals at 38°C; CaOx for 24 h (cat) and 2, 6, and 9 days (dog); MAP for 48 h (dog) and 6 days (cat). Samples were then filtered, acidified to pH 2 and frozen. 10 substances were analysed by HPLC and RSS values were calculated using EQUIL and SUPERSAT.

CaOx RSS (SUPERSAT): dog urine was initially supersaturated, whilst cat urine was undersaturated with the diets fed. Cat urine virtually reached the solubility product (Ksp; RSS=1) after 24 h incubation, whereas dog urine had not reached Ksp even after 9 days. MAP (SUPERSAT): urine from both species was undersaturated and increased towards Ksp during incubation. Final RSS values were compared for the two programmes. SUPERSAT resulted in values close to 1 for both CaOx and MAP, EQUIL 2 gave similar values for CaOx RSS, however, MAP RSS values were considerably higher than 1.

It was concluded that EQUIL 2 and SUPERSAT both calculated reasonably accurate RSS values for CaOx, whereas only SUPERSAT provided an accurate measure of MAP RSS.

References:
Polyunsaturated fatty acids of the n-3 or n-6 type are not synthesized de novo in animal tissues. When fed, displacement of endogenous fatty acids (20:3n-9 and 20:4n-7 types) occurs resulting in enrichment of n-3 and n-6 highly unsaturated fatty acids (HUFA; 20:5n-3, 22:5n-3, 20:3n-6, 20:4n-6). Competition between the n-3 and n-6 fatty acid precursors for these conversions exists. Because the 20 carbon HUFA play important roles as precursors and antagonists of eicosanoid biosynthesis, dietary modification of their metabolic HUFA products may result in an alteration of certain eicosanoid related disorders. Thus, understanding the nature of these effects forms the basis for making dietary recommendations. Dietary studies with rat plasma, tissues, and human plasma have been published in which a quantitative relationship between diet and tissue fatty acids were described (Lands et al 1990, 1992). The purpose of the present study was to examine the extent to which similar relationships exist in canine species.

In this study clinically normal, adult dogs were fed known basal diets plus beef tallow, safflower oil, linseed oil, or menhaden fish oil (40 en % fat) or plus beef tallow or safflower oil (20 en % fat). Plasma was collected on Day 0 and plasma and whole blood were collected on Day 28 during the supplement period. Neutrophils were isolated via gradient centrifugation. Total lipids were extracted and lipid classes subfractionated via thin layer chromatography. Lipid subfractions (total phospholipid (PL) in plasma and neutrophils and triglyceride (TG) in plasma) were derivatized to fatty acid methyl esters and gas chromatography used to generate fatty acid profiles. The 18:2n-6 and 18:3n-3 TG fatty acids were expressed as weight % while the PL fatty acid results were expressed as n-6 and n-3 HUFA as a percentage of total HUFA. Dietary n-3 and n-6 fatty acid ranged from 0.26-19.6 for 18:3n-3; 2.5-27.4 for 18:2n-6; 0.0-8.8 for n-3 HUFA; and 0.0-0.09 for n-6 HUFA (all en %). Algebraic equations resembling the competitive hyperbolic relationship commonly used to describe rate-limiting processes (Lands et al, 1992) were modified to fit the canine PL data and second-order polynomial regression analysis was used to fit the plasma TG data. Regression of the TG data revealed r2 values of 0.999 and 0.997 at p<0.05 for the 18:3n-3 and 18:2n-6 acids respectively allowing accurate prediction of plasma TG fatty acids from known dietary amounts. Constants for the PL hyperbolic equations were determined using those developed in other species using trial and error modifications. The magnitude of constants representing the effective concentrations of dietary 18:3n-3 and 18:2n-6 were low (0.29 and 0.036 respectively) consistent with the long-standing notion that adequate amounts of dietary essential fatty acids may be no greater than 0.5 en %. Further, the 8-fold relative difference between these constants supports low metabolic conversion rates of 18:3n-3 vs 18:2n-6 seen in other studies. The quantitative relationships determined here will help predict plasma and tissue enrichment of the n-3 and n-6 fatty acids from their dietary concentrations.

References:
“RED HAIR” IN BLACK CATS IS REVERSED BY ADDITION OF TYROSINE TO THE DIET.
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Five experiments were conducted to elucidate the nutritional basis for “red hair” in black cats.

Exp.#1: Four black kittens from the same litter were given a purified diet based on casein and lactalbumin from weaning to 12 wk of age. Kittens were then divided into two groups: one group received a 380 g/kg gelatin + amino acids (AA) diet that exceeded NRC (1986) recommendations, and the other group was given a similar diet based on casein and lactalbumin. After 4 months the kittens given the gelatin-AA diet had reddish-brown hair, whereas the kittens given the diet of casein-lactalbumin maintained their black hair color. Hair from the kittens given the gelatin-AA diet contained less melanin on microscopic examination than the hair from the other kittens.

Exp.#2: Four black kittens were divided into two groups: one group received the gelatin-AA-based diet (containing 3 g tyr/kg) and the other group received the same diet supplemented with L-tyr to give a total concentration of 19 g/kg diet. Both kittens receiving the unsupplemented diet developed reddish brown hair, while the supplemented kittens maintained their black hair, demonstrating that tyr supplementation prevented the formation of “red hair”.

Exp.#3: Four complete AA-based diets containing the following concentrations of phe/tyr (g/kg): 12/0, 12/4.5, 24/0, 24/4.5 were given to four black kittens. Kittens given the 12/0 and 12/4.5 diets developed reddish brown hair, whereas the kittens given the 24/0 and 24/4.5 diets maintained black hair.

Exp.#4: Two black-haired queens were given the gelatin-AA-based diet for 3 mo, which produced a hair coat change to reddish brown. They were mated to a black tom and gave birth to black kittens with reddish brown hair. The hair was predominately black by 8 wk of age. The restoration of hair color was attributed to the phe and tyr present in the queens’ milk proteins.

Exp.#5: 20 black weaned kittens were divided into five groups each of four kittens and given a gelatin-based diet supplemented with AA to provide >1.5 times the amino acid recommendations of NRC (1986). The total phe conc. in the supplemented diet was 11g/kg (7.8g from gelatin and 3.12 g from AA) and the tyr conc. was 2.8 g/kg from gelatin. To the basal gelatin-AA diet were added either 0, 1, 2, 3, or 6 g L-tyr/kg diet at the expense of starch. After 4 mo none of the dietary treatments were able to prevent the development of reddish brown hair in all cats in the group.

As phe is obligatorily metabolised to tyr, the total sum of both aromatic AA (TAAA) should be used to assess dietary adequacy of tyr. The AA diet with 24 g of phe (assumed to be totally available) maintained hair color, but the gelatin-AA-based diet supplemented with 6 g tyr (TAAA of 19.7g/kg) was inadequate. Small intestinal digestibility of phe and tyr from gelatin is probably not > than ~0.8, which would represent an available TAAA of not > than ~17.6 g/kg. We suggest that the requirement for expression of maximal melanin synthesis in the hair of cats is > 17.6 g and < 24 g of available TAAA/kg diet. This value exceeds twice the requirement determined by Williams et al (1987) for maximal growth rate and nitrogen balance. We are not aware of another example, where a secondary function requirement is so much greater than the growth requirement.

References
The numerous changes which occur during the natural aging processes of the skin are becoming increasingly well characterised. These encompass changes to skin associated lymphoid tissue (SALT), a reduction in the numbers of cells such as fibroblasts and mast cells, generalised extracellular matrix degeneration and thinning of the epidermis. There is also an overall reduction in the levels of ceramides, fatty acids and cholesterol which together make up the epidermal lipid. In combination these changes lead to a progressive deterioration in the ability of the skin to operate as a barrier, be this in terms of resisting disease, retaining water or self-repair.

A commonly employed measure of skin barrier function is transepidermal water loss (TEWL) which measures the rate of water egress through the epidermis. An elevated TEWL is generally regarded as an indicator of compromised barrier function and can be associated with conditions such as contact dermatitis, psoriasis and atopic skin disease. Somewhat paradoxically however, human studies have shown that TEWL declines in aged individuals despite the general trend towards poorer physiological skin health with age.

The aim of this study was to determine what changes, if any, occur to canine skin during three main lifestages using TEWL as an indicator. The investigation was performed by taking measurements from labrador retrievers which were grouped as follows; 0-12 months (young), 2-7 years (adult) and 9+ years (senior). Prior to taking measurements all dogs were trained to stand still for the entire 45 seconds of a TEWL reading. Readings were taken from the lumbar region using a Servo Med Evaporimeter (EP2). This work was performed following validation of the Evaporimeter by the authors for use on the dog.

It was found that the mean diffusion rate for the young dogs is 5.19g/m2.h, 0.35 (mean / CV) compared to 10.96g/m2.h, 0.27 (mean / CV) for the adult (P=0.041). These compared to a mean TEWL for the senior dogs of 25.61g/m2.h, 0.30 (mean / CV) giving a significant difference between the senior and young dogs (P=0.031) and also the senior and adult dogs (P=0.037).

From these results it would appear that canine skin does show age related changes. TEWL is lowest in the young canine, in this case before one year old, suggesting that the epidermal barrier is optimal at this stage resulting in excellent skin health and hydration characteristics. It is possible that the better organisation and smaller average size of the keratinocytes observed for young human skin is also true for the dog and contributes to better barrier properties. Whereas increases in TEWL between infant and adult skin have been similarly reported in human, the further increase in water loss for the senior dog is at variance with human trends. The present study demonstrates a significant rise in canine TEWL between adult and senior (2-7 years to 9-plus years). This may reflect differences in the way that dog skin ages and may offer opportunities to augment barrier function in older dogs through nutrition.
WEIGHT LOSS IN OBESE CATS: EVALUATION OF A HIGH PROTEIN DIET.

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Recent studies showed that more than 25 % of cats are overweight or obese. In cats moderate energy restriction (60 % of adult maintenance requirement) resulted in a weight loss of about 1 %/wk over a 18-wk period. While loss of lean body tissue appears to be an inevitable or obligatory physio-logic response to weight reduction in humans, it has been shown that increased dietary protein level spares lean body mass in dogs fed for weight loss. To balance hypoenergetic diets would then be an important challenge, moreover when a reduction of metabolic rate occurs in response to energy restriction, as we showed in dog. The aim of this study was to test a new diet with a high protein level and to evaluate its ability to induce weight loss while preserving lean body mass.

Seven neutered healthy obese adult European shorthaired cats, 6.5 ± 0.7 (range 3.2-8.6) years old were used. Estimated excess body weight (BW) was about 35 % (27-44). Hormonal and biochemical analyses did not show any primary endocrine disorder. Animals were allotted to 2 groups and were then fed either the experimental high protein diet (HP ; CP 45.2%, 3050 kcal ME/kg as is) or a commercial hypoenergetic food (MP ; CP 34.0%, 2600 kcal ME/kg). Initially each cat was given the same amount of food it consumed when a premium food (CP 30.8%, 4280 kcal ME/kg) was given ad libitum during a 4-w preliminary period. After a 4-w observation period the food allowance was adjusted to obtain a 1-2 % BW loss per w. Animals were individually fed using electromagnetic doors. Body weight and body condition score were recorded every w. Body composition (body water pool, free-fat mass and fat mass) was determined from the dilution of a single dose (1.5 g/kg) of deuterium oxide. The resting energy expenditure (REE) was assessed 4 times (initial, final and 2 intermediary measurements) from 6-h continuous monitoring of respiratory gas exchanges in a ventilated metabolic cage. Nitrogen fluxes were assessed through 13C-leucine kinetics method in overnight unfed animals, at the beginning and the end of the experimental period.

Initial BW and body fat content (BF%) were (mean ± S.E.) 6.6 ± 0.4 kg, 46.3 ± 2.4 % and 7.2 ± 0.3 kg, 41.4 ± 0.8 %, for HP and MP groups respectively. Initial BW was 139 ± 2 and 131 ± 2 % Target BW. Time to reach Target BW was 142 ± 10 and 121 ± 13 d and mean weekly BW loss 1.61 ± 0.08 and 1.58 ± 0.15 %. Final BW and BF% were 4.3 ± 0.2 kg, 29.9 ± 4.1 % and 5.0 ± 0.3 kg, 25.9 ± 6.7 %. BW loss was 2.3 ± 0.2 and 2.2 ± 0.3 kg and its fat con-tent 77.0 ± 4.5% and 73.9 ± 10.1% for HP and MP groups respectively. The mean food con-sumption was 65 ± 4 % of the assumed normal energy requirement for an adult cat having an optimal body condition (60 kca/kg BW/d). Extrapolated daily REE was 45 ± 2, 45 ± 2, 44 ± 2 and 48 ± 3 kca/kg BW/d when BW was 100, 84 ± 2, 78 ± 3 and 70 ± 2 % of initial BW. Leucine oxidation rate was not significantly different before the initiation of weight loss and at the end of the experimental period (45 ± 9 vs. 55 ± 12 mmol/kg/h) in the HP group while it increased by 2-fold (42 ± 3 vs. 84 ± 4, p < 0.002) in the MP group.

Expected weight loss was reached in every case without any health problem using a 35 % restriction of energy allowance on the Target BW requirement basis. The REE did not decrease in response to energy restriction and weight loss. This may be an explanation to the success of slimming obese cats with moderate levels of energy restriction. The contribution of the free-fat mass to weight loss was important in both groups, without difference in its composition between the two groups. The highest protein level could have avoided an impaired nitrogen balance during weight loss.

References
MORPHOLOGY AND IMMUNOPATHOLOGY OF THE SMALL AND LARGE INTESTINE IN DOGS WITH DIETARY SENSITIVITY

Diverse diseases of the small and large intestine that can cause chronic diarrhoea have been characterized in dogs, including small intestinal bacterial overgrowth (antibiotic-responsive enteropathy) and steroid-responsive inflammatory bowel disease. A mild colonic inflammation associated with reduced absorptive function has been observed in dogs with dietary sensitivity, which exhibit intermittent loose faeces on some diets. The aim of this study was to assess intestinal morphology and immunopathology in the small and large intestine to determine whether dietary sensitivity is associated with mucosal abnormalities throughout the intestinal tract.

Eight healthy control dogs (Beagles) were compared to 10 dogs with ‘non-specific dietary sensitivity’ (6 Labrador Retrievers, 2 Large Munsterlanders, 2 English Springer Spaniels), that had previously been proven to produce wet faeces compared to control dogs fed the same diet. All dogs were fed a canned diet for 3 weeks before sampling. Small and large intestinal endoscopic biopsies were investigated by light microscopy (H&E staining) and graded by the severity of lesions (0: normal - 3: severe damage). Immune cell populations were quantified per unit area in the lamina propria (villus tip and base and crypt areas in the duodenum, crypt area in the colon) or as percentage of the epithelial cells by (1) computer aided immunohistochemistry (CD3+, CD4+, CD8+, CD45+, IgA+ and IgM+ plasma cells; immunoperoxidase staining) and (2) flow cytometry (CD3+, 4+, 8+, 21+, MHCII+; using two colour fluorescence and gating on CD45+ cells; differentiation of upper (mainly epithelial) cells by EDTA treatment and subsequent collagenase digestion to release mainly lamina propria cells.

Dogs of the control group tolerated the diet without any apparent adverse effects, while the ‘sensitive’ dogs had wet faecal quality. The histological investigation revealed mild diffuse abnormalities in the small and large intestine of the ‘sensitive’ dogs (mean grade 0.9±0.8 and 1.7±0.3), while the duodenum of controls appeared histologically normal (grade 0) and only 2 dogs of this group had changes in the colon (grade 1 and 2, group mean 0.4±0.7). By immunohistochemistry, lymphocyte and plasma cell populations showed only minor differences between the groups. Numbers of intraepithelial CD45+ and CD3+ cells were increased in the ‘sensitive’ group in the colon (p<0.05) and in the duodenum (p<0.05 for CD45). Lamina propria cell populations showed a tendency for a higher CD4+:CD8 ratio in the ‘sensitive’ dogs, while IgA- and IgM-producing plasma cells were found in comparable numbers. Flow cytometry confirmed only minor differences between the lymphocyte populations in the 2 groups.

The current study showed evidence for mild inflammation in the small and large intestine of sensitive dogs, and was associated with increased intraepithelial lymphocyte numbers and an altered lamina propria CD4+:CD8 ratio. These findings indicate that dietary sensitivity can be associated with mucosal abnormalities throughout the intestinal tract, but the role of specific dietary ingredients in the pathogenesis of these changes needs further investigation.
VALIDATION OF SINGLE-CELL GEL ELECTROPHORESIS ASSAY (COMET ASSAY) FOR ASSESSING LEVELS OF DNA DAMAGE IN CANINE AND FELINE LEUKOCYTES

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Increasing evidence suggests involvement of free radical species in the development of oxidative DNA damage, the consequences of which have been implicated in a number of degenerative disorders such as cancer and ageing. As such, the need to accurately assess levels of DNA damage has received renewed attention. Here we report the development and validation of a single-cell gel electrophoresis assay (comet assay) for assessing levels of DNA damage in canine and feline leukocytes.

Leukocytes were collected from 12 healthy adult cats (9.2 ± 2.2 years) and 12 healthy adult dogs (5 ± 2 years) at the WALTHAM Centre for Pet Nutrition, and subjected to DNA damage ex vivo by exposing the leukocytes to a range of hydrogen peroxide (H2O2) concentrations (0-250mM). Levels of DNA damage were assessed and quantified by visual and computer image analysis for validation purposes. DNA damage was scored on an arbitrary scale of 0-4 (0 = no DNA damage to 4 = extensive DNA damage). The results obtained showed high correlations between visual scoring and computer image analysis for feline samples (percentage DNA in tail = R2 > 0.99, tail moment = R2 > 0.95, and tail length = R2 > 0.91), and canine samples (percentage DNA in tail = R2 > 0.97, tail moment = R2 > 0.95, and tail length = R2 > 0.91).

Advantages of using the comet assay include: (1) requirement of small numbers of cells per sample, (2) sensitivity of detecting low levels of DNA damage, (3) potentially high-throughput assay, (4) ease of application, (5) flexibility and low cost. With the capacity of the comet assay to measure end products of free radical reactions, this is the first study to validate an assay for the assessment of DNA status that can be used to determine the protective effects of dietary antioxidants on oxidative stress in dogs and cats.
ASSESSING AGE-RELATED CHANGES IN PERIPHERAL BLOOD LEUKOCYTE PHENOTYPES IN LABRADOR DOGS USING FLOW CYTOMETRY.
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WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire, LE14 4RT, UK

Whole blood specimens were taken from a total of 122 Labrador Retriever dogs at the WALTHAM Centre for Pet Nutrition, ranging from 0.6 to 14.2 years of age in order to establish immunological baseline data on age-related changes in peripheral blood leukocyte subsets. Leukocyte populations were phenotyped using lysed whole blood staining and triple-colour flow cytometric analysis. Commercially available monoclonal antibodies were used to identify cell surface markers for CD3, CD4, CD8, B-cells (CD21-like), CD14, and propidium iodide was used to establish lymphocyte viability. Relative percentage levels of lymphocytes, monocytes and granulocytes were also assessed.

Linear regression analysis was used to identify significant trends in the data obtained. Analysis identified a significant decrease in CD4 (R2 = 0.03, p<0.05), a significant increase in CD8 (R2 = 0.24, p<0.001), with a corresponding decrease in the CD4:CD8 ratio (R2 = 0.15, p<0.001) with increasing age. Percentages of CD3 demonstrated a significant increase (R2 = 0.06, P<0.008) with increasing age. No significant differences were identified with increasing age in relative percentages of CD14, B-cell surface markers, lymphocyte viability, lymphocytes, monocytes and granulocytes.

These results illustrate a significant correlation between ageing and changing relative percentage leukocyte phenotype in Labrador Retriever dog populations. Using discriminant analysis on the CD3 and CD8 data, this is the first study able to define two statistically distinct groups, adult dogs (0.6 to 8 years) and senior dogs (8+ years), with an overall correct classification of 83% (cross-validated). Previous research has shown that a decline in immune function in relation to age can generally be characterised by an increase in CD8 percentages, a decrease in CD4 percentages and an inverted CD4:CD8 ratio. These trends are supported by the baseline data acquired from our Labrador Retriever dog population. Therefore, it is imperative that age be considered in any study where the interpretation of leukocyte subset phenotype is utilised. The data from this study also provide a reference of leukocyte subset populations for the assessment of immune status in Labrador Retriever dogs.
ANTIOXIDANT STATUS OF ELDERLY BEAGLES AS AFFECTED BY DIETARY ANTIOXIDANT INTAKE.

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Hill’s Pet Nutrition, Inc. Topeka, KS.

A large body of evidence exists which indicate reactive oxygen species (ROS) may play a major role in degenerative diseases such as cancer, cardiovascular disease, cataracts, brain dysfunction and aging. Little is known about the benefits of various antioxidants (AOX) in petfoods. Thus, it was our objective to assess the effect of varying AOX combinations and concentrations on antioxidant status. Forty dogs (mean age = 7.6 yr; range = 3.3-10.2) were fed an AAFCO- tested adult food (i.e., 18%CP/ 10%CF/ 64% NFE) for 4 wk adaptation, and then assigned to one of five treatments in a randomized complete block design: 1) a control (Ctrl) formula, 2) Ctrl + 1X inclusion of AOX vitamins consisting of vitamins E, C & beta carotene, 3) Ctrl + 2X inclusion of AOX vitamins, 4) Ctrl + 2X inclusion of AOX vitamins + fruits & vegetables, 5) Ctrl + fruits & vegetables only. All test foods were fed for 4 wk. Measures of antioxidant status included serum alpha tocopherol, ascorbate, and oxygen radical absorbance capacity (ORAC) and urinary 8-hydroxy-2’-deoxyguanosine (8OHdG).

Results showed a significant increase in ORAC with increasing AOX inclusion (trt effect, P=.06; linear (P<.05)). All treatments containing supplemental vitamin E increased serum E concentrations relative to the Ctrl (P<.01). Only the treatments containing fruits & vegetables increased serum vitamin C (P<.01). Results suggest that the addition of various AOX packages improved AOX status of elderly dogs as measured by ORAC and serum vitamin E & C.

<table>
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<th>Diet</th>
<th>, serum ORAC µM</th>
<th>, serum Vitamin E µg/mL</th>
<th>, serum Vitamin C µg/mL</th>
<th>, urinary 8OHdG/Cr µ/mg</th>
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<td>(.0003)</td>
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, represents change in measurement between d-29 and d-0 (post – pre)
THE EFFECT OF SUPPLEMENTAL VITAMIN E ON MITOCHONDRIAL STRUCTURE IN RACING GREYHOUNDS BEFORE AND AFTER RACING

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Racing greyhounds are withdrawn from racing by five years of age. During exercise, increased production of reactive oxygen species may overwhelm the body's natural antioxidant defenses and it is possible that cellular damage resulting from this oxidative stress may shorten a greyhound's active career. In a preliminary experiment, examination of mitochondria from one dog showed marked disruption 5 minutes after a race, which was not completely resolved 24 hrs after racing. A study was, therefore, performed to determine whether administration of supplemental vitamin E affected performance or the severity of mitochondrial disruption in trained racing greyhounds. Sixteen greyhounds (2-5 yrs old, both sexes) fed a standard dry diet and raced over 500 m twice weekly were randomly assigned to two groups. The control group of 8 dogs were not supplemented throughout the study. The treated group of 8 dogs were not supplemented for 8 weeks then were supplemented with alpha tocopherol acetate (100 IU daily for 8 weeks then 1000 IU daily for 8 weeks). Serum alpha tocopherol was measured in the blood before and 5 min, 60 min and 24 hrs after a race at the end of each diet period. Visual evidence of oxidative damage at the mitochondrial level was also measured at the same time-points. Five dogs were removed from the study because of minor injuries. Among the remaining dogs, alpha tocopherol concentrations increased markedly with supplementation. Changes in mitochondria were not as dramatic as in the preliminary experiment and differences between dogs were difficult to distinguish. Mitochondrial disruption may, therefore, be greater in untrained dogs and provide a better model for changes due to oxidative stress related to aging.
THE EFFECT OF NEUTERING ON FOOD INTAKE, BODY WEIGHT, PLASMA LEPTIN AND INSULIN CONCENTRATIONS IN NORMAL AND LIPOPROTEIN LIPASE DEFICIENT MALE CATS.

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The practice of neutering increases the incidence of obesity in both male and female domestic cats. Few controlled studies have examined the post-operative physiologic consequences of neutering on hormonal regulation and body condition. Our experiment examined the effects of neutering on food intake, body weight, and plasma leptin and insulin concentrations in normal and lipoprotein lipase (LPL) deficient adult male cats (2-5 yrs). Additionally, body composition and energy expenditure were evaluated by D2O and H218O-water isotope dilution methods. All cats were acclimated to a commercial dry diet for 8 weeks and fed ad libitum for the duration of the experiment. Body weights were recorded weekly. Eight normal and 8 LPL deficient cats were neutered by the standard open technique. Eight normal and 8 LPL deficient animals were used as controls. Body composition and hormonal analysis of serum and plasma samples were evaluated on the day of neutering (0) and at weeks 2, 4, 7, 12, 16, and 34. Food intake was determined daily during the weeks of sampling. Energy expenditure was determined during two 14-day periods, 3 weeks prior to and following neutering, in 5 intact and 5 neutered normal cats. Food intake increased immediately following neutering. Neutered cats consumed 12 % more (p<0.05) diet than intact cats during the first week and the food intake remained elevated throughout the trial. Compared to intact control animals, the neutered animals had a significant (p<0.05) increase in body weight by week 9 and 13 in normal and LPL lines, respectively. The weight gain continued for 25 weeks, then plateaued when the neutered normal and LPL deficient cats had increased their body weight by 24 and 26 %, respectively. In the normal neutered cats, plasma leptin concentrations, as determined by RIA, increased with body weight from means of 1.9 to 3.3 ng/ml. The increase in leptin concentration was less than expected as obese male cats of similar body weight were found to have leptin concentrations of 8.5 to 18.2 ng/ml. Plasma insulin concentrations increased (p<0.05) with neutering by week 2 in LPL deficient cats and by week 4 in normal cats. Plasma insulin concentrations remained elevated and did not increase further with gains in body weight.

These findings support the hypothesis that the increase in body weight after neutering is driven by an immediate increase in food intake. Deposition of lipid in adipose, that results from neutering, is apparently not controlled by the hypothesized gatekeeper function of LPL. These results indicate that the feedback control of leptin in body fat mass regulation may be altered by neutering.
THE USE OF THE SERVO MED EP-2 EVAPORIMETER TO MEASURE THE TRANSEPIDERMAL WATER LOSS (TEWL) IN CANINE SKIN.

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TEWL describes the total amount of water lost through the skin, a loss that occurs constantly via passive diffusion through the epidermis. The rate of TEWL depends directly on ambient relative humidity and temperature as well as the integrity and thickness of the stratum corneum. TEWL is a normal physiological phenomenon, however, if it rises too high the skin can become dehydrated, disrupting form and function and potentially leading to infection or transepidermal passage of deleterious agents. Conversely, if TEWL drops too low there may be perturbation of the critical balance between differentiation and proliferation in epidermal cells of the skin. As such, TEWL is a useful parameter to assess the health status and barrier function of the skin.

Previous studies at WCPN have highlighted a potential problem with standard deviations and coefficients of variance (CV) when using TEWL on dogs. It was hypothesised that some of the variation may be due to slight movement of the dogs during measurements. The aim of this study was to compare TEWL measurements in trained and untrained dogs. These values were then compared to those obtained for dogs under general anaesthesia and therefore completely still.

TEWL assessment was conducted using an EP-2 Evaporimeter (ServoMed). Measurements were made by applying the probe to an area lateral to the lumber spine. The probe was placed onto an animal using minimal pressure, first parting the haircoat to ensure good skin contact. A 30s stabilisation period was permitted, before recordings were made over the subsequent 15s. The probe was moved slightly between each reading to avoid local effects caused by the application of the probe head. A minimum of 6 readings were taken on each dog. Results from untrained dogs, dogs trained to stand still for the entire 45s reading period, and dogs anaesthetised for routine dental procedures were compared.

CVs in the trained and untrained dogs were similar (25 and 26%), but were lower in the anaesthetised animals (16%). TEWL values were 42% lower in the trained (10.96 g/m2h), compared with the untrained (18.99 g/m2h) dogs. Values were 11% lower again in the anaesthetised dogs (9.43 g/m2h).

The results of the study demonstrate the requirement for an absolutely still subject when performing TEWL measurements. It is likely that slight movement can cause fluctuations in the distance between the skin and the probe sensors leading to erroneous readings. The fact that the TEWL values are increased under these circumstances suggests that changes in the probe position increase the rate of vapour flow over the sensors probably due to the creation of localised air currents. The slightly lower values detected during anaesthesia may be due to the further reduced movement under these conditions. However, an alternative explanation may have been a small drop in skin temperature.

These results indicate the importance of having a compliant subject when conducting TEWL measurements.
INFLUENCE OF CALCIUM- AND PHOSPHORUS SUPPLY ON THE APPARENT DIGESTIBILITY OF THESE MINERALS IN GROWING DOGS.
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Skeletal diseases in growing dogs are often diagnosed, mostly in large and giant breeds. The connection between mistakes in feeding regime and disturbances of bone development is shown by various authors: the negative effects of a lack in calcium (Ca) supply (MAREK a. WELL-MANN 1931) and the consequences of an oversupply with energy and/or Ca for skeletal health (MAREK a. WELLMANN 1931, HEDHAMMAR et al. 1974, HAZEWINKEL et al. 1991, MEYER a. ZENTEK 1992). The inability of puppies to decrease the digestibility of Ca in order to compensate a three-fold oversupply was already shown by HAZEWINKEL et al. (1985). There are only few information about the digestibility of Ca and Phosphorus (P) in puppies with an insufficient supply with those minerals in the literature.

To determine and quantify the ability of growing dogs with malnutrition of Ca and P to alter the apparent digestibility (aD) at various degrees of over- and undersupply at different ages during growth, a feeding trial with 24 Beagles was carried out from weaning until 6.5 months of age. 5 groups were formed to carry out 3 trials of around 6 weeks duration each. The Ca-supply was adjusted to an amount between 15 and 300% of the requirement, the P-supply between 28 and 300%. A diet based on rumen, rice and cellulose supplemented individually with minerals and vitamins was fed. All but the nutrients in question were supplied after the requirement figures of MEYER (1992). 5 dogs were raised as a control group, i.e. all nutrients met the requirements. As intended no clinical signs of disturbances of skeletal development were noticeable. The weight curve was not influenced systematically by the feeding regime. At the age of 6.5 to 13 weeks of age no statistically significant differences in the aD of Ca were seen between the groups. Only in the 15% group of the 2. trial a significant increase of the aD of Ca compared to the 300% group could be measured. The 3. trial revealed a highly significant effect of an oversupply with P on the aD of Ca. At this age the dogs of the group with 300% P increased the aD of both Ca and P significantly. The aD of P was already influenced significantly in the youngest dogs (trial 1) when the Ca intake or the Ca/P-ratio did not fit the requirements: the aD of P decreased in cases of Ca-oversupply and Ca/P-ratios below 1:1 in trial 1 and 2. The oversupply with P and the Ca/P-ratio below the recommended range in the 300% P group of the 3. trial led to significant (p<0.001) higher rates of aD for Ca and P compared to the control group (100%).

The results demonstrate that dogs younger than 4 to 5 months are not able to adjust the digestibility of Ca in relation to the Ca-intake. Not only a missing ability to down-regulate the aD but also the incompetence to increase the uptake in case of an insufficient supply in young growing dogs makes it necessary to adjust the daily supply of Ca to the requirements precisely. In contrast, an ability to alter the apparent digestibility of P was already observed in very young dogs.

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MEYER, H., J. ZENTEK (1998) Ernährung des Hundes, Parey Verlag, Germany
BIOPROTEIN – A NEW POTENTIAL FEED INGREDIENT IN DOG DIETS

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BioProtein is a new high-protein meal produced by continuous bacterial fermentation using a defined mixture of four different bacteria and natural gas as the carbon and energy source (Skrede et al., 1998). The BioProtein is a reddish/brownish non-dusty meal containing 70% crude protein on a dry matter basis and a nutritionally well-balanced amino acid composition. The amino acids of BioProtein are well digested by several animal species, including mink, Atlantic salmon, pigs and young chicks (Skrede et al., 1998). Recent studies have shown that BioProtein is a suitable protein source in diets for weanling and slaughter pigs (Øverland et al., 2001). Previous studies of digestive capacity have shown a close similarity between dogs and blue foxes (Ahlstrøm & Skrede, 1998). The present study was conducted to extend the knowledge of BioProtein as an ingredient of diets for dogs, using the blue fox (Alopex lagopus) as model animal.

Four isonitrogenous dog diets containing 0%, 4%, 8% and 12% BioProtein were produced at Centre for Feed Technology, Ås, Norway. The diets contained 93% dry matter, 27% crude protein, 19% fat, 7% ash and 40% crude carbohydrate. The highest level of BioProtein corresponded to 30% of total dietary crude protein. Increasing levels of BioProtein balanced reduction in contents of fish meal, meat meal, and soybean meal. The diets were extruded using a twin-screw Bühler extruder.

Each diet was fed to 20 weaned blue fox cubs, 10 males and 10 females, with an initial body weight of approximately 2.8 kg. The main experiment was carried out in the growing-furriing period, starting August 8, when the animals were approximately two months old, and terminating December 5. A digestibility experiment of seven days duration was carried out using the same diets and three male blue foxes per diet.

All animals accepted the feed well and there were no significant differences in feed intake. The animals remained healthy throughout the experimental period and there were no casualties. Increasing levels of BioProtein tended to improve growth. The average overall body gain (in kg ± 1 standard deviation) of animals fed 0%, 4%, 8%, and 12% BioProtein were 6.79 ± 1.18, 6.87 ± 1.36, 6.95 ± 1.03, 7.16 ± 1.31, respectively. BioProtein appeared to improve feed conversion, since the increased growth was obtained without enhanced feed intake. Evaluation of fur quality showed no significant differences among treatment groups. Apparent digestibility of crude protein, crude fat and crude carbohydrate averaged 82.2%, 96.3% and 73.9%, respectively. There was no significant effect on digestibility of replacing conventional protein sources with BioProtein.

The data on digestibility and overall performance in blue foxes confirm earlier studies with other species and indicate that BioProtein is an attractive feed ingredient for use in feed for dogs.

References

MINERAL AND TRACE ELEMENT ABSORPTION IN DOGS FROM DRY DOG FOOD DETERMINED USING STABLE ISOTOPES.

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To date only very limited data on bioavailability of minerals and trace elements in dogs fed dog food are available although such information is essential for establishing correct mineral requirements (Morris et al 1994). We have determined absorption of Ca, Fe, Cu and Zn from a standard dry dog food in Beagles using the fecal excretion stable isotope technique. To compare standard methodology to the stable isotope technique, Ca absorption was also determined using a standard digestibility trial. The stable isotope technique allows researchers to obtain more precise results and minimize errors incurred as a result of endogenous excretion (Sandström, B., 1994). Fifteen beagles aged 9-15 y were fed a standard dry dog food for 4 weeks. The food supplied (dry matter basis): 20.1 % protein, 11.1 % fat, 1.5 % fiber, 5.49 kcal/g energy, 1.72 % Ca, 218 mg/kg Fe, 14.5 mg/kg Cu and 148 mg/kg Zn. At the end of the equilibration period apparent Ca, Fe, Cu and Zn absorption were determined from a single test meal extrinsically labeled with 44Ca, 58Fe, 65Cu and 70Zn. Dysprosium (Dy) was used as non-absorbable fecal marker to check for completeness of stool collections. Complete feces were collected for 5 days. A post sample was taken on day 6. Total Ca, Cu and Zn in pooled feces and post samples were determined by ICP-OES after dry ashing. Total Fe was analyzed by AAS and Dy by ICP-MS. Unabsorbed 44Ca, 58Fe, 65Cu and 70Zn tracer in feces was quantified by ICP-MS after separation of Ca (precipitation) and Fe and Zn (anion exchange) from matrix elements.

Mean Dy recovery (± SEM) in fecal pools was 100.8 ± 3.1 %. Dy found in post samples was negligible. Tracers amounts recovered in feces were corrected for a Dy recovery < 100 % if required according to Schuette et al., 1993. Mean apparent absorption for Ca, Cu, Zn and Fe were 10.1 ± 1.1 %, 23.1 ± 2.0 %, 11.5 ± 1.4 % and 8.8 ± 2.1 % respectively. Assuming a mean food intake of 250 g /day this would correspond to a total amount of absorbed Ca, Cu, Zn and Fe of 434 mg, 0.8 mg, 4.3 mg and 4.8 mg/day. Ca digestibility was approximately 5% lower than Ca stable isotope absorption, 4.8 vs 10.1%, respectively. This is most likely due to endogenous losses of Ca that are not corrected for in standard digestibility trials. The stable isotope technique also provided a more precise method of measuring Ca absorption in dogs (coefficient of variation = 41% for stable isotope technique vs 156% for standard digestibility).

With the exception of Cu, absorption values found were comparatively low. This might be due to high total element intake. Ca absorption in dogs was found to be quite variable (0-90 %) depending on Ca content and composition of food (Hazewinkel, 1989). The low absorption of minerals observed in our study should be taken in account when recommendations for requirements of these elements are established.

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EFFECT OF A SINGLE ORAL DOSE OF VITAMIN A ON THE RETINOL AND RETINYL ESTER RESPONSES IN THE BLOOD AND URINE OF DOGS.
Raila, J., Radon, R., Triepelschuch, A. and Schweigert, F.J.
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Dogs differ from other species with regard to the occurrence of lipoprotein bound retinyl esters in blood plasma and the excretion of vitamin A in the urine. The objective of the study was to determine the effect of a large single oral dose of vitamin A in dogs on the retinol and retinyl ester response in blood plasma and urine. For this purpose 8 male Beagle dogs (9 mo. of age; 10-12 kg body weight, BW) were fed a basal diet (Effem, Germany) that met the requirements for all essential nutrients, including vitamin A. The vitamin A supplement (10.000 IE/kg BW) was given once orally together with 5 ml cream (30 per cent fat). Blood was taken at -48, 1, 2, 3, 4, 6, 8, 24, 48, 72 and 96 h after dosing. Chylomicrons were separated by ultracentrifugation (d<1.006 g/ml). Urine was collected for 18 h each day until 96 h. Retinol and retinyl esters were separated by rp-HPLC. Triglycerides were measured by enzymatic methods.

Plasma retinyl ester (oleate, palmitate and stearate) concentrations peaked at 8 h (16.8 ± 4.7 mg/l; mean ± SD) and returned to baseline at 72 h (5.8 ± 1.9 mg/l), while plasma retinol concentrations increased slightly with a maximum at 6 h (1.43 ± 0.31 mg/l) and decreased to baseline at 48 h (1.01 ± 0.17 mg/l). The ratio between plasma stearate and palmitate changed as a consequence of total increase of vitamin A in plasma (Table 1) indicating a uptake of retinyl esters preferentially but exclusively as retinyl palmitate. As expected for lipid absorption, plasma triglycerides peaked between 2 and 4 h and returned to baseline by 24 h. Retinyl ester concentrations in the chylomicrons did not paralleled the increase of retinyl ester observed in plasma. In the urine, maxima in retinyl palmitate excretion occurred at 8 h and 48 h after vitamin A dosing. Levels of retinol in the urine were not affected.

This study shows that a large single oral vitamin A dose affected retinol and retinyl ester concentration in plasma as well as retinyl palmitate concentrations in urine of healthy dogs. Plasma as well as chylomicron kinetics, however, were different to those found in humans (Reinersdorff et al., 1996; Hadjadj, et al., 1999). Thus, differences in retinyl ester kinetics of chylomicrons and total plasma suggest species specific differences in the mechanism of intestinal vitamin A absorption in dogs.

**Table 1: Mean ratio of retinyl stearate/palmitate in the blood plasma of dogs after a single oral vitamin A dose (10.000 IU /kg BW)**

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>-48</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearate/Palmitate</td>
<td>2.59</td>
<td>1.44</td>
<td>1.31</td>
<td>1.44</td>
<td>1.51</td>
<td>1.68</td>
<td>1.76</td>
<td>2.02</td>
<td>2.12</td>
<td>2.06</td>
<td>1.99</td>
</tr>
</tbody>
</table>

This study shows that a large single oral vitamin A dose affected retinol and retinyl ester concentration in plasma as well as retinyl palmitate concentrations in urine of healthy dogs. Plasma as well as chylomicron kinetics, however, were different to those found in humans (Reinersdorff et al., 1996; Hadjadj, et al., 1999). Thus, differences in retinyl ester kinetics of chylomicrons and total plasma suggest species specific differences in the mechanism of intestinal vitamin A absorption in dogs.

**References:**
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INFLUENCE OF BODY SIZE ON INTESTINAL PERMEABILITY IN GROWING DOGS

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While the weight of the empty intestinal tract is 6-7% of the bodyweight in small dogs, it is only 3-4% in large and giant dogs. It could then be hypothesized that large and giant dogs have a limited digestive capacity compared to small dogs. This is supported by a tendency in the larger dogs towards a higher faecal water content and an increased frequency of soft stools (Meyer et al., 1999). Differences in the intestinal function could be part of the factors explaining the difference of digestive capacity and sensitivity in large dogs. A differential sugar permeability test using lactulose and rhamnose has been validated in the dog (Sørensen et al., 1993). According to the pore theory, small molecules, such as rhamnose (R), are believed to permeate transcellularly through the numerous small pores of enterocyte; whereas larger molecules, such as lactulose (L), could pass through infrequent large pores between cells (Johnston et al., 2001). Intestinal permeability, assessed by determining the ratio of urinary recoveries of L/R, may then be useful in the detection of mucosal damage. The aim of this study was then to compare intestinal permeability in growing dogs varying in body size.

Twenty four healthy female dogs were used in this study (6 Great Danes, 6 Giant Schnauzers, 6 Medium Schnauzers and 6 Miniature Poodles). A solution containing 25 g/L lactulose and 10 g/L rhamnose of total osmolality 422 mOsm/L was administered orally to dogs when they were 12, 22, 36 and 60 wk old. Urine was collected 8 hours later. Sugar concentrations were measured by use of HPLC and the ratio of urinary recoveries of L/R was calculated. During the same periods, the faecal quality was scored from grade 1 (hard and dry) to grade 5 (watery diarrhoea) for each animal.

An effect of age was observed in all dogs with a urinary L/R ratio that decreased significantly between 12 and 22 wk of age, then remained stable (mean ± SD, 0.40±0.13; 0.32±0.11; 0.30±0.10 and 0.33±0.12 at 12, 22, 36 and 60 wk of age respectively). Differences in permeability could also be related to dog size. The urinary L/R ratio was significantly higher in giant and large dogs than in small and miniature dogs (0.40±0.11 and 0.39±0.13 versus 0.28±0.08 and 0.28±0.10 respectively). This increased permeability was associated with a poorest faeces quality in giant and large dogs (3.2±0.1 and 3.0±0.2) than in small and miniature dogs (2.7±0.3 and 2.5±0.1).

Our results (1) are in agreement with those reported by Hill et al., (1998) that showed an effect of breed and (2) show that the high permeability in largest dogs could be a factor predisposing them to digestive disorders and explaining their digestive sensitivity.

References
EFFECTS OF DIETARY PROTEIN LEVEL ON PROTEIN METABOLISM IN DOGS

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Although estimates of the dog minimum protein requirement have been based on nitrogen balance studies, very little is known on the effect of dietary protein intake on rates of protein synthesis and oxidation in this species. The aim of this study was to evaluate the effects of graded dietary protein levels on leucine kinetics, assessed by the reference 13C-leucine method (Bier, 1989).

Seven adult Beagle dogs (BW = 14.2 ± 0.8 kg; mean ± SEM) were fed 4 different diets over 4 consecutive periods: i) a medium protein rich diet (MP ; 380 kcal ME/100g; 63 gCP/Mcal ME), ii) a low protein diet (LP ; 430 kcal ME/100g; 32 gCP/Mcal ME), iii) an high protein rich diet (GP ; 375 kcalME/100g; 100 gCP/Mcal ME), and iv) a very high protein rich diet (HP ; 380 kcal ME/100g; 148 gCP/Mcal ME. The MP diet was the maintenance diet that adult dogs usually fed in our kennel. Each diet was given according to NRC (1985) recommendation (132 kcal ME/kg BW0.75/d) for adult dogs. Animals were adapted to each diet for 2-w. After an overnight unfed period, a primed 3-h infusion of 13C-leucine at the rate of 10 µmol/kg/h was performed in order to determine whole body leucine both rate of appearance (Ra) and oxidation (Ox). This infusion was immediately followed by a primed 3-h continuous infusion of 13C-bicarbonate at the rate of 3 µmol/kg/h in order to determine total CO2 production rate which is necessary for the calculation of Ox. Blood samples were collected before and during the last hour of 13C-leucine infusion. Breath air samples were collected during the last hour of 13C-leucine and during the 13C-bicarbonate infusion. The 13C enrichment in plasma a-ketoisocaproate (an index of intracellular 13C-leucine enrichment) was assessed by gas chromatography-mass spectrometry, and the 13C enrichment in breath CO2 by gas chro-matography-isotopic ratio mass spectrometry. Protein synthesis was calculated from Ra and Ox. Protein breakdown, oxidation, and synthesis were expressed as mean±SE in gN/kgBW0.75/d.

For each dog, body weight did not significantly differ between the 4 experiment days. Whole body postabsorptive protein breakdown was significantly decreased by LP (3.21±0.17; p=0.05), GP (3.17±0.15; p<0.05) and HP (2.51±0.23; p<0.05) when compared with MP value (3.75±0.19). Whole body postabsorptive protein oxidation was not affected by dietary protein level (0.52±0.06, 0.66±0.05, 0.69±0.06 and 0.57±0.09, values for LP, MP, GP and HP, respectively; p=NS). Whole body postabsorptive protein synthesis was decreased by LP (2.69±0.12; p=0.06), GP (2.48±0.11; p=0.01) and HP (1.94±0.16; p<0.01) when compared with MP value (3.09±0.15).

These data suggest 1) that postabsorptive protein oxidation, an index of protein balance during postabsorptive state, was not affected by dietary protein level, and 2) that both postabsorptive protein breakdown and synthesis are decreased by either low or high protein diet. We conclude that in dogs, protein intake level could have a remnant effect on protein breakdown and synthesis until 24-h after the last meal, but in contrast could not have any remnant effect on the protein oxidation.

Reference

COMPATIBILITY AND DIGESTIBILITY OF MIXED DIETS WITH VARIOUS HYDROCOLLOID AND WATER CONTENTS IN 3 DIFFERENT BREEDS OF DOGS
Zentek, J., Kaufmann, D., Pietrzak, T.

Dogs can be susceptible to dietary dependent influences on faecal quality, leading to an undesired loss of consistency. 'Non specific' dietary sensitivities are seen frequently in larger breeds, but the underlying condition is not yet clear. The present study was designed to evaluate the potential effects of dietary hydrocolloids (guar and carrageen) and variations in water content in canned diets on digestibility, microbial metabolism and faecal quality.

Digestibilities and effects on the faecal quality of 4 canned foods with various contents of moisture (60 or 75 %) and carrageen or guar (table 1) and one dry diet were tested in three different breeds of dogs (Beagle, German Shepherd, German Shorthair Pointer). Two male and two female dogs were available from each breed.

| Table 1: Moisture, guar, carrageen and crude protein and crude fat in the experimental diets |
|------------------------------------------|-------|-------|-------|-------|-------|
|                                        | A     | B     | C     | D     | E     |
| Dry matter, %                          | 25.8  | 39.9  | 24.3  | 41.0  | 93.9  |
| Guar, % 1)                              | 0.40  | .8    | 0     | 0     | Not determined |
| Carrageen, % 1)                         | 0.4   | 0.8   | 0.4   | 0.8   | Not determined |
| Crude protein, % DM                     | 27.7  | 27.6  | 28.1  | 28.0  | 28.3  |
| Crude fat, % DM                        | 20.3  | 21.0  | 21.0  | 22.5  | 17.0  |

Beside digestibility trials the frequency of defecations and the consistency of the faeces were assessed (standard grading system, content of dry matter, unbound water). Dietary effects on the intestinal microflora were characterized by several microbiological and metabolic tests (faecal counts of Clostridium perfringens, hydrogen breath test, renal excretion of indican).

With the dry food the faecal consistency was firmer compared to the wet foods, although faecal dry matter was in a comparable range of 28 to 38 % in all periods. The amount of free water, determined by centrifugation, was higher in dogs fed the dry food (25 %) than in the periods with canned diets (12-15 %). Neither the addition of guar nor the various water contents in canned foods had a significant influence on faecal consistency. Soft or liquid faeces were detected in 15 % of the faecal samples of the Beagles and in about 40 % of the German shepherds and German shorthair pointers. Unfavourable faeces qualities occurred more frequently with the canned food and were independent of guar and water contents. Apparent digestibility of organic nutrients was comparable in all three breeds. Digestibilities of organic matter were between 85 and 87 %, of crude fat 96 %, of N-free extracts between 89 and 91 %. Crude protein digestibility varied around 80 % with dry food and 75 % with the wet diets. Faecal levels of Cl. perfringens were comparable in all dietary periods (108.9-109.4/g) The several indirect tests indicated only weak dietary influences on the microbial activities in the intestine. Fasting breath H2-concentrations and post prandial levels were lower with dry food compared to the canned diets. The renal indican excretion increased with the canned diets (11.9 – 16.7 µmol/kg BW/ d) compared with the dry food (7.7 µmol/kg BW/d).

In conclusion, effects of the canned diets on faecal quality and nutrient digestibilities were not influenced by addition of guar or by differences in moisture. Breed related differences in faecal quality were found, which have to be investigated in future studies.

1) concentrations as used in the recipe
INVESTIGATIONS ON INTESTINAL EFFECTS OF MANNOSE-OLIGOSACCHARIDES, TRANSGALACTOSYLATED OLIGOSACCHARIDES, LACTOSE AND LACTULOSE IN DOGS
Zentek, J., Marquart, B., Pietrzak, T.

Fermentable carbohydrates can influence the composition and metabolic activity of the intestinal microflora due to their 'prebiotic' effects which are of considerable interest for the formulation of standard and veterinary diets. In the present study mannose-oligosaccharides, transgalactosylated oligosaccharides, lactose and lactulose were added to a mixed diet for dogs and investigated for their effects on the faecal quality, digestibility and on some products of intestinal microbial metabolism. Four adult dogs were fed an experimental diet (composition: dry greaves 35 %, rice 35 %, fish meal 5 %, soya oil 20 %, cellulose 3 %, vitamin and mineral supplement 2 %) with the different carbohydrates added on top (1 g/kg BW/d). The experiment was designed as a 4x4 latin square and the dogs received the basic diet without any additives before and after the addition of the fermentable carbohydrates. The apparent digestibility of organic nutrients and minerals, the renal excretion of minerals and some traits in the faeces (consistency, weight, content of dry matter, un-bound water by centrifugation method, and pH) were assessed. Dietary effects on the metabolism of the intestinal micro-flora were characterized by concentrations of ammonia and volatile fatty acids in the faeces and renal indican excretion.

The faecal dry matter was decreased (31.6 %) when mannose-oligosaccharides were fed compared with the basic diet, while the faecal unbound water (5.5 %) decreased (significant compared to the lactulose period). The faecal pH was lowered, when mannose-oligosaccharides were added (6.6). Faecal ammonia concentration and -excretions were higher during the initial basic diet than during the final basic diet phase. The faecal ammonia-excretion during the mannose-oligosaccharide period was decreased, but this was significant only to the first control period with the basic diet. This could have resulted from reduced intestinal microbial protein degradation. The faecal volatile fatty acid content ranged from 139-209 mmol/ l without distinct dietary effects. Mannoseoligosaccharides tended to decrease the apparent digestibilities of organic matter, dry matter, crude protein and N-free extracts. The in vitro experiment revealed higher NH3-concentrations after anaerobic incubation of faecal suspensions over 24 hours, when the carbohydrates were added. Total gas production was lowest in the in the initial control period compared with the other experimental diets. The concentrations of hydrogen in the fermentation gas was reduced when the dogs were fed the transgalactosylated oligo-saccharides or lactulose (210/ 78.6 ppm; 1092 and 1021 ppm during the basic phases). The concentration of volatile fatty acids and the proportion of acetic acid increased du-ring the incubation, while the proportion of propionic acid and n-butyric acid decreased.

In conclusion these investigations showed, that lactose, lactulose or trans-galacto-sylated oligosaccharides had only weak effects on the several para-meters. Mannose-oligosaccharides (1 g/ kg BW/ d) resulted in a decreased faecal pH, a loss of faecal consis-ten-cy, reduced faecal NH3-concentration and a decrease of apparent digestibilities of organic matter, crude protein, nitrogen free extracts and dry matter.
DIETARY MANAGEMENT OF CALCIUM OXALATE UROLITHIASIS IN DOGS
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Epidemiological evidence suggests that calcium oxalate (CaOx) urolithiasis occurs more commonly in older dogs. One survey showed that, although this stone type can occur across all ages, the mean age of detection was 8.5 ± 2.9 years with 60% of affected dogs between 6 and 11 years of age (Lulich et al., 1999). Diet can influence the concentration of a number of substances present in the urine that are thought to be involved in CaOx urolith formation and may thus play a role in prevention of recurrence of this disease.

This study was conducted to assess the effect of feeding a commercially available Diet* on the urine of 9 confirmed CaOx stone-forming dogs (mean age at start 8.8 ± 2.5 years). All uroliths were surgically removed and quantitatively analysed by infrared spectroscopy. Dogs with uroliths composed of >70% CaOx were recruited to the study. 24-hour urine samples were collected at or close to the time of stone formation while dogs were maintained on their normal food (pre-trial), and after 1 month, 3 months, 6 months and 12 months of receiving the Diet. Urinalysis was conducted using previously described methods (Markwell et al., 1999). RSS values were calculated using a modified version of the SUPERSAT computer program (Robertson, 1969). Results were compared analysis of variance. P<0.05 was taken as significant.

CaOx RSS and urinary concentrations of calcium were significantly reduced by feeding the Diet. Oxalate concentration tended to decrease although the difference was not significant. Struvite RSS remained unchanged compared with pre-trial values.

Pre-trial mean calcium oxalate RSS was above the predicted formation product (RSS~12) falling within the zone of oversaturation; comparable with data from human stone-formers. After feeding the Diet for 1 month the mean CaOx RSS was significantly reduced to within the zone of metastable supersaturation (RSS 1-12). A significantly lower level of supersaturation was maintained throughout the 12 months, when compared with the pre-trial samples. Spontaneous homogenous crystallisation will not occur at this level of supersaturation, therefore, feeding this diet would be expected to reduce the recurrence of stone formation in these dogs.

* WALTHAM Canine Lower Urinary Tract Support Diet (canned)

References
WEIGHT LOSS IN OBESE EXPERIMENTAL DOGS - EVALUATION OF A HIGH PROTEIN, LOW CARBOHYDRATE DIET

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2National Veterinary School of Nantes, France
3Royal Canin, Centre de Recherches de Saint Nolff, Vannes, France

Obesity is the most common nutritionally-related health problem in companion animals (Sloth 1992). Successful treatment of obesity implies not only a reduction of food intake and weight loss but also owner compliance and motivation, behavioural changes, physical exercise and follow-up of the animal after the weight loss program. Whatever diet is chosen, the principle of any weight loss program is to provide a limited amount of energy in order to induce weight loss whilst providing sufficient nutrients, and especially protein to minimize deficiency and losses of fat free mass (Hannah 1999).

The aim of this study was to compare a high protein, low starch and high fibre diet -DP-(crude protein 44.1 %, fat 8.7 %, crude fibre 10.0 % -as is) with a low protein, high starch and high fibre diet -HF- (crude protein 21.6 %, fat 7.7 %, crude fibre 21 % -as is) during the weight loss program of 8 adult chronically obese Beagles, 4 neutered males and 4 intact females, 5.5 (range 4-7) years old, showing at least 30 % (30-72) excess body weight (BW). The dogs were allotted to 2 comparable groups according to sex and body weight. During a baseline period, dogs underwent hormonal and biochemical evaluation in order to rule out any primary hormonal or metabolic disorder. Body composition was determined using deuterium labelled water dilution method (Son et al 1998), before and after the energy restriction. Initially, dogs were fed the same amount of food that they were eating on the maintenance baseline diet (crude protein 24.0 %, fat 16.1 %, 3810 kcal/kg). Those amounts were progressively decreased to induce a rate weight loss of around 2 % weekly. During the weight loss period, BW, food consumption, body score and pelvic as well as thoracic circumference were regularly monitored. One month after the beginning of the energy restriction, dogs were placed in metabolism cages for a week to assess their nitrogen balance.

Results. A moderate energy level –80 % of the maintenance energy requirement (MER) for optimal BW in males and 65 % MER in females- induced weight loss but was not sufficient to keep it up. Energy allowance was thus gradually decreased to reach 65 % MER for males and 45 % MER for females in order to reach the target weight. Those levels of restriction led to a rate of weight loss of 2 and 2.4 % a week for the DP and HF diets respectively. Target weight and optimal body condition were reached within 12 to 24 weeks for the HF diet and 21 to 26 weeks for the DP The proportion of lean tissue in total weight loss was 30 % and 20 % for the HF and the DP diets respectively. The apparent digestibility coefficients of crude protein were 79 and 83 % for HF and DP diets respectively.

In conclusion, a higher protein level allows a better conservation of the lean body mass. Energy restriction must be stricter in females than in males in order to induce and maintain weight loss. Energy restriction must be regularly adapted in order to keep a constant rate of weight loss up.

References
Obesity has been recognized as the most common form of malnutrition in small animal practices in the Western World (Sloth, 1992). Faced to an obese dog, the practitioner has the choice between several low-energy diets. Higher protein diets have been shown to better maintain muscle mass during weight loss (Hannah, 1999). This paper describes a clinical trial in which a high protein diet has been offered to client-owned dogs starting a weight loss program. The purpose of the study was to evaluate the efficiency of such a diet in field conditions.

From April to June 2000, nine privately owned obese dogs -8 females and 1 male- were recruited in 4 practices to test the efficiency of a high fibre, low carbohydrate and high protein diet (44.1 % crude protein, 28.6 % total dietary fibre, 8.7 % ether extract and 2800 kcal ME/ kg) in the management of canine obesity. The mean age, weight and body condition scores of the dogs were respectively 8 years (range 3-10), 30 kg (13.5-48) and 4.6/5 (4.5-5). In order to exclude all forms of secondary obesity, clinical examination, complete blood count, routine serum biochemistry, urinalysis and evaluation of thyroid and adrenal function were performed in every case. Mean excess body weight was 30 % (11-58). History and clinical examination showed inactivity or lethargy in 5 dogs, impaired breathing in 3 dogs and locomotion problems in 2 dogs but all other parameters were within normal limits. After he had agreed to enter the study, each owner was given sufficient amount of food for a month of test and was asked to weigh his dog weekly at home and to bring him back monthly at the clinic for physical exam, body weight measurement and getting more food. Clinical examination, weight, thoracic and pelvic circumference, fecal score and aspect of the coat were performed or recorded monthly. Owners were also called monthly by an investigator to follow compliance.

Treatment consisted of feeding 40 -55 % of maintenance energy requirements for the dog’s estimated ideal body weight until it reached optimum body condition. Dogs were fed twice daily and exercise was recommended to minimize loss of muscle mass.

Results. All the dogs completed the whole study. The time necessary to reach the target weight ranged from 4 to 38 weeks (mean : 19). This trial demonstrated a huge disparity in the rate of weight loss : from 0.9 to 3.14 % (mean : 2 %) per week. This variation is explained in part by the intensity of rationing and the degree of owner’s compliance with the vet’s recommendations. Improvement in body condition score was obvious and owners reported that their pets were much more active and playful.

Conclusion. The high protein experimental diet was successfully tested in field conditions and all dogs reached optimal body condition. Treatment of obesity involves a suitable diet formulation and continual patient monitoring to maintain the owner’s enthusiasm and compliance. As weight loss program can be very long, effective client motivation plays a key role in the success of the treatment. Their efforts are rewarded by a more lively and playful pet.

References
Cataract normally seen in older dogs were diagnosed by an experienced ophthalmologist in two litters of Newfoundland puppies after the breeder observed abnormal behavior. Both litters of 8 pups each came from a single kennel but the parent-pairs were not related and previous litters showed no signs of any cataract. Different degrees of lens opacity were diagnosed in both litters, described as usually beginning with a cloudy, equatorial opacification in the rear of the lens. But often no nuclear opacity could be seen in the examined eyes, meaning that the center of the lens was clear. In one of the litters, the first symptoms were observed as a opacity of the posterior "Y"-sutures, usually invisible in a healthy puppy. Subsequently both litters were diagnosed with the characteristic, serrated annular cataract in the rear of the lens.

Reviewing the literature, nutritional cataracts are observed in different species, due to a deficiency of certain vitamins and essential amino acids or an excess of particular sugars. It is known, that a deficiency of the essential amino acid arginine and phenylalanine produces a cataract in dog, cat and wolf puppies raised motherless with commercial, as well as experimentally produced milk replacer (Vainisi et al. 1981; Remillard et al. 1993; Glaze and Blanchard 1983). The risk of cataract formation seems to increase with an earlier onset of supplementary feeding of the milk replacer. A cataract could be produced experimentally in Foxhound puppies with a very low protein diet.

In our case both litters needed to be supplemented with a commercial milk replacer (declared as a single food) due to agalactia of the bitches every two hours from the second day post partum. The breeder mixed the milk precisely according to the formula. An analysis of the milk replacer showed, that the protein and amino acid composition is according to the guidelines for complete foods supporting growth of the American and European organizations NRC, AAFCO and FEDIAF. The protein, arginine and phenylalanine content in dry matter was 31.60 %, 1.11 % and 1.61 %. According to Meyer et al. (1985), milk of bitches contains 37.80 %, 2.12 % und 1.89 %, respectively.

The breeder started to feed a canned complete puppy food in each litter at the age of 3 weeks. After the discovery of the cataracts in 8 week- and 3 week-old pups the milk replacer was replaced by the moist complete puppy food and at the same time the essential amino acids arginine (200 mg/day) and phenylalanine (500 mg/day) were supplemented additionally to the food of every puppy for 15 weeks.

An ophthalmological examination 15 weeks after the discovery of the cataracts showed a total disappearance of the opacities in a few pups and slight improvement in the rest of the pups. The clinical picture of the characteristic cataract and the development are concurrent with the literature citations of nutritional cataract in other canines.

These findings indicate that the current recommendations for the supply of certain amino acids might be insufficient for very young puppies of certain breeds.

References
A PLACEBO CONTROLLED DOUBLE BLIND STUDY ON THE EFFECT OF NUTRACEUTICALS (CHONDROITIN SULFATE, MUSSEL EXTRACT) IN DOGS WITH JOINT DISEASES AS PERCEIVED BY THEIR OWNERS

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In our nutrition consultation practice we are often given anecdotal reports on the effect of nutraceuticals on lameness in dogs. Even in cases where such products did not even contain a substance with a potential chondroprotective effect but mainly calcium carbonate some owners reported enormous improvements of the lameness of their dogs. These observations initiated the idea to compare dog owners’ perception of the effects of either chondroitin sulfate or New Zealand green-lipped mussel extract (Perna canaliculus) to a placebo in a double blind field study. 70 dogs of different breed, age and sex with degenerative joint disease of the shoulder, elbow, hip joint and/or stifle were included in this study and randomized into three groups. The first group received 22 mg chondroitin sulfate per kg body weight and day (n=21), the second group received 11 mg mussel extract per kg body weight and day (n=18) and the third group was fed a placebo (n=19). Changes in clinical symptoms during a 12 week oral application period were verified separately by dog owners and the attending veterinarians using standardized questionnaires at the beginning and the end of the study. In those questionnaires the subjective assessment of the selected parameters and their development were systematically verified. Clinical signs were classed by a scale from 1 to 7; 1 = much improved, 4 = unchanged and 7 = much worse. 83 % of the participants finished the trial; the rest was excluded for different reasons such as refusal to eat the substances. None of the tested substances led to distinct improvement of symptoms or to a total recovery in general. Within the clinical signs evaluated by dog owners, the symptoms ‘the dog is lame’ and ‘pain’ improved most. In the above mentioned scale the mean value for the symptom ‘the dog is lame’ was 3.19±1.5 in the chondroitin sulfate group, 2.72±1.4 in the mussel extract group and 2.37±1.2 in the placebo group. The mean for the symptom ‘pain’ was 3.62±1.5 in the chondroitin sulfate group, 3.17±1.5 in the mussel extract group and 2.63±1.2 in the placebo group. In conclusion dog owners as well as the attending veterinarians who generally agreed with the owners only perceived a slight improvement in all three groups including the placebo group. However, when we looked at single data then in all three groups some dog owners stood out who observed enormous improvements which may have been caused by a coincidence of the onset of treatment and a spontaneous transient improvement of the lameness or by a optimistic perception of the effects.
Ingredients used to manufacture pet foods vary in their ability to deliver nutrient to the animal. Nutrient digestibility of the final pet food, therefore, is important to ensure that an animal can absorb sufficient nutrients from the diet to meet its nutrient requirements. Pet food manufacturers typically measure protein digestibility (uncorrected for endogenous losses) over the entire gastrointestinal tract, something that may not be accurate due to microbial fermentation in the large intestine. The aim of the present study was to compare protein and amino acid digestibility at the end of the ileum and over the entire digestive tract of adult dogs.

Five healthy adult dogs (3 males, 2 females) of mixed breed with an initial body weight range of 15-25 kg (mean ± SEM, 20 ± 2.4 kg), scheduled for destruction, were housed individually in outdoor concrete kennels. The dogs were fed a premium commercial dry dog food containing 26.6 % protein to energy requirements, to which chromic oxide was added as an indigestible marker. Each day's total food allowance was given in 10 equal portions, beginning hourly at 08:00. The diets were fed for 10 d and water was available at all times. Faecal samples of each dog were collected on day 9. On day ten, 4 h after the start of the hourly feeding, the dogs were euthanised with an intravenous injection of pentobarbitone, their body cavity opened and 20 cm of the distal ileum dissected out. The ileal content was gently flushed out and freeze-dried. Ileal, faecal and diet samples were analysed for dry matter, organic matter, crude protein, and amino acids.

All dogs remained healthy throughout the trial period. Table 1 presents the apparent ileal and faecal digestibilities of several dietary compounds. There were significant differences in the digestibility of several dietary components when measured at the distal ileum and over the entire digestive tract.

Table 1: Apparent digestibilities of various dietary components measured at the distal ileum and over the entire digestive tract of adult dogs

<table>
<thead>
<tr>
<th>Component</th>
<th>Ileal</th>
<th>Faecal</th>
<th>Significance</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>78.6±1.2</td>
<td>86.8±0.2</td>
<td>***</td>
<td>+8.2</td>
</tr>
<tr>
<td>Organic matter</td>
<td>81.8±1.1</td>
<td>85.4±0.3</td>
<td>**</td>
<td>+3.6</td>
</tr>
<tr>
<td>Crude protein</td>
<td>76.9±1.2</td>
<td>85.4±0.4</td>
<td>*</td>
<td>+8.5</td>
</tr>
<tr>
<td>Amino acid N</td>
<td>83.9±1.5</td>
<td>85.9±0.6</td>
<td>NS</td>
<td>+2.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>83.6±1.4</td>
<td>78.7±3.5</td>
<td>NS</td>
<td>-4.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>89.3±0.9</td>
<td>84.6±5.5</td>
<td>NS</td>
<td>-4.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>84.1±1.2</td>
<td>83.2±0.7</td>
<td>NS</td>
<td>-0.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>86.3±1.0</td>
<td>82.7±0.6</td>
<td>**</td>
<td>-3.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>73.5±2.4</td>
<td>80.1±1.0</td>
<td>*</td>
<td>+6.6</td>
</tr>
</tbody>
</table>

NS = non significant, * = P<0.05, ** = P<0.01, *** = P<0.001.

Similar results to those found in the present study have been found by Muir et al. (1996) and Murray et al. (1998) using ileal cannulated dogs. These studies reported higher dry matter, organic matter, crude protein and energy digestibilities when measured at the distal ileum. Although, the large intestine of the dog is relatively short, there appears to be a significant metabolism of nutrients. The apparent faecal digestibility method is not an accurate method for the measurement of the absorption of crude protein and amino acids from canine diets.

References
Thirty-two dogs were fed the same variety of canned dog food for 8 weeks. Dogs ranged in age from 2 to 14 years. Four breeds (Labrador Retriever, Beagle, Fox Terrier and Manchester Terrier) were on trial. Skin pH, sebum production rate, skin hydration, skin trans-epidermal water loss (TEWL), skin elasticity and skin thickness were assessed on the right dorsal side of lumbar region at 0, 4, and 8 weeks. Skin thickness decreased with increased age. Labrador Retrievers had thicker skin than the other breeds. There were significant differences between breeds and periods and a significant breed-period interaction for skin hydration and skin pH. Labrador Retrievers had higher skin hydration values than the other breeds and Manchester Terriers had higher skin hydration values compared to beagles. Skin hydration values decreased with time. Manchester Terriers had higher skin pH than the other breeds. The skin pH also decreased with time. Beagles and Manchester Terriers did not show changes in sebum levels with time, whereas Fox Terriers and Labradors sebum values decreased with time. Manchester Terriers had higher skin elasticity compared to the other breeds. Beagles had significantly lower TEWL values compared to Fox Terriers. Also, the TEWL values generally decreased with time. Age, breed, sex and period appeared to influence various skin parameters in this study.
A study was conducted to compare the nutritional adequacy of home-prepared diets in young and adult dogs using data gathered from a population of dogs and their owners in Vienna, Austria. Home-prepared diets were compared to commercial pet foods and to recommendations set by the American Association of Feed Control Officials (AAFCO). Both young and adult animals were studied with participants selected based on their responses to an animal ownership questionnaire in which dietary histories were obtained. Young (< 1 yr) and adult (1-7 yr) dogs were recruited and divided into commercial diet (CD) or home-prepared diet (HPD) fed groups. The HPD included those diets based on the self-selected use of table scraps and more carefully prepared recipes using human grade foodstuffs. No nutritional advice was directly given to any pet owner as to diet preparation. The CD group comprised only those animals fed commercially available complete and balanced diets. Dogs fed greater than 15% commercially manufactured complete and balanced diets mixed with home-prepared foods were excluded. Seven-day weighed food records, laboratory analyses of sub-samples of daily feedings during the seven-day period, and serum samples collected after a 30 day period of feeding the respective diets were analyzed and compared. Compared to AAFCO recommendations, mean energy and fat contents of both the HPD and CD were higher with a trend that HPD were somewhat higher than CD. The HPD were significantly lower in calcium and phosphorus and Ca:P ratio than both CD and AAFCO recommendations. The HPD were also lower in potassium, copper, zinc, and the fat soluble vitamins A, D, and E. Dietary essential fatty acid content of the HPD were adequate. Dogs fed the HPD exhibited normal serum vitamin A and parathyroid hormone concentrations, normal serum chemistries, and complete blood counts. Differences between the young and adult animals were unremarkable and could be explained as those ascribed to growth and development.

The HPD were higher in total saturated fat and lower in total polyunsaturated fat compared to a selected group of American and Austrian commercial dry extruded type diets but similar to canned types from both countries. Relative fatty acid content of serum phospholipid fractions of the HPD were significantly lower in 18:2n-6 and 20:4n-6 than serum of dogs fed the American commercial dry extruded type diets in a colony at Texas A&M University, U.S.A. In some cases these differences in phospholipid 20:4n-6 in dogs fed dry vs canned type diets may be important in inflammatory or other prostanoid mediated cellular responses. Also of interest is that some of the HPD dogs’ serum triglyceride fractions contained no 18:3n-3 even though all home-prepared diets contained this fatty acid. This finding along with the low dietary vitamin E in the HPD may indicate increased PUFA peroxidation and possible vitamin E depletion. Also, beta-oxidation and utilization of the diet 18:3n-3 may have also occurred because this fatty acid is generally poorly converted to longer carbon chain n-3 fatty acids. Consistent with this latter possibility are the low relative amounts of long chain n-3 PUFA in the serum PL fractions.

The home-prepared diets were energy dense, but without an increased nutrient density. However, no clinical signs of deficiency were noted. Reasons for these findings include that the AAFCO recommendations provides a sufficient margin of safety above absolute physiological requirements. The possibility also exists that the analyzed diets may not have been representative of long-term diet intakes of the animals in spite of owner assurances.
CONVERSION OF ESSENTIAL FATTY ACIDS BY DELTA-6 DESATURASE IN DOG LIVER MICROSONES

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Long Chain Polyunsaturated Fatty Acids (LCPUFA) are physiologically important precursors for eicosanoids, leukotrienes, and prostanoids. Desaturation of essential fatty acids (EFAs) by D6-desaturase is considered the rate-limiting step in conversion of EFAs to LCPUFA. This study was designed to study the conversion of EFAs by D6-desaturase in dog liver microsomes.

Liver microsomes were prepared using fresh liver from healthy dogs. Microsomes were incubated with 14C labeled 18 carbon EFA substrates. Following incubation, lipids were extracted, saponified and phenacylated. The resulting fatty acid phenacyl esters (FAPES) were separated by HPLC. Radioactivity in the FAPES was measured with a liquid scintillation counter. Accumulation of radioactive product was converted to enzymatic activity and expressed as pmol/min/mg protein. Using 18:3n-3 as substrate, the apparent D6-desaturase maximal velocity was 50.9 pmol/min mg protein and using 18:2n-6 substrate, the maximal velocity was 5.4 pmol/min mg protein. Apparent Km values were 20.8 mM for 18:3n-3 and 41.8 mM for 18:2n-6. Maximal velocities were lower than those previously reported in dogs and other species. Possible explanations for the low values include the presence of high endogenous fatty acid concentration (especially 18:2n-6) inherent in the dog liver microsome preparations providing high amounts of competitive non-radioactively labeled substrate, or methodological differences used in other studies. After accounting for endogenous 18:2n-6 fatty acids, the corrected Vmax and Km for the a-LA substrate was 62.4 pmol/min mg protein and 12.4 mM, respectively. Corrected values of Vmax and Km for LA substrate were not calculated due to interference from high endogenous LA substrate concentrations inherent in the liver microsomes.

These data show that dog liver microsomes have the ability to desaturate EFAs. Also, the maximal velocity of D6-desaturase of 18:3n-3 is considerably higher than that of 18:2n-6 in vitro. The Km value for 18:3n-3 is at least 2-fold lower than that for 18:2n-6. Physiologically, 18:3n-3 concentration in liver (2.4 mM) may never exceed the Km for desaturation in the absence of high dietary amounts. Conversely, 18:2n-6 amounts are readily converted because their concentration (64.4 mM) easily exceeds the Km. These phenomena may explain the low in vivo conversion rate of 18:3n-3 in dogs and other species. These findings suggest that high levels of 18:3n-3 supplementation may be necessary to exceed the D6-desaturase Km and significantly affect physiological levels of n-3 LCPUFA in the dog.
THE USE OF SORGHUM AND CORN AS ALTERNATIVES TO RICE IN DOG FOODS

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2 University of New England, Armidale, Australia

Rice is commonly used in premium Australian dog foods due to its highly digestible and hypoallergenic nature (Costa, 1997). Sorghum and corn are grains available in Australia which are considerably less expensive than rice. Sorghum and corn are known to contain starch that is less digestible in the intestinal tract due to a strong starch-protein matrix (Murray et al., 1999), however the extrusion process involved in the manufacture of dog food is likely to gelatinise the starch and make it more digestible (Camire 1998). The purpose of this study was to evaluate faecal nutrient digestibility of diets containing rice, sorghum and corn, and to determine the effect these diets had on faecal quality through evaluation of faecal score.

Eighteen-mixed breed dogs were fed extruded dry dog foods containing 50% rice, sorghum or corn. The remaining ingredients included equal inclusions of sugar beet pulp, maize gluten and poultry meal. The trial was conducted over 12 days, following an adaptation period on a commercial dry dog food for 4 weeks. The diets were introduced over the first 4 days, and faecal samples were collected on the final 5 days. Faecal scores were measured with a score of 1 indicating hard dry faeces, and a score of 5 indicating diarrhoea. Total starch was determined enzymatically and energy was determined using a bomb calorimeter. Nitrogen was determined and protein was calculated as N x 6.25. Faecal nutrient digestibilities were calculated by adding the marker celite (2%) to the diet, which was measured as acid insoluble ash.

The mean faecal scores (± standard deviation) for the rice, sorghum and corn diets were 2.4 ± 0.3, 2.0 ± 0.1 and 2.1 ± 0.1 respectively, with the rice diet being significantly higher than the sorghum and corn diets (P<0.05). The faecal starch digestibility was 100% for each diet (P>0.5). Faecal energy digestibility coefficients for the rice, sorghum and corn diets were significantly different at 0.90± 0.006, 0.87 ± 0.007 and 0.85 ± 0.012 respectively (P<0.01). The faecal protein digestibility coefficients for the rice, sorghum and corn diets were significantly different at 0.87 ± 0.006, 0.85 ± 0.007 and 0.83 ± 0.009 respectively (P<0.001).

There were mild reductions in both energy and protein digestibility with the sorghum and corn diets compared to the rice diet, however, these small differences are not likely to be biologically significant. Starch digestibility was complete and faecal scores were ideal for each of the diets. Therefore, sorghum and corn are good alternatives to rice as the primary cereal grain in dog foods.

References
DETERMINATION OF INSULIN SENSITIVITY IN THE DOG: AN ASSESSMENT OF THREE METHODS
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The prevalence of diabetes in dogs is increasing and has been estimated at approximately 1.5%.
It is particularly prevalent in the older, obese, female dog. By comparison with type 2 diabetes in humans, it is likely that insulin resistance is important in the aetiology of diabetes in dogs. However it generally remains hidden, and early disease often goes undetected until the animal is severely diabetic. Although dogs have often been used as an experimental model for human diabetes, there is little information available regarding insulin resistance and its consequences in the dog, due, in part, to the lack of well-evaluated methods for the estimation of insulin sensitivity. This study evaluates the suitability of three methods of measuring insulin sensitivity, the oral glucose tolerance test (OGTT), the intravenous glucose tolerance test (IVGTT), and the insulin tolerance test (ITT), for determining insulin sensitivity on the adult dog.

Five non-obese adult Labrador retriever dogs (3 female, 2 male, age 7.0 (SD 1.2) years) were studied. They were placed on a standard maintenance diet (kJ metabolic energy/day = 460 x body weight (kg)0.75) and were given similar exercise levels throughout. They received the 3 tests on separate days, following an overnight fast, in random order with a 2 week rest period between tests. The OGTT, as described for dogs by Kaneko, 1989, consisted of an oral glucose bolus of 4g/kg body weight given as a 50%w/v solution. Venous blood was collected at frequent intervals for 180min. The IVGTT consisted of an IV glucose bolus of 500mg/kg body weight as a 50% solution, administered over 30 seconds. Venous blood was collected at frequent intervals for 120min. The ITT consisted of an IV bolus of quick-acting insulin (Velosulin, Nordisk Wellcome), 0.1U/kg body weight. Venous blood was collected every minute for 20 minutes.

Following the OGTT, plasma glucose profiles did not compare with those previously reported. A biphasic, delayed glucose profile was observed, which was difficult to interpret, with mean glucose levels remaining elevated 3h following the glucose load (fasting glucose 4.8(SE 0.06), at 3h, 5.5(SE 0.32) mmol/l). This was probably due to delayed gastric emptying and altered gut motility following the larger hyperosmolar glucose load. In this form, the OGTT was therefore considered unsuitable.

The ITT was quick and easy to perform. It correlated well with SI, the index of insulin sensitivity in the IVGTT in 4 out of the 5 dogs (r = 0.95, p<0.05). However it had to be terminated prematurely in 3 of the 5 dogs, as they were showing clinical signs of hypoglycaemia.

The IVGTT was also easy to perform, without risk of hypoglycaemia. Kinetic modelling could also be applied to the insulin and glucose data to give direct measures of insulin sensitivity (SI) and glucose effectiveness (SG). Reproducibility studies on 3 further animals (3 large breed dogs, 6 repeated tests) showed a mean intraindividual Coefficient of Variation for SI of 15.8%.

Although not applicable to the routine measurement of insulin sensitivity in routine veterinary practice, we conclude that the IVGTT is the method of choice in a research environment, as it provides a safe, reproducible and informative measure of insulin sensitivity.

Reference
THE EFFECT OF DIETARY SUPPLEMENTATION WITH n-3 POLYUNSATURATED FATTY ACIDS ON INSULIN SENSITIVITY IN HEALTHY LABRADOR RETRIEVERS

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*University of Surrey, Guildford, U.K.

Obesity is becoming increasingly common in older dogs, with a current incidence in the UK estimated at between 11-44%. This is linked to overfeeding and a sedentary lifestyle, although in certain pure-bred dogs, such as Labrador retrievers, who have a greater risk of becoming obese, there appears to be a genetic link to obesity. As with human subjects, obesity in dogs is associated with fasting hyperinsulinaemia, impaired glucose tolerance and an increased insulin response to a glucose load. It is considered to be the single most important factor in the development of diabetes. Recent human studies have shown that dietary supplementation with n-3 polyunsaturated fatty acids (PUFA) can improve insulin sensitivity. The present study was therefore carried out to investigate the effect of dietary n-3 PUFA supplementation in the healthy Labrador retriever.

Six healthy, non-obese Labrador retriever dogs (mean age 5 years (SD 2.5)) were placed on a standard maintenance diet (kJ metabolic energy/day = 460 x body weight (kg)0.75), enriched with marine fish oil containing 0.08g eicosapentaenoic acid (EPA) and 0.06g docosahexaenoic acid (DHA)/100g, for 7 months. During an equivalent control period, dogs were fed the standard diet without marine fish oil supplementation. Dogs received similar exercise levels throughout the study. Insulin sensitivity was assessed at the end of each dietary period, with an intravenous glucose tolerance test (IVGTT) (300mg glucose/kg body weight as a 50% solution, administered over 30 seconds). Frequent venous blood samples were taken for 120min, and analysed for glucose and insulin. Indices of insulin sensitivity (SI) and glucose effectiveness (SG) were calculated using minimal model analysis of IVGTT data. Red blood cell fatty acid profiles were measured following each dietary period.

<table>
<thead>
<tr>
<th></th>
<th>Fasting glucose (mmol/L)</th>
<th>Fasting insulin (pmol/L)</th>
<th>SI (x10^-4 min^-1 pM^-1)</th>
<th>SG (x10^-2 min^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean±SEM)</td>
<td>4.56±0.2</td>
<td>47±11.4</td>
<td>0.88±0.21</td>
<td>3.81±0.86</td>
</tr>
<tr>
<td><strong>n-3 PUFA Diet</strong></td>
<td>4.39±0.1</td>
<td>57±8.6</td>
<td>1.12±0.34</td>
<td>2.8±0.69</td>
</tr>
</tbody>
</table>

Total RBC n-3 PUFA was significantly higher in n-3 PUFA supplemented diets compared to control (6.2+1.2 vs 1.7+0.4 mol% respectively, p<0.001). There was no significant difference between saturated fatty acid profiles between the diets. Insulin sensitivity was good in 5 of the 6 dogs. There were no significant differences in any parameters of insulin sensitivity between the diets. However one dog, with a degree of insulin resistance, had greatly improved insulin sensitivity on the n-3 PUFA supplemented diet (SI control diet 0.53, n-3 PUFA diet, 0.94).

A diet enriched in n-3 PUFA at this level therefore had little effect on insulin sensitivity in healthy, insulin-sensitive dogs. However, there is some indication that it might be beneficial in insulin resistant animals. Further studies are necessary to confirm this.
Several studies have suggested that insulin resistance is a factor causing dyslipidemia (Laasko et al 1990). The aim of the present study was to determine in dogs the effect of insulin resistance on the lipoproteins metabolism.

Five healthy male Beagle dogs, aged 2-7 years old, weighing 8.80-15 kg at the beginning of the study were used. In order to develop insulin resistance, a high fat diet (55% fat calories) was given at hyperenergetic level (twice the NRC 1985 recommendation) for 7 months. Body weight increased by 20% to 65%. To assess insulin sensitivity in vivo, we applied the euglycemic-hyperinsulinemic clamp technique (De Fronzo et al, 1979) before and after the diet. A primed insulin infusion (2mU/kg/min) was performed. A simultaneously glucose infusion was adjusted to maintain euglycemia. The amount of glucose infused is a measure of tissue sensitivity to insulin. We also used this technique in combination with stable isotopes, hot-glucose-infusion method (Finegood et al 1987), for measurement of glucose production. Plasma lipoproteins were separated by fast protein liquid chromatography (FPLC). Concentrations of cholesterol, triglycerides, phospholipids and non esterified fatty acids in the different lipoproteins were measured by enzymatic methods.

Dogs were found to be insulin-resistant by the euglycemic hyperinsulinemic clamp (p < 0.05). In healthy and insulin-resistant dogs, hepatic glucose production was between 1.74 and 2.45 mg.kg-1.min-1 while it was completely suppressed in response to hyperinsulinemia during the clamp. In healthy as well in insulin-resistant dogs, high density lipoproteins (HDL) were the predominant lipoproteins and the major cholesterol-carrying particles in plasma. No significant increase in triglycerides-HDL was showed after diet. Very low density lipoproteins (VLDL) increased in insulin-resistant dogs as a consequence an increase in both cholesterol (p<0.05) and triglycerides (p<0.05). Low density lipoproteins (LDL) data showed no significant difference between healthy and insulin-resistant dogs.

Thus, the high fat diet induced insulin resistance which carried out dyslipidemia with the similar changes in lipoprotein profile as it has been described in man (Fresnais et al 1997), i.e. an increase in cholesterol- and triglycerides-VLDL and in triglycerides-HDL.

References:
SIMULTANEOUS DETERMINATION OF TOTAL BODY WATER AND PLASMA VOLUME IN CONSCIOUS DOGS BY THE INDICATOR DILUTION PRINCIPLE

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In normal dogs, body weight and body composition vary considerably between breeds, sex and age and the relative size of the body compartments changes throughout the life cycle of individual dogs. Furthermore, the size of the body fluid compartments may be severely affected in several physiological and pathological conditions such as heavy exercise, pregnancy, malnutrition and acute or chronic diseases. Therefore, repeated determination of total body water (TBW) and plasma volume (PV) is of major interest in many physiological and clinical situations.

In this study, TBW and PV were determined simultaneously in five 2-year old female Beagle dogs by means of the indicator dilution principle (Expt. 1). The dogs were placed in a sling and a sterile catheter, inserted into the external jugular vein, was used for bolus injection of deuterium oxide (D2O; 100 mg/kg) and Evans Blue dye (EB; 0.5 mg/kg). Another catheter was placed in the exteriorised carotid artery for repeated blood sampling. The dogs were maintained on a standard diet and the experiments were repeated after 2 months (Expt. 2) following the same protocol. All experimental procedures were carried out according to Danish national legislation. A total of five samples (~3 ml) of heparinized arterial blood were obtained from each dog at exactly –10 (blank), +60, +120, +180 minutes and 6 days after the injection. Packed cell volume (PCV), determined at 10,000 ¥ g for 15 min., was corrected for 2% trapped plasma. The plasma samples were analysed for EB within 24 h by two-wavelength (627 and 740 nm) spectrophotometry, or stored at -20 °C for subsequent determination of deuterium enrichment by gas isotope ratio mass spectrometry (Brand et al. 1996). PV was calculated from the intercept (=Y0) of the plasma EB disappearance curve in a semi-logarithmic plot versus time, following correction of the optical density of each sample by its own blank value. Blood volume (BV) was calculated as: \( BV = PV \times (1-PCV) \). TBW was calculated in a similar way by semi-logarithmic extrapolation of the plasma deuterium curve. All values are given as mean±SE and, for each measure, the repeatability coefficient was calculated according to Bland & Altman (1999).

<table>
<thead>
<tr>
<th></th>
<th>Expt.1</th>
<th>Expt.2</th>
<th>Repeatability coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>1.8±0.4</td>
<td>12.1±0.4</td>
<td>83.4</td>
</tr>
<tr>
<td>Total body water (ml/kg)</td>
<td>564±25</td>
<td>555±19</td>
<td>5.1</td>
</tr>
<tr>
<td>Plasma volume (ml/kg)</td>
<td>48.7±3.4</td>
<td>48.0±3.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma volume (% of TBW)</td>
<td>8.6±0.4</td>
<td>8.6±0.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Blood volume (ml/kg)</td>
<td>80.7±4.6</td>
<td>82.2±4.1</td>
<td></td>
</tr>
</tbody>
</table>

Rates of body water turnover, derived from the data obtained at days 1 and 6, ranged from 63 to 95 ml/kg per day. The results presented in the Table are in accordance with data published by Andersen (1970) confirming that reliable measures of total body water, water turnover and plasma and blood volumes may be obtained with high precision in normal conscious dogs by means of the indicator dilution techniques described in this paper.

References
SELECTED GELLING AGENTS IN CANNED DOG FOOD AFFECT DIGESTIBILITIES AND FECAL CHARACTERISTICS IN ILEAL CANNULATED DOGS

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2 TNO Food and Nutrition Research Institute, The Netherlands

Most canned dog foods contain a gelling agent to provide texture and body to the food. While gelling agents improve the appearance of canned foods, little is known about the effects of gelling agents on nutrient digestibilities and fecal characteristics. The effects of three gelling agents [wheat starch, a guar gum/carrageenan mixture (50:50) and a locust bean meal (LBM)/carrageenan mixture (50:50)] were evaluated. Dogs were fed a canned diet containing chunks in gravy with either no gelling agent (control) or one of two levels (0.2 and 0.5% of the diet on a wet weight basis) of the gelling agents. Six ileal cannulated purpose bred dogs were randomly assigned to diets in a 6 x 7 Youden square design. Dogs fed the diets containing gelling agents had higher ileal digestibilities of organic matter (P = 0.05), fat (P < 0.01), and gross energy (P = 0.02) when compared to dogs fed the control diet. Ileal digestibilities of total amino acids and total nonessential amino acids were lower (P = 0.04) in dogs fed the control diet than in dogs fed diets containing gelling agents. Dogs fed the guar gum/carrageenan-containing diets had higher (P < 0.05) ileal digestibilities of all amino acids except histidine, methionine, and cysteine in comparison to dogs fed the control diet. Dogs fed the control diet had increased (P < 0.01) total tract dry matter (DM) digestibilities compared to dogs fed the gelling agent-containing diets. After correcting for differences in DM intake, dogs fed the control diet had lower (P < 0.01) fecal output (0.27 g wet feces/g DM intake) vs. dogs fed the gelling agent-containing diets (0.36 g wet feces/g DM intake). Dogs fed the control diet had lower (P < 0.01) fecal DM percentages and higher (P = 0.02) fecal scores than dogs fed the gelling agent-containing diets. The addition of a gelling agent, in particular the guar gum/carrageenan mixture, appears to improve ileal digestibilities, but also increases fecal output.
EFFECTS OF SUPPLEMENTAL FRUCTOOLIGOSACCHARIDES AND MANNANOLIGOSACCHARIDES ON COLONIC MICROBIAL POPULATIONS, IMMUNE FUNCTION, AND FECAL ODOR COMPONENTS IN THE CANINE

Division of Nutritional Sciences and Department of Animal Sciences, University of Illinois, Urbana, IL, USA.

Some information exists on the nutritional effects of fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) in various species. However, very little information currently exists on the effects of feeding these prebiotics to companion animals. A 4 ¥ 4 Latin square design was used to examine whether supplemental FOS and/or MOS influenced microbial populations, immune function, and fecal odor components in dogs.

Four purpose-bred adult female dogs were surgically fitted with ileal cannulas at least two weeks prior to the initiation of the experiment. Dogs were offered 200 g of a dry, extruded kibble diet twice daily (0800 and 2000 h). Fresh water was available at all times. At the time of feeding, dogs were dosed with four treatments: 1) Control (no FOS or MOS); 2) 1 g FOS; 3) 1 g MOS; or 4) 1 g FOS + 1 g MOS. Each 14 d period consisted of a 10-d adaptation phase and 4-d collection phase of feces and ileal effluent. On d 14 of each period, blood samples were collected for the analysis of serum immunoglobulins (Ig) and complete blood count (CBC). A fresh fecal sample also was collected on d 14 for the analysis of fecal IgA, fecal odor components, and bacterial enumeration. Data were analyzed using the GLM procedure of SAS. Treatment means were compared using pre-planned orthogonal contrasts.

Dogs supplemented with MOS had decreased (P = 0.05) total aerobes and increased (P = 0.13) Lactobacillus populations in feces, positive indicators of colonic health. A decrease in total anaerobes was detected in dogs supplemented with FOS + MOS. No differences were observed in E. coli, Clostridium perfringens, or Bifidobacterium populations. Although no differences were observed in fecal IgA levels, ileal secretory IgA concentrations were increased in dogs supplemented with FOS + MOS (P < 0.05) vs control. Total white blood cell number and neutrophil % were not different among treatments. However, lymphocytes (% of WBC) were increased (P < 0.05) in dogs supplemented with MOS. Serum IgA concentrations also tended to increase in dogs supplemented with MOS (P = 0.13) and FOS + MOS (P = 0.16). No differences were observed in serum IgG or IgM concentrations among treatments. Dogs supplemented with FOS and FOS + MOS had significant decreases in fecal concentrations of total indoles and phenols. Numeric decreases in total BCFA and ammonia concentrations were observed in dogs supplemented with FOS and FOS + MOS.

The results of this study suggest that the supplementation of FOS and MOS may have beneficial effects on colonic health. Two grams of MOS appeared to be sufficient in enhancing local and systemic immune capacity as well as beneficially influencing microbial populations. Although 2 g FOS/d decreased certain fecal odor components, a higher daily dose may have resulted in greater changes in these components and microbial populations. Because variation was high for certain measurements taken in this study, a larger number of observations per treatment would have been beneficial for the detection of differences among treatments.
AGE-RELATED DIFFERENCES IN LEUCOCYTE POPULATIONS, LYMPHOCYTE SUBSETS, AND IMMUNOGLOBULIN (Ig) PRODUCTION IN THE CAT

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

To assess age-related differences in feline immune function, 51 domestic cats were assigned to two groups, Adult (n=26) and Senior (n=25). Analyses of leucocyte populations, lymphocyte subsets and antibody production by two-sample t test revealed significant differences between the two groups. The Senior group had lower total leucocyte (p<0.05), lymphocyte (p<0.001), and eosinophil (p<0.05) counts. Neutrophil counts were significantly higher in the Senior cats (p<0.05). Analysis within the lymphocyte population, revealed that the overall percentage of cells staining for pan T-cell marker was similar in both groups. Within the T-cell population, the percentage of CD4+ cells was lower (p<0.001) and CD8+ cells higher (p<0.05) in the Senior group, resulting in a lower CD4+/CD8+ ratio (p<0.001). The percentage of cells staining positive for the natural killer cell marker, CD56 was not different, but the older cats had a lower percentage of CD21+mature B cells (p<0.05). Plasma IgG and IgA levels were higher in the senior group (p<0.05 and p<0.001 respectively). Our findings are in agreement with age related changes observed in other mammals, including humans, and suggest that these species share similar mechanisms of immunosenescence.
PREVALENCE OF LOWER URINARY TRACT DISORDERS OF DOGS AND CATS IN THE UNITED STATES

1Hill’s Pet Nutrition Inc., Topeka, KS and University of Minnesota, St. Paul, MN, USA

Studies describing the prevalence of lower urinary tract disease in dogs and cats indicate a prevalence rate between 0.85-1.5% for cats and 0.53-1.6% in dogs. Thus, the relative number of animals at risk for LUTD would appear quite low despite a perception by owners and veterinarians that urinary tract disease constitutes a significant health risk to companion animals. The purpose of this study was to 1) determine the overall prevalence of lower urinary tract disease in dogs and cats; 2) define the relative distribution of specific diagnoses within this category of disease; and 3) identify risk factors associated with lower urinary tract disease.

A cross-sectional study was conducted in 1995 on dogs and cats within the United States seen at private practices using a computerized practice management system. Information on age, breed, sex, body condition score, diet and assigned diagnostic codes were collected electronically from participating practices distributed throughout 7 major regions of the United States. Prevalence estimates and frequencies for population description were generated using a statistical software program. A univariate analysis was used to screen for the associations between urinary tract diseases and age, breed, gender, diet, body condition score, and region. All disorders reported at a prevalence of 0.1% (cats) or 0.04% (dogs) or higher were screened for an association with increased or decreased risk using a chi-square and prevalence odds ratio (OR) as a measure of relative risk. Multivariate models were used to evaluate the association of risk while controlling for other confounding variables included in the study.

Records were completed for 15,226 cats and 31,484 dogs. The prevalence for all cases of upper and lower urinary tract disease were 6.6% and 2.8% for cats and dogs, respectively. Lower urinary tract disease was diagnosed in 4.6% of cats and 2.0% of dogs. Cystitis was the most common diagnosis in both cats and dogs (Table 1). Risk factors associated (P<0.05) with lower urinary tract disease by chi-square analysis included increasing age, diet type, diet form, gender, breed and increased body condition scores for dogs. Associated risk factors were similar in cats with the surprising exception of diet form (i.e. dry, canned, semi-moist), which was not associated. The association of lower urinary tract disorders with advanced age and increased body condition score and gender remained significant (P<0.05) following multivariate analysis.

<table>
<thead>
<tr>
<th>Disease Description</th>
<th>Canine (%)</th>
<th>Feline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystitis</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>UTI</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Feline Urologic Syndrome</td>
<td>Na</td>
<td>1.3</td>
</tr>
<tr>
<td>Urolithiasis</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Bacterial cystitis</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Obstruction</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Urethritis</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

In summary, lower urinary tract disease diagnosed by private veterinary practitioners is a common disorder in the United States. The prevalence of disease in our study is greater than previously reported, particularly in cats. Although several associated risk factors were identified, advancing age and elevated body condition score were consistently associated with disorders of the lower urinary tract.
PREVALENCE OF RENAL DISORDERS OF DOGS AND CATS IN THE UNITED STATES

1 Hill’s Pet Nutrition Inc., Topeka, KS and University of Minnesota, St. Paul, MN, USA

Renal disease is a common cause of morbidity and mortality in aging dogs and cats. The prevalence of renal disease in cats in 1992 was reported at 3.05%. The overall prevalence in dogs in unknown but is approximately 20% in dogs over the age of 5 yr. Recent evidence suggests the prevalence may be increasing. The purpose of this study was to 1) determine the overall prevalence of renal disorders in dogs and cats; 2) define the relative distribution of specific diagnoses within this category of disease; and 3) identify risk factors associated with renal disease.

A cross-sectional study was conducted in 1995 on dogs and cats within the United States seen at private practices using a computerized practice management system. Information on age, breed, sex, body condition score, diet and assigned diagnostic codes were collected electronically from participating practices distributed throughout 7 major regions of the United States. Prevalence estimates and frequencies for population description were generated using a statistical software program. A univariate analysis was used to screen for the associations between renal disorders and age, breed, gender, diet, body condition score, and region. All disorders reported at a prevalence of 0.02 or higher were screened for an association with increased or decreased risk using a chi-square and prevalence odds ratio (OR) as a measure of relative risk. Multivariate models were used to evaluate the associate of risk while controlling for other confounding variables included in the study.

Records were completed for 15,226 cats and 31,484 dogs. The prevalence of all-cause renal disease was 2.2% in cats and 0.8% in dogs. A general diagnosis of chronic or acute renal disease/failure accounted for the majority of diagnoses with few animals having further definition of the renal pathology (Table 1). Risk factors associated (P< 0.05) with renal disease by chi-square analysis included increasing age, diet type, diet form, gender, breed and decreased body condition scores for dogs and cats. Himalayan, Persian, mixed breed and Siamese cats were at increased risk for renal disease and failure (OR: 5.8, 4.8, 5.8, 8.2, respectively). Of dogs, the ShiTzu was at increased risk for uremia (OR 3.6). The average age of pets with renal disease was greater than the reference population for both cats (13.2 vs. 5.3 yr., respectively) and dogs (10.2 vs. 5.5 yr., respectively). The association of renal disorders with increasing age and decreased body condition score remained significant (P < 0.05) following multivariate analysis.

<table>
<thead>
<tr>
<th>Disease Description</th>
<th>Canine (%)</th>
<th>Feline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute/Chronic Renal Disease</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Nephrolithiasis</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Nephritis</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>0.013</td>
<td>0.007</td>
</tr>
<tr>
<td>Pyeloephritis</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>0.003</td>
<td>0.01</td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>–</td>
<td>0.02</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>0.003</td>
<td>–</td>
</tr>
</tbody>
</table>

In summary, renal disease was commonly diagnosed by private veterinary practitioners in the United States. Our study did not support the observation that the prevalence of renal disease is increasing. Advancing age and decreased body condition score were major risk factors.
CALCULATION OF GROSS ENERGY IN PET FOODS: DO WE HAVE THE RIGHT FIGURES ON HEAT COMBUSTION?
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Ludwig-Maximilians-University Munich, Germany, Waltham Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

In a previous study considerable discrepancies between measured and calculated heat combustion or gross energy (GE) of pet foods in data from several laboratories (Kienzle et al. 1998) were observed. Heat combustion for all nutrients is covered by a range. Picking one figure from that range for calculation of gross energy may result in errors. This might be the case for fiber containing materials in pet foods such as gelling agents, whose nature and form differ somewhat from those typically present in feeds formulated for agricultural animals. There is limited information on the heat combustion of these materials and it is unclear how these relate to the different methods used for determining fiber. The present investigation was carried out to clarify whether such potential sources of error in the calculation of gross energy are quantitatively relevant in pet foods. The following categories of materials were investigated: i) Food ingredients providing relatively purified sources of nutrient groups which included 7 pectin samples, 6 galactomannan sources, 5 carrageen samples, 4 alginate samples, 1 sample of gum tragacanth, agar agar and gum arabicum, 2 xanthan samples, 22 cellulose samples, 6 lignin samples, 9 protein samples, 6 fat samples, ii) food ingredients providing a combination of nutrient groups which included 3 meat samples, 2 lung samples, 2 samples of skim milk powder, iii) 67 prepared complete pet foods. Samples were analyzed for heat combustion (adiabatic bomb calorimetry), crude nutrients, acid detergent fiber (ADF), and acid detergent lignin (ADL). Some of the non-starch polysaccharides which gave low levels of crude fiber and ADF were also analyzed for total, insoluble and soluble fiber. The heat combustion of cellulose ranged between 17.0 and 17.5 kJ/g organic matter (OM). The variation was larger for other non-starch polysaccharides (14.0-18.2 kJ/g OM). The heat combustion of lignin ranged between 17.0 and 29.2 kJ/g OM. Starch had a narrow range (17.2-17.3 kJ/g OM). Heat combustion of protein samples varied between 22.0 and 24.6 kJ/g, and of fat samples varied between 38.0 and 39.6 kJ/g OM. When cellulose was analyzed for crude fiber only between 62 and 85 % OM were detected. ADF analyzes of cellulose ranged between 75 and 93 % OM. The crude fiber content of all other non-starch polysaccharides did not exceed 13 % OM. With the exception of pectins (ADF 0.7-37 % OM) and alginites (ADF 39-66 % OM) the ADF content was also below 13 % in these samples. In contrast the total fiber content was above 80 % OM in all non-starch-non-cellulose polysaccharides and the percentage of soluble fiber was high (25-93 % OM). Unprocessed lignin gave high readings for crude fiber (39-61 % OM) and ADF (96-99 % OM), while processed lignin had low crude fiber content (< 1% OM) and low ADF content (< 32 %). ADL determined unprocessed lignin (78-91 % OM), but again processed lignin was analyzed incompletely (< 29 %). Pectin and alginate gave false positive ADL readings of up to 31 % OM, other non-starch polysaccharides were not determined by ADL. When gross energy was calculated with the factors (kJ/g) 24 for protein, 38 for fat and 17 for carbohydrate including fiber there was a good agreement between calculated gross energy and heat combustion.

References
FURTHER DEVELOPMENTS IN THE PREDICTION OF METABOLIZABLE ENERGY (ME) IN PET FOOD

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Ludwig-Maximilians-University Munich, Germany

Predicting digestible or metabolizable energy (DE, ME) in prepared dog food by factors for nutrients is possible with reasonable accuracy only on condition that the digestibility of nutrients is rather uniform throughout the products to which such equations will be applied. The wide variation of products on the market can be covered more precisely by equations that predict digestibility of energy. In a previous study (Kienzle et al. 1998) a method was presented to predict apparent digestibility of energy (ad GE %) in pet foods by the content of crude fiber in dry matter. ME was predicted in three steps: Gross energy (GE) determined by bomb calorimetry was multiplied with the predicted digestibility to calculate DE, which was then corrected for energy losses by urine which were predicted from protein content. When this method was tested in an independent data set it showed close agreement for all products with a fiber content below 5 % dry matter. In dog foods with a higher fiber content, however, the prediction of ME was not satisfactory. A consecutive investigation (Kienzle et al. 2001) showed that the impact of fiber (cellulose) on energy digestibility in the dog is stronger in high fat than in high carbohydrate diets. Consequently the 234 observations on digestibility of energy in dogs available for our first study (Kienzle et al. 1998) were divided into two groups with a Nfe-content above or below 40 % dry matter, respectively, and re-evaluated. The following equations for the prediction of energy digestibility (ad GE %) in dog food were obtained:

Nfe < 40 % dry matter: ad GE (%)=90.9-0.98*crude fiber in % dry matter
Nfe > 40 % dry matter: ad GE (%)=91.2-2.21*crude fiber in % dry matter

ME can be predicted accordingly as follows:

DE = GE * ad GE (%)/100
with ad GE (%) predicted by the appropriate use of the above equations
ME = DE - 1.04 kcal/g protein

References
Cellulose is used as dietary treatment for dogs with chronic diarrhea but evidence on the effect on feces quality is contradictory. In the present investigation the influence of cellulose origin and fiber length or particle size on feces quality was tested. Ten Beagle dogs (BW: 11.7 kg - 16.1 kg, 5 female, 5 male) were fed with a ration consisting of cooked greaves (55.7 % DM), cooked corn starch (38.5 % DM) and sunflower oil (5.8 % DM) according to energy requirements. Six different cellulose types were added (crude fiber 10 % DM) to the basal diet which was fed as a control in a latin square design. The cellulose types were: 1) microcrystalline cellulose (more than 50 % of particles <32 µm2 and 30 % <75 µm2), 2) mixed cellulose with fibers (200-300*20 µm) of different origin, 3) and 4) two celluloses from beech-tree (60*20 µm and 300*20 µm, respectively), 5) and 6) two celluloses from pine-wood material (60*30 µm and 300*36 µm, respectively). After 10 days adaptation, feces were collected for seven days. Feces quality was classified as either liquid, or soft and pasty or solid, well formed. Dry matter and pH were determined. Fecal pH was not affected by cellulose. Dry matter in the feces showed a trend to decrease with increasing fiber length or particle size. The quality of the feces increased with increasing fibre length (table 1). While the intake of microcrystalline cellulose led to diarrhea feces were well-formed and solid when cellulose with a fibre length of 300 µm was fed. The origin from either beech tree or pine-wood had no effect on any of the parameters measured.

<table>
<thead>
<tr>
<th>cellulose, type/fiber length</th>
<th>liquid</th>
<th>soft</th>
<th>solid</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>49.2</td>
<td>50.8</td>
<td>0.00</td>
<td>63</td>
</tr>
<tr>
<td>microcrystalline</td>
<td>33.9</td>
<td>57.2</td>
<td>8.9</td>
<td>56</td>
</tr>
<tr>
<td>60µm</td>
<td>6.2</td>
<td>39.3</td>
<td>54.5</td>
<td>112</td>
</tr>
<tr>
<td>200-300µm</td>
<td>0.0</td>
<td>7.1</td>
<td>92.9</td>
<td>56</td>
</tr>
<tr>
<td>300µm</td>
<td>0.0</td>
<td>0.9</td>
<td>99.1</td>
<td>112</td>
</tr>
</tbody>
</table>
EVALUATION OF BODY COMPOSITION THROUGH ISOTOPIC DILUTION METHOD BY A LOW-COST TECHNIQUE: FOURIER-TRANSFORM INFRARED SPECTROSCOPY

1Ferrier, L., 2Robert, P., 1Dumon, H., 1Nguyen, P
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2 INRA Macromolecules Physics and Chemistry Unit, Nantes Research Center, Nantes, France

Total body water (TBW) measurement is useful for the evaluation of body composition, which give an indication of the nutritional status in animals. TBW is measured by the isotopic dilution method, which consists to inject a known dose of an isotopic tracer (2H-, 3H-or 18O-labelled water) assayed in the plasma after a period of equilibration. The pool of body water is then simply deduced from the dilution rate of the tracer. Because of proton exchange between water and organic compounds, the dilution of 18O-labelled water is usually considered as the best evaluation of TBW, but the cost of 18O-enriched water is prohibitive. Isotopes are usually assayed by isotope ratio mass spectrometry (IRMS), a very expensive and time-consuming technique. The aim of this study was to evaluate the Fourier-Transform Infrared spectroscopy (FTIR) as a low-cost technique to measure total body water (TBW) by isotopic dilution of deuterium oxide (D2O). Indeed, D2O is cheaper than 18O-enriched water, and D2O assays by FTIR are quick and quite easy to perform.

TBW was measured using the isotopic dilution of D2O and 18O-enriched water. Seventeen dogs, from different breeds and weight (from 5 to 57 kg) were included in the study. After blood collection, they received a subcutaneous injection of D2O (150 mg/kg) and 18O-enriched water (40 mg/kg). Five hours later blood samples were collected and a second injection of D2O was performed (500 mg/kg). Blood was then collected five hours later. Tracers were assayed by IRMS (D2O and 18O) and FTIR (D2O).

We first made a comparison between the results obtained through IRMS and FTIR. Results did not show any difference when D2O is injected at 500 mg/kg (NS, paired Student’s t-test). However, the 150 mg/kg dose is too weak for good assay conditions by FTIR. Then, we established a correlation ($r^2=0.99$) between TBW deduced from D2O assays by FTIR and 18O assays by IRMS. The equation deduced was applied to data obtained from a new group of animals. Results show no difference between 18O-IRMS-derived TBW, and D2O-FTIR-derived TBW after this equation was applied.

Thus, TBW calculation from FTIR assays of D2O can be considered as a quick, easy-to-use and low-cost method to evaluate body composition.
ASSESSING AGE-RELATED DIFFERENCES IN LYMPHOCYTE SUBSETS AND DEFINING LIFE-STAGE CLASSIFICATIONS BASED ON IMMUNOLOGICAL STATUS IN CATS.

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Studies to assess age-related differences in feline immune phenotype in 51 Domestic Shorthaired cats (Adults; n=26 and Seniors; n=25) at the WALTHAM Centre for Pet Nutrition revealed significant differences between the two groups. The senior group had lower total leukocyte (p<0.05) and lymphocyte (p<0.001) counts. Analysis within the lymphocyte population, revealed that the overall percentage of cells staining for pan T-cell marker was similar in both groups. Within the T-cell population, the percentage of CD4+ cells was lower (p<0.001) and CD8+ cells higher (p<0.05) in the senior group, resulting in a lower CD4+:CD8+ ratio (p<0.001).

Further studies utilising 288 Domestic Shorthaired cats at the WALTHAM Centre for Pet Nutrition, ranging from 0.2 to 15.9 years confirmed these significant changes in immune parameters with age. Linear regression analysis identified a significant decrease in CD4 (R2 = 0.12, p<0.001), a significant increase in CD8 (R2 = 0.15, p<0.001), with a corresponding decrease in the CD4:CD8 ratio (R2 = 0.23, p<0.001) with increasing age. Using discriminant analysis on the CD4:CD8 ratio data we were able to define two statistically distinct groups, kittens (2 to 8 months) and adults (8 months to 15.9 years), with an overall correct classification of 77% (cross-validated). These findings are in agreement with age-related changes observed in other mammals, including humans, suggesting that these species share similar characteristics of immunosenescence. The data also show the potential for using markers of immune status for defining life-stage classifications.
Prior to the determination of the activity of antioxidant enzymes in *in vivo* samples, samples are frequently stored under varying conditions and time periods. The effect of storage conditions on antioxidant enzyme activities however are not well documented. Thus the purpose of this study was to investigate the impact of freezing samples for different time periods prior to analysis on the activity levels of two enzymes, erythrocyte superoxide dismutase (SOD) and whole blood glutathione peroxidase (GPx), under controlled conditions. These two enzymes play an important role in the primary defence system *in vivo* protecting against the potential damaging effect of excess free radicals.

Colorimetric commercial assay kits (Randox Laboratories, UK.) were validated for the determination of erythrocyte SOD (Kit No. SD125) and whole blood GPx (Kit No. RS505) in the domestic cat. The intra- and inter-assay coefficients of variation for both assays using fresh samples being <10% demonstrated an acceptable level of variation. The effect of freezing on the level of SOD activity was evaluated for 10 individual feline samples (EDTA) at 1, 3, 7, 14, 21 and 28 days after freezing. Initially, the fresh erythrocytes after isolation were washed 4 times by repeated suspension with 0.9% saline solution, and centrifugation at 3000rpm for 10 minutes at 4°C. The remaining pellet was then stored at -80°C until required for analysis. The activity level of whole blood GPx was determined in 8 feline individual samples (heparinised) when fresh and at 2, 4, 7, 14, 21, 31 and 63 days after storage at -80°C. Enzyme activity per unit of haemoglobin was determined for both SOD and GPx samples.

A significant effect of storage time on both SOD and GPx activities were observed (ANOVA P < 0.05). The SOD activity of the samples were significantly decreased after 14 days of storage at -80°C, however no significant differences were determined between the activities of the other time points measured. For whole blood GPx activity, significant differences were seen between the fresh samples and those stored for a period of 63 days, however GPx values were relatively stable with no significant differences up to 31 days of storage. These data need to be taken into consideration when evaluating the antioxidant status of cats. Clearly care needs to be taken regarding the storage time of samples without affecting the antioxidant activity of erythrocyte SOD or whole blood GPx.
ARE CHANGES IN APPARENT DIGESTIBILITY IN AGEING CATS LINKED TO
CHANGES IN FEEDING BEHAVIOUR?

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2 WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire,
UK

There is a body of evidence indicating that ageing in cats is associated with a decline in macronutrient digestibility. The aim of this investigation was to determine whether adult cats exhibit changes in their feeding behaviour as they age and whether this could be related to decreased macronutrient digestibility. In the first study 6 young cats (3.0 + 0.9 y) and 6 senior cats (11.6 + 1.4 y) were fed to appetite with a standard canned cat food for 15 d, and feeding behaviour monitored in both groups during the final 5 d. Measurements were made of the number of meals consumed daily, the amount consumed at each meal and the duration of each meal. In the second study the same 12 cats were allocated to 1 of 3 groups, each group comprising 2 young and 2 senior cats offered 3 diets enriched with beef tallow, olive oil or sunflower oil in a 3*3 Latin square design. Each diet was fed for 21 d at a level of 300kJ ME/BW0.75 and feeding behaviour was monitored during the last 5 d. In both studies no significant differences in number of meals consumed, the amount consumed at each meal or the duration of each meal were observed. Typically the older cats consumed slightly more meals per day (12-14 compared to 11-12). However, the younger cats tended to consume larger meals than the senior cats (28.98 compared to 19.05 kJ ME/kgBW0.75), which resulted in longer meal times for the younger cats (1.80 compared to 1.34 min). The rates of feeding, however, were very similar for both age groups; 19.97 and 18.13 kJ/kgBW0.75/min. This study indicates that cats of all ages exhibit a pattern of consuming several small meals throughout the day. There is no evidence that the age-related decline in macronutrient digestibility in cats is linked to changes in feeding behaviour.
The primary cause of periodontal disease is the accumulation of plaque on the tooth surfaces, which incites an inflammatory response in the periodontium (gingivitis). Gingivitis is reversible if plaque is removed from the tooth surface by for example, regular toothbrushing. This study reports the effects of daily toothbrushing on periodontal disease in cats.

Twenty-four cats were divided into two age and gender matched groups; one group received daily toothbrushing on the buccal tooth surfaces only from ten weeks of age (toothbrushing group) and the other group (non-toothbrushing group) received no form of oral care. Cats were assessed for periodontal disease at one and two years of age. Gingivitis Index (GI) was assessed on the buccal, palatal and lingual surfaces of all teeth. GI was scored using a modification of the Löe and Silness method1. A score of 0 to 4 was given for each tooth surface based on the degree of inflammation. Differences in GI between the groups were determined by statistical analysis (T-test).

Both groups of cats had low levels of gingivitis (Table 1). At 1st and 2nd assessments, the buccal GI appeared to be lower in the toothbrushing group, but this difference was not statistically significant (un paired T-test, p>0.05). There was no significant difference in the palatal/lingual GI between the toothbrushing and non-toothbrushing group. At the year 2 assessment, GI was significantly higher (paired T-test, p<0.05) in the palatal/lingual than the buccal surfaces in both the toothbrushing and non-toothbrushing groups. There was an apparent increase in the palatal/lingual GI in year 2 compared to year 1 in both groups of cats, although this difference was only significant (p<0.05) in the non-toothbrushing group (Table 1). There was a slight decrease in buccal GI in the toothbrushing group of cats between year 1 and 2, although this decrease was not significant. The buccal GI in the non-toothbrushing group remained the same for year 1 and year 2.

Toothbrushing reduced gingivitis on the buccal tooth surface to some degree, although the effect was not statistically significant. This may be because the cats are young so the level of gingivitis in the non-toothbrushing group is not high enough to detect a significant difference. Alternatively, as it is very difficult to brush cats’ teeth it is possible that the toothbrushing may not have been rigorous enough to reduce the degree of gingivitis to a low level.

Reference
1: Löe H (1967). The gingival index, the plaque index and the retention index systems. J. Periodontol 38:610-616.

| Table 1: GI Results in Toothbrushing and Non-Toothbrushing Groups |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Year 1          | Year 2          |                  |                  |
|                                 | Toothbrushing   | Non Toothbrushing | Toothbrushing   | Non Toothbrushing |
| Buccal                          | Mean 0.93 SD 0.25 | Mean 1.01 SD 0.22 | Mean 0.89 SD 0.18 | Mean 1.00 SD 0.22 |
| Palatal                         | Mean 0.98 SD 0.31 | Mean 1.00 SD 0.22 | Mean 1.10 SD 0.25 | Mean 1.12 SD 0.16 |
| Lingual                         | Mean 1.00 SD 0.22 | Mean 1.10 SD 0.25 | Mean 1.12 SD 0.16 | Mean 1.12 SD 0.16 |
Changes in the activity of the cells which form bone (osteoblasts) and cells which resorb bone (osteoclasts) can be monitored using biochemical markers measured in serum and urine. To date, there have been no studies to determine systemic levels of markers of bone turnover in cats. The first objective of this study was to assess a number of established human assays of markers of bone formation and resorption for their potential use in the cat. The second objective was to establish the relationship between markers of bone turnover with age and with the presence of Feline Osteoclastic Resorptive Lesions (FORL) of the tooth root and alveolar bone.

Serum (n = 128) and urine (n = 81) samples were collected from cats between 4 months and 14 years of age. Cats aged 1 year and older were radiographically screened for FORL at the time of serum and urine collection. Osteoblast activity was assessed by measuring bone alkaline phosphatase BAP levels in serum by ELISA [BAP(E), Alkphase B™] and by a wheat germ lectin assay [BAP(W)]. Osteoclast activity was assessed by measuring deoxypyridinoline crosslinks (DPD) in serum by ELISA [DPD(E), Total Serum Dpd™], and in urine by High-Performance Liquid Chromatography [DPD(H)], and by measuring collagen telopeptide fragment levels (b-CTX) in serum (Elcys™). Inter-assay agreement and the relationship between marker levels, age, and FORL was assessed using CMDT™ (v. 1.0b), and SPSS™ (v. 10).

Analysis showed significant correlation between the DPD(E) and DPD(H) assays (rs = 0.691, p < 0.01) and the BAP(E) and BAP(W) assays (rs = 0.872, p < 0.01). Significant correlations were not established between the b-CTX and DPD(H) assays (rs = 0.034) and between the b-CTX and DPD(E) assays (rs = 0.237). Among cats unaffected with FORL, and cats under 1 year of age, a significant inverse relationship between marker concentrations and age was observed [BAP(E) rs = -0.663; BAP(W) rs = -0.884; DPD(H) rs = -0.558; b-CTX rs = -0.437 p < 0.01]. DPD(E) was not significantly correlated with age (rs = -0.12). In general, the correlation between bone turnover marker concentrations and age were higher among cats & 2 years old than in older cats. No difference in bone turnover marker concentrations were observed between cats without FORL and those affected with the disease. Regression analysis showed no significant relationship between the number of FORL in an individual animal and bone turnover markers. ROC analysis showed that markers could not distinguish between diseased and non-diseased states.

In conclusion, the results of this study show: (i) that BAP measured by a human immunoassay or a wheat germ lectin precipitation method may be a useful marker of bone formation in the cat. DPD, measured by human immunoassay or by HPLC could potentially be used as a marker of bone resorption in cats, (ii) that bone turnover decreases with age in the cat, which is consistent with findings in other mammals, and (iii) that the presence of FORL does not appear to be associated with systemic changes in bone cell activity.
Feline Odontoclastic Resorptive Lesions (FORL) are one of the most common oral diseases in domestic cats. Teeth affected by FORL are characterised by root resorption with progressive destruction of the tooth structure and alveolar bone as a consequence of osteoclastic activity. The aim of this study was to identify potential risk factors associated with FORL in a population of ageing domestic cats housed at the WALTHAM Centre for Pet Nutrition. Also, a group of cats were assessed on more than one occasion to investigate whether the progression of FORL varies between cats.

Three hundred and eighty two (382) adult cats were assessed for the presence of FORL by means of a full clinical oral examination and intra-oral radiography. The mean age of the cat population was 4.74 years and the age range was from 5 months to 14 years (median age 4 years). The progression of FORL was also determined in 31 cats which were examined on more than one occasion, at 12, 18, 24 or 30 months after the first examination.

Teeth affected by FORL were detected in 138 of the 382 cats, resulting in a prevalence rate of 36%. The prevalence rate was found to increase with age (Figure 1); the youngest cat with FORL was 2 years old and the oldest cat with no lesions was 10 years old. FORL were identified in all teeth, but the teeth most commonly affected by FORL were the mandibular 3rd premolars and maxillary 2nd premolars.

Teeth affected by FORL were found to increase with age (Figure 1); the youngest cat with FORL was 2 years old and the oldest cat with no lesions was 10 years old. FORL were identified in all teeth, but the teeth most commonly affected by FORL were the mandibular 3rd premolars and maxillary 2nd premolars.

The progression of FORL was found to vary between cats. After 12 months cats had developed lesions in 0 to 5 more teeth. After 18 months, cats had developed lesions in 2 to 12 additional teeth, after 24 months, cats had developed lesions in 1 to 3 more teeth, and after 30 months, cats had developed lesions in 0 to 8 additional teeth. The progression of FORL varies between individual cats, however, the reasons for this difference remain unclear and warrant further investigation.
RICE BRAN DECREASES PLASMA AND WHOLE BLOOD TAURINE IN CATS
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Taurine is a sulfur containing amino acid that is required in the diet of cats. Although cats can metabolize a limited amount of taurine from cystine, they require taurine for the conjugation of bile acids. Without adequate dietary supplementation, the amount of taurine lost in the feces as fecal bile acids will exceed the amount of taurine that is formed, and the animal will become taurine deficient. Rice bran, a common ingredient in commercial pet foods, is moderately soluble, contains a higher amount of fat than other bran products, and increases the excretion of fecal bile acids in rats (Gestel et al., 1994). It is expected that cats will have an increased taurine requirement when they are fed products containing whole rice or rice bran, and that additional taurine supplementation will be required in these diets. This study was performed to determine if a purified diet that contains 26% rice bran (dry matter (DM) basis) alters taurine metabolism and increases taurine requirements in cats.

Sixteen young adult male specific-pathogen-free cats were randomly assigned to two study groups, and were fed a purified diet with 0.5g/kg dietary taurine in which 26% DM of corn starch (C), was replaced with 26% DM full fat stabilized rice bran (RB). Food intake, and animal weights were measured throughout the duration of the project. Whole blood and plasma samples were obtained before the start of the project and during weeks 1, 2, 4, 6, 8, 12, 16 and 22. Blood was collected by jugular venipuncture in heparinized syringes, and whole blood was separated for taurine analysis. Plasma was obtained after centrifugation of the heparinized blood sample at 10,000 x g for 15 minutes. The plasma was immediately deproteinized with an equal volume of 60g/L sulfosalicylic acid and centrifuged at 10,000 x g for 15 minutes at 4°C. Whole blood and plasma samples were frozen at –70°C until analysis. Whole blood and plasma taurine analysis was performed using an amino acid analyzer (Model 121-M amino acid analyzer). Statistical analysis was performed with a one-way ANOVA using SYSTAT.

The mean plasma taurine at the start of the project was 156.3 + 12.9 nmol/ml (mean + SEM) for the RB group, and 113.4 + 10.7 nmol/ml for the C group (p=0.23). Plasma taurine concentrations were lower in the RB group cats after 6 weeks, with a mean plasma taurine of 34.0 + 5.2 nmol/ml versus 49.0 + 4.5 nmol/ml in the C group (p=0.03). Plasma taurine levels in the RB cats remained significantly lower than the C group cats throughout the remainder of the sampling period. At 22 weeks plasma taurine in the RB group was 12.4 + 1.8 nmol/ml while the C group plasma taurine was 50.8 + 7.71 nmol/ml (p<0.001). Mean plasma taurine in the C cats decreased to deficient levels after 8 weeks (27.7 + 3.6 nmol/ml), however after week 12 the levels were again in the normal range. This result was expected because taurine from the reserve pools in skeletal muscle will move into the plasma pool to increase the plasma levels of taurine (Pacioretty et al., 2001). A significant difference in whole blood taurine was measured between the RB (162.9 + 18.9 nmol/ml) and the C (363.4 + 29.3 nmol/ml) cats at 22 weeks (p<0.001). Based on the whole blood and plasma taurine concentrations measured in this study, rice bran fed at 26% dietary dry matter, causes taurine deficiency in cats.

References
EFFECT OF PROCESSING OF DIETARY PROTEIN ON LYSINE BIOAVAILABILITY IN GROWING KITTENS
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Lysine is one of several essential amino acids with chemical characteristics that increase sensitivity to moist heat processing damage, and this leads to a decreased bioavailability relative to other amino acids. The purpose of this study was to examine the relationship between lysine bioavailability and growth response in kittens fed heat damaged casein and to validate the growth assay method for quantifying amino acid bioavailability in the kitten.

Sixteen male 7 to 9 week old specific-pathogen-free domestic shorthair kittens were sorted by age and weight and were assigned as pairs to one of eight dietary groups in a Latin square design, with each pair of kittens rotating through all dietary treatments, one at a time, for eight 10 day periods. Throughout the 80 day study period, the kittens were offered food and water ad libitum and body weights and food intakes were measured daily. All diets supplied crystalline amino acids in excess of requirements (except for lysine). Casein was included in some diets either as the unadulterated powdered form, or as a product treated to simulate processing damage. Casein was damaged by mixing with 5% dextrose and autoclaving for 2 hours at 121 degrees Celsius. Experimental diets supplied graded levels of lysine at 4, 5.5, and 7 grams per kilogram of diet (diets 6, 7, and 8 respectively). Processed or unheated casein was added to two lysine free formulations (diets 4 and 2, respectively) and to two complete formulations (lysine was supplied at 16 grams per kilogram of diet, diets 3 and 1, respectively) at 80 grams per kilogram of diet. Diet 5 contained crystalline amino acids as the sole nitrogen source, and together with diets 1 and 3 served as controls. The casein added to lysine free diets 2 and 4 provided the only lysine in the diet, and this allowed determination of the bioavailability of lysine for supporting growth in the kittens.

Statistical significance was set at p<0.05. Least squares mean daily gains and food intakes for control groups (diets 1, 3, and 5) were not significantly different from each other. Addition of heated casein to diets as the sole source of lysine resulted in significantly lower average daily gains compared to untreated casein (diet 4, 2.69 grams per day and diet 2, 14.9 grams per day, respectively). The casein used in the experiment contained 7.85% lysine. Linear regression analysis of the growth data from kittens fed diets 6, 7, and 8 (R2=0.985) allowed for estimation of the amount of bioavailable lysine supplied by casein in diets 2 and 4. At equal weight gains, the ratio of the amount of lysine in the purified diet to the amount of lysine in the casein diet was taken as the bioavailability of lysine in casein for the growing cat. Lysine bioavailability was calculated as 56.5% and 96.2% in the processed and untreated casein, respectively.

The addition of heated or untreated casein had no effect on the growth response or food intake of the kittens when added to the basal amino acid diets. Heat damaged casein showed a decreased bioavailability of lysine in the kittens. The growth assay method of estimating amino acid bioavailability is a satisfactory technique for use in the cat.
The role of iodine in feline hyperthyroidism, which is the most common endocrine disorder of middle-aged to old cats in the USA, is discussed by several authors. Johnson et al. (1992) report a wide range of iodine concentrations in cat foods purchasable in the USA and New Zealand. Therefore, we determined the iodine content of commercial cat foods available in Germany. We also fed different amounts of iodine to cats in a feeding study and measured iodine excretion in urine and faeces to provide information about iodine balance and to find out whether urinary iodine excretion is an accurate indicator of dietary iodine intake in cats.

Concerning the iodine content, there are differences in iodine concentration of up to thirty fold among commercially-prepared complete foods for cats available in Germany (218 – 6356 µg iodine/kg dry matter).

Feeding study: eight adult, healthy cats (European Shorthair) kept separately, were fed diets containing known amounts of iodine (six feeding periods of seven days each: 0, 12, 25, 50, 75, 150 µg iodine/kg body weight/d in addition to their normal diet containing 40.8 µg iodine/kg BW/d). Samples (urine and faeces, 24h each) were taken on the last day of each period. Iodine analysis (method: Sandell-Kolthoff) was performed on samples of urine and faeces.

Urinary iodine excretion as determined by the 24 h collections in the feeding study is highly correlated with alimentary iodine intake (R=0.969; p<0.001). Therefore, we suggest that the estimation of iodine intake in cats can be made by measuring urinary iodine excretion. Whereas faecal iodine excretion did not depend on the amount of iodine consumed in the diet: During the complete study an average of 13 ± 4 µg iodine/kg BW/d was determined.

Reference

Cats are believed to have evolved on a diet consisting mainly of animal tissues and as a result have developed a specialised metabolism like other true carnivores such as mink and ferrets. Urine characteristics are directly related to the diet of cats (Burger and Smith, 1987) and as a result it is difficult to establish ‘normal’ reference values. One approach to obtain ‘normal’ biological reference values is to determine the composition of feral cat urine, as these animals are ingesting a ‘natural diet’. Characterising the composition of feral cat urine will provide base line data to evaluate the urinary characteristics of domestic cats fed commercial petfoods.

Standard predator trapping techniques were used to catch a total of 85 adult feral cats in four areas, two in the lower North Island and two in the upper South Island of New Zealand. The cats were killed and urine was manually expressed immediately upon death. pH was immediately determined using a calibrated mobile pH-meter whereafter each cat was weighed. The urines were frozen, transported to the laboratory and analysed in duplicate for specific gravity, osmolality, and protein, urea, ammonia, magnesium, calcium and creatinine using commercially available kits. Data were analysed using ANOVA.

The average body weight (mean ± SEM) of the 53 adult male feral cats was 3.76 ± 0.19 kg (range 2.30 to 5.50), significantly heavier (P<0.001) than the 32 adult females at 2.86 ± 0.11 kg (2.00 to 3.85). Table 1 presents the measured urine compositional data for 71 cats. Significant differences were observed between males and females for urine pH and creatinine. Expressing the results on a creatinine basis, significant differences were observed for magnesium (P<0.05), with specific gravity, osmolality, calcium and total nitrogen all tending towards significance (P<0.10), with females having higher levels compared to males.

### Table 1: Urine characteristics and composition of male and female feral catsa

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>Range</td>
</tr>
<tr>
<td>pH</td>
<td>6.38±0.09*</td>
<td>5.73 – 7.39</td>
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<tr>
<td>Specific gravity (g/ml)</td>
<td>1.048±0.002</td>
<td>1.017 – 1.065</td>
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<tr>
<td>Osmolality (mOsm/kg)</td>
<td>2062±104</td>
<td>665–3178</td>
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<tr>
<td>Creatinine (mM)</td>
<td>16.6±1.1*</td>
<td>4.8–32.3</td>
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<tr>
<td>Total nitrogen (mM)</td>
<td>3050±194</td>
<td>634–5411</td>
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<tr>
<td>Protein (mg/l)</td>
<td>380±36</td>
<td>30–1152</td>
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<tr>
<td>Urea (mM)</td>
<td>1394±90</td>
<td>253–2340</td>
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<tr>
<td>Ammonia (mM)</td>
<td>106±9.3</td>
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<tr>
<td>Calcium (mM)</td>
<td>0.67±0.09</td>
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<tr>
<td>Magnesium (mM)</td>
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<td>0.65–7.22</td>
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</table>

*a=n=45, 26 (male, female) except for ammonia and Mg (30, 21).
+ = P<0.10, * = P<0.05.

Reference

The purpose of this study was to further validate the multifrequency bioelectrical impedance analysis (MF-BIA) method for the assessment of total body water (TBW) and extracellular water (ECW) in healthy cats. Fifty six adult domestic shorthair cats (35F, 5FS, 11M, 5MC) were evaluated after food and water had been withheld for 24 hours. Body weight, body condition score (5-point system), and morphometric measurements (scapula height, pelvic height, head to tail, nose to tail, and eye to tail) were recorded. Venous blood samples for deuterium and bromide were obtained prior to and 90 minutes following intravenous injection of 0.4g/kg BW of D2O and 30 mg/kg BW of NaBr. Concentrations of deuterium were determined using Fourier transform infrared spectrophotometry and bromide concentrations were assessed by high pressure liquid chromatography. Each cat was anesthetized with ketamine and diazepam, to effect. Ten simultaneous MF-BIA spectral data measurements were recorded at 50 frequencies (5kHz to 1000kHz) from three electrode configurations (the occipital protuberance and lumbosacral junction [body], the right elbow and right knee [ipsilateral], and the left elbow and right knee [contralateral]) with the cat positioned in both sternal and left lateral recumbency using intradermal tetrapolar platinum electrodes. Path length between the tetrapolar electrodes was recorded for each of the lead configurations. Impedance spectral data were fit to an enhanced version of the Cole-Cole model. ECW and intracellular water (ICW) volumes were predicted using model electrical resistance terms RE and RI in equations derived from Hanai mixture theory. Descriptive statistics, Pearson’s product moment correlation (r), least square linear regression, and standard error of estimate (SEE) were computed. Bland-Altman plots were constructed to display differences between the dilution and MF-BIA methods. All data are expressed as mean ± SE unless otherwise stated. A p-value <0.05 was considered significant.

The mean age of the 56 healthy cats was 4.40±0.38 years, mean body weight was 4.69±0.19 kg, and mean body condition score was 3.5±0.1. There was a significant difference using the sternal ipsilateral path length MF-BIA predicted TBW and the TBW determined by deuterium oxide dilution. There were no significant differences between the remaining MF-BIA predicted TBW at any configuration and the TBW determined by deuterium oxide dilution. The MF-BIA configuration with the smallest SEE for the prediction of TBW compared to deuterium space was the left lateral recumbent body path length configuration (r = 0.86, SEE = 0.24L). The difference between deuterium dilution and MF-BIA TBW was 0.16±0.04 L, and the limits of agreement (mean difference ± 1.96 SD) were -0.41 to 0.74 L. There were no significant differences between the MF-BIA predicted ECW and that determined by bromide space. The MF-BIA configuration with the smallest SEE for ECW prediction of bromide space was the left lateral recumbence body path length configuration (r = 0.93, SEE = 0.07L). The difference between bromide space and MF-BIA predicted ECW was -0.003 ± 0.01L, and the limits of agreement were -0.17 to 0.17L. The results of this study confirm that MF-BIA is a valid method to assess TBW and ECW in healthy cats without the need for laborious dilution techniques.
EVALUATION OF MULTIFREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS FOR THE ASSESSMENT OF EXTRACELLULAR WATER AND TOTAL BODY WATER IN HEALTHY CATS
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Multifrequency bioelectrical impedance analysis (MF-BIA) is an electrical method of assessing body composition that has the potential to quantify total body water (TBW), extracellular water (ECW), and intracellular water (ICW), and therefore enable prediction of fat free mass (FFM), fat mass (FM), and body cell mass (BCM). The purpose of this study was to develop scaling constants and assess the effect of animal position, animal length measurement, and electrode configuration on the volume prediction accuracy of the Hydra ECF/ICF Bio-Impedance Analyzer (Model 4200, Xitron Technologies, San Diego, CA) compared to TBW estimated by deuterium water space and ECW estimated by bromide space.

Twenty adult domestic shorthair cats (5F, 5FS, 5M, 5MC) were evaluated after food and water had been withheld for 24 hours. Body weight (BW), body condition score (5-point system), and morphometric measurements (scapula height, pelvic height, head to tail, nose to tail, and eye to tail) were recorded. Venous blood samples for deuterium and bromide were obtained prior to and 90 minutes following intravenous injection of 0.4g/kg BW of deuterium oxide and 30 mg/kg BW of sodium bromide. Concentrations of deuterium were determined using Fourier transform infrared spectrophotometry and bromide concentrations were assessed by high pressure liquid chromatography. Each cat was anesthetized with ketamine and diazepam, to effect. Ten simultaneous MF-BIA impedance spectral data measurements were recorded at 50 frequencies (5kHz to 1000kHz) from three electrode configurations (the occipital protuberance and lumbosacral junction [body]; the right elbow and right knee [ipsilateral]; the left elbow and right knee [contralateral]), with the cat positioned in both sternal and left lateral recumbency using intradermal tetrapolar platinum electrodes. Path length between the tetrapolar electrodes was recorded for each of the lead configurations. Impedance spectral data were fit to an enhanced version of the Cole-Cole model. ECW and ICW volumes were predicted using model electrical resistance terms RE and RI in equations derived from Hanai mixture theory. Descriptive statistics, least square linear regression, Pearson’s product moment correlation (r), and standard error of the estimate (SEE) were computed. Bland-Altman plots were constructed to display differences between the dilution and MF-BIA methods. All data are expressed as mean ± SE unless otherwise stated. A p-value < 0.05 was considered significant.

The mean age of the 20 healthy cats was 5.51 ± 0.69 years, mean body weight was 5.28 ± 0.24 kg, and mean body condition score was 4.0 ± 0.2. There were no significant differences between the lead and height configurations on the MF-BIA predicted TBW or ECW. The MF-BIA TBW (sternal contralateral path-length) prediction of deuterium space was r = 0.84, SEE = 0.26 L. The difference between deuterium oxide dilution and MF-BIA TBW (sternal contralateral path-length) was -0.0001 ± 0.06 L, and the limits of agreement (mean difference ± 1.96 SD) were -0.50 to 0.50 L. The MF-BIA ECW (sternal body head to tail) prediction of bromide space was r = 0.91, SEE = 0.07 L. The difference between bromide space and MF-BIA ECW (sternal body head to tail) was 0.0001 ± 0.02 L, and the limits of agreement were -0.16 to 0.16 L. MF-BIA is a practical, safe, non-invasive, rapid, and portable technique to assess TBW and ECW in healthy cats eliminating the need for laborious dilution techniques.
BREATH HYDROGEN RESPONSES OF CATS GIVEN COMMERCIAL DRY AND CANNED DIETS INDICATE INTESTINAL MICROFLORAL ACTIVITY VARIES WITH DIET TYPE.

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Inflammatory bowel diseases are most commonly observed in middle-aged and old cats and are suggested to result from hypersensitivity reactions to bacterial antigens. Research on dietary taurine requirement of cats indicates that intestinal microbial growth and metabolic activities vary with diet composition and processing. Therefore, predisposition of cats to developing inflammatory bowel diseases may be increased by dietary factors which induce microbial growth. As an initial step to investigate this possibility, breath hydrogen concentration, an indicator of abundance of intestinal microbes, was measured in cats given diets expected to variably promote intestinal microbial growth. All cats were adapted to diets for at least two weeks and trained by restricted-interval feeding (4 hr/day) to consume within 20 min of diet presentation approximately 25% of their maintenance energy requirement. Breath hydrogen concentrations as measured with a dedicated gas chromatograph (Quintron, Model 12) were determined before a test meal (4-6 g diet dry matter/kg body weight) and then every 20 min for 8 hr or hourly for 10 hr. A clear rise above baseline hydrogen concentrations, 1-2 ppm, was not observed in 6 male cats given a casein-based purified diet. Whereas, in 6 other male cats given a commercial canned diet containing macronutrient proportions similar to the purified diet, a mean (± SEM) peak concentration of 22 ± 4 ppm was observed at 6.3 hr following test meal ingestion. Mean (± SEM) area-under-the-curve (AUC ppm×hr) breath hydrogen response to the canned diet (69 ± 8) was substantially greater (p<0.05) than responses to the purified diet (14 ± 5) and a standard commercial dry-type diet (21 ± 6, n=5). The response to the canned diet was not significantly different than responses to uncooked forms of the canned diet with (57 ± 10, n=6) and without gamma irradiation treatment (54 ± 18, n=6) to inactivate microbes present in ingredients. Breath hydrogen responses to 2 canned and 2 dry popularly-fed, AAFCO-tested all stages, premium and standard diets were determined in 8 adult female cats using a 4 x 4 Latin-square design. AUC and peak responses to the canned diets were similar and approximately 2 times greater (p<0.05) than responses to the dry diets. Relative to dry diets, canned diets although typically low in carbohydrate induce a greater breath hydrogen production and therefore appear to support a greater intestinal microfloral population. Variations in the magnitude and timing of peak breath hydrogen responses to the canned diets examined might have resulted from differences in protein digestibilities and interactions with agents used in gel formation, such as rice flour, soy flour, corn starch, or carageenan.
IN VITRO FERMENTATION CHARACTERISTICS OF MANNAN OLIGOSACCHARIDES BY DOGS AND CATS
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An in vitro experiment was conducted to determine the fermentation characteristics of mannan oligosaccharides (MOS) by dog and cat colonic bacteria. A total of 192 50-mL centrifuge tubes (capped with stoppers equipped with one-way gas release valves) were used. Of these tubes, 96 were without [blanks; to correct for inoculum contribution] and 96 were with the substrate (0.5 g of MOS dry matter [DM]). Four dogs and four cats (donors) were fed diets containing fiber for 2 wk before their fresh feces were used to prepare the inocula for the in vitro (anaerobic) fermentations (Sunvold et al., 1995). The tubes were incubated at 39°C for 6, 12, 18, or 24 h and processed for determination of DM and organic matter (OM) in the residue (AOAC, 2000) and volatile fatty acids (VFA; Erwin et al., 1961) and lactate (Barker and Summerson, 1941) in the supernatant. The experimental design was a completely randomized design with treatments being arranged as a 2 × 4 factorial (Steel et al., 1997). Data were analyzed by using the General Linear Model Procedures of SAS (SAS, 1999) and the linear, quadratic, and cubic effects of incubation time were tested by using orthogonal contrasts (Steel et al., 1997).

No interactions (P > 0.05) between species and incubation time were detected for any of the measurements evaluated. There were no differences (P > 0.05) between dogs and cats in digestion of MOS (averaging 58.6 and 61.3% for DM and OM, respectively). Digestion of DM (54.3, 57.9, 60.7, and 61.3%, respectively) and OM (56.8, 60.7, 63.7, and 64.1%, respectively) increased linearly (P < 0.05) with extending incubation time. Concentrations (mM) of acetate (21.4 vs 16.9), propionate (8.4 vs 4.5), total VFA (35.0 vs 26.1), and lactate (8.3 vs 6.7) were higher (P < 0.05) for dogs than for cats. The proportions of lactate in the total acids produced, however, were not different (P > 0.05) between dogs (19.1%) and cats (20.4%). Extending incubation time increased (P < 0.05) concentrations of acetate (7.7, 18.8, 22.6, and 27.6 mM, respectively), propionate (0.9, 4.0, 7.3, and 13.8 mM, respectively), and total VFA (10.1, 26.8, 36.7, and 49.7 mM, respectively) linearly. The response of lactate concentrations (7.7, 8.7, 7.6, and 5.9 mM, respectively) to extending incubation time, however, was quadratic (P < 0.05). Results indicate that MOS is highly fermentable in the colon of dogs and cats. Because MOS fermentation produced significant amount of lactate (20% of total acids), results suggest its prebiotic potential for dogs and cats.

References
HOW DOES AGE INFLUENCE OBJECT PLAY IN DOMESTIC HORSES?

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It is recognised that play is more prevalent in juveniles than adults of many species of animal, including the domestic horse (Equus caballus). Play has also been reported to change during the development of foals. Play behaviour is similar between colts and fillies for the first month after birth, but after this time play differs markedly between colts and fillies as play fighting becomes more prevalent in colts. Levels of solitary play are reported to drop dramatically two months after birth. Object play has not previously been extensively studied, so the aim of the following studies was to gain a better understanding of this behaviour of the domestic horse.

A study of ten foals (six males and four females of mixed breeds) was conducted to determine how object manipulation and play, as well as social play develop over the first three months of life in the domestic horse. Each foal was filmed for 30 minutes on alternate days from two days of age until three months of age. A Jolly Ball (Horseman’s Pride, Ravenna, OH, USA) was placed in the field or the stable during observations to ensure that all foals had the opportunity to exhibit object play. Exhibition of object manipulation and play, as well as social play was recorded. Due to differences in management conditions and the differences in breed specific behaviour each foal was analysed as a single case. Object play was significantly greater in the first and/or second month of life than in the third month for five of the ten foals studied (P<0.05). This suggests that object play may follow the same development pattern as other solitary play.

Object play in adult domestic horses has not previously been studied. A study was conducted with 12 adult horses (six male and six females of mixed breeds, between 6 and 20 years of age) to test object stimuli associated with eliciting object play and manipulation. Five sacks constructed of different materials (bubble wrap, textured surface and smooth surface; anaglypta wallpaper, textured surface and smooth surface; brown paper and cotton). The horses were introduced to the sacks for five minutes in a stable containing only wood-shaving bedding and the horses behaviour was recorded on videotape. Object play was only exhibited twice throughout the study and levels of object manipulation were low (average 1.06% of observation time).

Due to the low levels of object play observed, the above study was repeated using a population of mainly juvenile horses (two females aged two years, one male and one female aged three years, one female aged five years and one female aged 20). Significantly higher levels of object manipulation and play were observed in this population (U=535, P<0.001), than the previous adult group. The aged horse in this population showed no object play during the trial.

These studies suggest that object manipulation and play change during aging in the domestic horse. After the first two months of life exhibition of object manipulation and play begins to decrease. Also, juvenile horses appear to display greater levels of object manipulation and play than adult horses. This could have implications for the use of object manipulation and play and environmental enrichment for the domestic horse.
IODINE BALANCE IN RELATION TO IODINE INTAKE IN PONIES
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Iodine balance trials (iodine intake: 3, 23, 43 and 83 µg iodine/kg body weight/day) were performed with 4 ponies (2 mares and 2 geldings, body weight 230-360 kg, 4-11 years). The duration of each period was 14 days, samples of blood and urine were taken every 7 days. Six hours after feeding, blood samples were taken. At the same time urine samples were taken, as spot-urine in the geldings and via catheter in the mares (urine quantification by creatinine). 24 hours feces were collected every 14 days.

The renal and fecal excretion of iodine were determined. The hormones of the thyroid T3, T4, FT3 and FT4, as well as the protein-bound iodine in serum were determined.

To determine the iodine content of feed and feces a modified analytical method was used. This method is based on an alkaline ashing procedure and a subsequent iodine determination using the Sandell-Kolthoff-Reaction. After precipitation of the protein-bound iodine, the same method was used to determine iodine in serum. For the urine samples, a WHO recommended method (1) for the iodine analysis of human urine was used. This method was slightly modified to accommodate a wider range, so it supplied reliable results for the iodine content of equine urine. The accuracy of our methods was assessed by neutron activation analysis (NAA).

The majority of iodine was excreted via urine, the renal excretion was nearly equivalent to the iodine intake. A significant correlation was found between the iodine intake and the renal excretion of iodine (R=0.912; p<0.001). Therefore, the renal excretion of iodine is considered to be a suitable parameter to estimate the iodine supply of ponies.

In contrast to dogs (2) and cats (3), in ponies the iodine fecal excretion increased slightly with higher intake.

The thyroid hormones T3, T4, FT3, FT4 and the protein-bound iodine in serum did not change in relation to the iodine uptake.

Fecal and renal endogenous losses calculated by regression analysis are in a similar range as the recommendations (4).

References
In the last years in horse nutrition zinc was discussed in connection to hoof horn quality. In this context the bioavailability of different zinc compounds became interesting. In the present investigation the serum response after oral intake of different zinc compounds was investigated. Four ponies (body weight between 200 and 350 kg) were fed with a hay/oats diet with a low zinc content (27 mg/kg dry matter). The following zinc compounds were tested in each pony in a latin square experimental design in two single dose experiments with 10 mg and 20 mg zinc/kg body weight (BW), respectively: zinc oxide, zinc sulphate, and a zinc sulphate glycin chelate. Zinc lactate was given only as a 10 mg/kg BW single dose. Blood samples were taken before and 2, 4, 6, 8, 10 and 24 hours after the zinc dose. Zinc was determined by atomic absorption spectrometry. In both experiments the zinc plasma content increased significantly and dose dependantly when zinc was applied either as zinc sulphate or as zinc sulphate glycin chelate (table 1) indicating a higher bioavailability of these compounds compared to zinc oxide or lactate.
A METHOD TO ESTIMATE DIGESTIBLE ENERGY IN HORSE FEED

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Predictive equations for digestible energy (DE) of horse feed based on crude nutrients are often only valid under limited conditions, however, in general these conditions are not clearly defined. This may lead to considerable errors. Important points in this context are i) multiple co-correlations between nutrients and energy may limit the validity of equations derived by multiple regression analysis in rations which are somewhat different from those which were applied to derive the equations ii) in rations for horses crude fiber encompasses a multitude of substances with a very different digestive physiology. Detergent fiber would be much more precise (Zeyner, 1995), however, in practice there are not enough data, and detergent fiber is not declared on mixed feed iii) the situation is further complicated by interactions between nutrients. For instance the digestibility of straw may be increased when it is given in combination with concentrates. The exchange of carbohydrate for fat may lead to an increase in digestibility whereas very high fat addition may decrease digestibility again. The present study was designed to calculate a predictive equation that uses crude nutrients and is valid for a large number of practical rations and for most mixed feed and to define clearly the limits of its validity. This concept was applied to a total of 199 observations on apparent energy and nutrient digestibility (120 from Leipzig, 16 from Munich and 63 from literature, see Fehrle, 1999; Kaden, 2000; Zeyner, 2001). Because it is impossible to estimate digestibility of fiber based on crude nutrients mean fiber digestibility (47 %) was used. Consequently the range of application of the equation was limited to rations with a crude fiber content below 35 % dry matter (dm). Rations with more then 8 % crude fat in dm were also excluded from our calculations because of a possible decrease of digestibility. Crude protein, crude fat and N-free extract were tested for nutritive uniformity by the Lucas-test (r = 0.908***, 0.966*** and 0.809***) before their digestibility was estimated by the content of each nutrient. Estimated digestibility and heat combustion of crude nutrients were then used to establish a target function. The resulting predictive equation DE [MJ/kg dm] = -3.66 + 0.211 protein + 0.421 fat + 0.015 fiber + 0.189 N-free extract [crude nutrients in % dm; r = 0.992***, ±s = 1.236] can be applied to either rations or mixed feed or single feed stuffs and so can be used for additive diet calculation on condition that – in rations - the crude fiber content does not exceed 35 % in dm and fat does not exceed 8 % in dm. DE in fat supplemented diets with a crude fat content between 4 and 8 % in dm and in mixed feed which are part of such rations may be slightly underestimated. Further research should explore the possibility of a mathematical description of the interaction between fat addition and crude nutrient digestibility.

References:
Beta-carotene is used in the feeding of mares to improve fertility. It may also be used as a potential source of vitamin A in high-quality mixed feeds or supplements in order to prevent excessive vitamin A intake in horses fed high amounts of such feeds. However, the bioavailability of synthetic β-carotene for horses has been questioned. In the present investigation the serum response after the intake of β-carotene either from grass meal or a synthetic beadlet preparation (Lucarotin™) added to a balanced ration was compared in four β-carotene depleted adult ponies. Both carotene sources were each given in an amount of 0.8 mg β-carotene/kg body weight with or without added dietary vegetable fat (2.5 and 4 % crude fat in dry matter, respectively) in a latin square design. Each feeding period was followed by a wash-out period of 4 weeks with low intakes of β-carotene (0.4 mg/kg body weight). Serum β-carotene, cholesterol and triglycerides were analysed after 1 and 4 weeks in each test and wash-out period. Fecal recovery of β-carotene was determined, and random samples of urine were analyzed for β-carotene. Repeated measure analysis was used to test for the homogeneity of serum responses over time, and the paired T-test was used to quantify significant differences. Within 4 weeks of supplementation, serum β-carotene increased about 10-fold from a mean initial concentration of 0.05 µMol/L to 0.53 µMol/L, the response being significant from week 1. There was no effect of β-carotene source and of fat addition, respectively. Fecal excretion of β-carotene ranged from 55 to 81 % of intake. No β-carotene was detected in any urine sample. Overall serum cholesterol was 2.75 µMol/L and was not affected by carotene source. However, fat addition resulted in a significant decrease of serum cholesterol. Serum triglycerides were not affected by carotene nor by fat addition.

<table>
<thead>
<tr>
<th>zinc dose mg/kg BW</th>
<th>control</th>
<th>zinc oxide</th>
<th>zinc sulphate</th>
<th>zinc lactate</th>
<th>zinc sulphate chelate</th>
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<tr>
<td>10</td>
<td>860±220</td>
<td>100±360</td>
<td>2080±800*</td>
<td>1810±630</td>
<td>2120±670*</td>
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<tr>
<td>20</td>
<td>721±106</td>
<td>1136±436</td>
<td>4134±1323*</td>
<td>-</td>
<td>4895±1497*</td>
</tr>
</tbody>
</table>
SUPPLY WITH THE TRACE ELEMENTS ZINC, COPPER AND SELENIUM IN HORSES IN BAVARIA
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The supply with trace elements in 106 horses from 13 farms in Bavaria was investigated. The horses belonged to different breeds: Thorough-bred, Standard-bred, Arabian horse, Quarter horse, Icelandic pony, Shetland pony, Connemara Pony, Tinker pony, Andalusian horse, Huzule horse, Lusitanian horse, Peruvian Paso, Trotter, Draft horse and Haflinger. Some of the horses were kept for breeding, others for competing but the majority was kept as a hack. The 49 mares, 41 geldings and 16 stallions were aged between less than one and more than twenty years. Blood samples and samples of fodder were taken twice (once in summer and once in winter). Samples of hair and hoof horn were taken once. All samples were analyzed for zinc, copper and selenium. Trace element intake of all horses was calculated from analyzed content in feed and compared to the requirements. Many horses ate diets very low in zinc and selenium. About 25% of the horses got less than half of the recommended zinc intake of 1 mg/kg BW/day. 52% of the horses ate less than half of the recommended selenium intake of 2.5 µg/kg BW/day. Copper intake met the requirements of 0.1 mg/kg BW/day in 85% of the horses. This was even the case in some diets which contained mineral supplements and mixed feed. The selenium plasma level with an average of 76±34 µg/l was clearly lower than the references (100-250 µg/l for adult horses). Plasma levels of zinc and copper as well as the content of all three elements in hair and hoof horn were similar to those reported hitherto in horses without known nutritional deficiencies. There were no specific clinical signs of trace element deficiencies in any of the horses participating in the study. The results invite speculation whether the safety margin of the requirements especially for zinc and selenium content in diets for adult horses are excessive.
INTERACTIONS BETWEEN MIXED FEED AND ROUGHAGE ON APPARENT ENERGY AND NUTRIENT DIGESTIBILITY IN HORSES

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Interactions between roughage and concentrates such as improvement of roughage digestion after addition of mixed feed have been described in horses. However, so far only few quantitative data are available on such effects. In the previous study interactions between roughage and concentrates were investigated in four adult ponies. Grass meal was either fed alone or in combination with either mixed feed, or hay or straw. The mixed feed was also fed in combination either with hay or straw. Each experiment consisted of a 5 day adaptation period and 10 days of feces collection.

Apparent digestibility of energy (ad GE %) was highest when grass meal and mixed feed were combined (ad GE 76.9 % ). The combination of hay and mixed feed (ad GE 71.6 %) and grass meal fed alone (ad GE 68.5 %) were also highly digestible. Digestibility decreased when mixed feed was combined with straw (ad GE 60.2 %) or grass meal with hay (ad GE 62.4 %) or hay alone (ad GE 63.1 %). The combination of grass meal with straw had a rather low digestibility (ad GE 53.3 %). Straw fed alone (only eaten by two ponies) showed a very low energy digestibility (ad GE 29.4 %). Assuming a constant digestibility of straw, hay or grass meal the respective digestibility of the feed stuffs used in combinations were calculated by difference. This resulted in a digestibility by difference of more than 100 % for the mixed feed in combination with straw, a result that indicates an enormous improvement of straw digestibility by combining it with mixed feed. A similar but quantitatively smaller effect was observed for grass meal in combination with straw (ad GE by difference 92.1 %, ad GE grass meal fed alone 68.5 %). When it was calculated other way round with a constant digestibility of grass meal the digestibility of straw by difference amounted to 56.4 % compared to 29.4 % when fed alone. Interactions between concentrates and hay were much less marked. These results indicate that in energy evaluation and ration calculation for horses interactions between feed stuffs should be taken into account.
Anti-oxidant status has been implicated as a modulator of inflammation. We have recently demonstrated that ascorbic acid (AA) is quantitatively the most important antioxidant in equine pulmonary lining fluid and that both pulmonary and plasma concentrations of AA are reduced in horses affected by recurrent airway obstruction or heaves (formerly known as equine chronic obstructive pulmonary disease (COPD)). The plasma bioavailability of AA from the diet has been previously demonstrated, but there appear to be no reports in the literature of pulmonary bioavailability of dietary AA.

Six healthy ponies free of respiratory disease on the basis of endoscopy of the respiratory tract and cytological and bacteriological analysis of tracheal wash and bronchoalveolar lavage (BAL) were studied in a 3 x 3 Latin square design. Ponies were stabled in pairs and fed a diet of haylage to maintain bodyweight and condition. The ponies were allowed access to paddocks whilst muzzled during the day. Ponies were studied in stable pairs and each pair received three treatments: 1) Control (C); 2) Ascorbyl palmitate (AP); 3) Stabilised ASCORBIC ACID (SAA). Doses chosen to ensure equivalent activities of ascorbic acid were fed. Each treatment lasted two weeks and was followed by a washout period of two weeks before the next treatment period. Every two weeks BAL was performed in the right and left lung. On the day following BAL, blood samples were collected before and every hour following feeding up to 8 hours to determine plasma bioavailability.

Both AP and SAA produced elevations in plasma AA concentration similar to those reported previously in the literature. Peak plasma AA concentrations following feeding of AP or SAA were seen at around 6-8 hours post-feeding. There was no change in plasma AA over the corresponding period in the control treatment. Data for concentrations of AA in lung lining fluid will be presented.
THE SOMATOTROPIC AXIS IN THE THOROUGHBRED YEARLING: DIETARY INFLUENCES ON PLASMA INSULIN-LIKE GROWTH FACTOR I

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Equine Studies Group
3WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, U.K.

Developmental orthopedic (DOD) disease is a major source of economic loss to the equine industry (Jeffcott and Henson, 1998). Osteochondrosis, one form of DOD, is characterized by the abnormal maturation in the epiphyseal growth plate. Insulin-like growth factor-I’s (IGF-I) role in the normal maturation of growth and articular cartilage has been well documented (Olney and Mougey, 1999). The objective of this study was to determine if the composition of a pasture supplement designed to increase energy density of the ration would affect plasma levels of IGF-I.

Twenty-four Thoroughbred foals were fed one of two supplements differing in carbohydrate profiles for 16 months. The first (SS) contained 57 ± 6 % NSC and 18 ± 1 % NDF. The second (FF) contained 19 ± 1 % NSC and 37 ± 1 % NDF. Fat was incorporated into FF to equalize DE about 3.3 Mcal/kg DM. Body weight was measured on an electronic scale every 28 days. Plasma IGF-1 concentration was measured by radioimmunoassay.

Average daily gain in the SS group was not different (P = 0.86) from that in the FF group (0.79 ± 0.03 kg/d; 0.80 ± 0.03 kg/d; respectively) over the whole period. Weight gains observed during the summer months began to decrease in October and November. Reasons for this decline may be due; first, to cooler temperatures and increased energy demands for thermoregulation; second, foals were weaned in November; and third, pasture quality begins to decrease at this time of year, supplying the weanlings with less of the energy they need to grow. In April, a considerable increase in ADG was observed in both treatment groups. This change was attributed to milder temperatures, improving pasture conditions, and presumably some compensatory growth after depressed gains during the winter months. Average daily gain was correlated with plasma IGF-I concentrations (r = 0.34, P < 0.001).

Plasma IGF-I concentrations over the whole study period were higher in the SS group (223 ± 1.8 ng/ml) than in the FF group (217 ± 1.5 ng/ml) (P = 0.0001). Plasma IGF-I was higher in the SS group than in the FF group in May and June of 1998 (P = 0.04, and 0.03; respectively), and February, April and May of 1999 (P = 0.05, 0.003, and 0.03; respectively). Plasma IGF-I concentrations were 7.3 – 27.3 ng/ml higher in the SS group than the FF group during these months. Differences of this magnitude have biological significance in both in vitro and in vivo systems (Henson et al., 1997; Dahl et al., 1997).

The results show that plasma IGF-I concentration is decreased by replacement of soluble carbohydrates with fiber and fat. This decrease is probably associated with subdued plasma glucose and insulin responses to FF, which represent the diurnal feeding-fasting cycle of metabolites and hormones. Plasma IGF-I responses provide a link between the diet and studies of IGF-I’s control of cartilage maturation in cell culture. Others have suggested that the risks of DOD and OCD are increased by high intakes of feed energy. The plasma IGF-I data emphasize that the chemical form of the feed energy affects the somatotropic axis.

References:
Expired breath condensate has been used to monitor airway inflammation and oxidative stress in man. The concentration of hydrogen peroxide in condensate ([H2O2]c) has been shown to correlate with airway neutrophil activation and is elevated in human patients with asthma. To the best of our knowledge the collection of expired breath condensate in the horse has not been reported.

A collection method for horses has been developed and validated using a 5 litre stainless steel collection tube surrounded by ice and water. For collection, the horse was fitted with a mask and a two-way valve, which caused no signs of distress. A flexible pipe (volume 5l) heated to 30°C connects the expiratory port of the valve to the collection tube. Samples have been collected from several healthy horses and hydrogen peroxide, hydrogen ions, nitrite and nitrate have been detected in condensate, whilst vitamin C, glutathione, uric acid and protein have not been detected in any samples. In healthy horses at rest, [H2O2]c is in the range 0.5-1.0 umol/l.

To test the reproducibility of the technique, [H2O2]c was measured from three consecutive collections repeated on three healthy horses at rest. The %CV ranged from 3 to 14%. In two horses following administration of nebulised hay extract, breath condensate was collected hourly for 5 hours. No clinical signs of dyspnoea were seen but [H2O2]c increased post-challenge, peaking at four hours whilst no change was detected using nebulised saline as a control. No clinical signs of respiratory distress were observed in either group.

The main advantage of this technique is that, as it is non-invasive, it can be used repeatedly to follow the time course of an inflammatory response. A limitation to endoscopy of the respiratory tract and collection of tracheal wash or bronchoalveolar lavage samples for such purposes being that the actual process of sampling causes changes in cell populations and biochemical components of the lung lining fluid. Collection of equine expired breath condensate appears to be useful for the detection of airway inflammation and permits monitoring of the time course of an inflammatory response.
OXIDATIVE STATUS OF ENDURANCE HORSES.
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*Equine Studies Group, WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leics., U.K

Aging, cancer and cell membrane damage, including that induced by strenuous exercise, are effects of oxidative stress, which is a detrimental imbalance in the oxidative-antioxidative system of cells. Metabolic advantages of increased oxygen supply during exercise may paradoxically be implicated in oxidative injury to muscle cells by free radicals and other reactive oxygen species produced by oxidative reactions (Sjodin et al., 1990; Sen, 1995). We propose that the antioxidant defenses of endurance horses are severely tested during prolonged and strenuous endurance exercise and the degree of oxidative stress may be related to muscle damage. This study was conducted to determine the level of oxidative stress that develops in horses during an endurance race, and establish the relationship of oxidative stress to muscle damage, hydration status and animal welfare.

Thirty-five horses were studied during the 80 km or 160 km Old Dominion endurance race, which must be completed within 12 and 24 h, respectively. Measurements included pre- and post-race BW, blood levels of packed cell volume (PCV), total plasma protein (TPP), ascorbic acid (VIT C), a-tocopherol (VIT E), cellular glutathione (GSH), glutathione peroxidase (GPX), aspartate aminotransferase (AST), and creatine kinase (CK), at 0, 40, 80 km, and after 60 min of recovery (REC) in the 80 km race, and at 0, 64, 106, 142, 160 km and REC in the 160 km race. A portable laboratory was set up at the race and blood samples were analyzed on site or frozen on dry ice for later analyses.

In the 80 km race, pre- and post-race BW were 453 ± 12 and 421 ± 12 kg, respectively. Mean PCV levels were similar (P > 0.05) at 0, 40, 80 km and REC. Mean TPP concentrations were higher (P < 0.05) at 80 km and REC than at 0 km. Mean plasma VIT C concentrations decreased (P < 0.05) from 0 km to 40, 80 km and REC. Mean plasma VIT E concentrations were similar (P > 0.05) for the duration of the race. Mean GPX concentrations increased (P < 0.05) at 80 km from 0 km, and mean AST and CK concentrations increased at 40, 80 km and REC.

In the 160 km race, pre- and post-race BW were 446 ± 8 and 425 ± 10 kg, respectively. Mean PCV and TPP levels increased (P < 0.05) at 64 and 106 km compared to 0 km. Mean plasma VIT C concentrations decreased (P < 0.05) from 0 km to 142, 160 km and REC. Mean plasma VIT E concentrations increased (P < 0.05) from 0 km to 142 km and were similar (P > 0.05) to pre-race levels at all other times. Mean GSH concentrations decreased (P < 0.05) from 0 km to 64, 106, 142, 160 km and REC and mean GPX concentration increased (P < 0.05) from 0 km to 142 and 160 km. Mean AST and CK concentrations increased (P < 0.05) at 160 km and REC compared to pre-race levels.

The results demonstrate that oxidative stress develops during endurance competition and encourage the testing of antioxidant supplements to improve performance and welfare.

References:
ASCORBIC ACID IS REDUCED IN EQUINE PLASMA AND EPITHELIAL LINING FLUID IN HORSES AFFECTED BY RECURRENT AIRWAY OBSTRUCTION (RAO)

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2 Equine Studies Group, WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, U.K;
3 DBAMS, Imperial College, London.

Anti-oxidant status has been implicated as a modulator of inflammation. Unlike man, the horse is able to manufacture ascorbic acid endogenously. Previously in horses, glutathione has been considered as quantitatively the most important lung lining fluid anti-oxidant. Therefore we were interested to determine ascorbic acid concentrations in plasma and bronchoalveolar lavage (BAL) of healthy horses and horses affected by recurrent airway obstruction (RAO; previously referred to as equine chronic obstructive pulmonary disease (COPD)).

BAL was performed in the right lung of 4 healthy horses with no history of respiratory disease and 10 horses suffering from RAO whilst in clinical remission. All horses had been kept continuously at grass for at least 2 months. BAL was performed using two 100 ml aliquots of saline at 37°C. A jugular venous blood sample was obtained immediately prior to BAL. Ascorbic acid (AA) in BAL and plasma were analysed by HPLC using electrochemical detection and UV detection, respectively. BAL concentrations of ascorbic acid were corrected to mmol.l⁻¹ of epithelial lining fluid (ELF) using plasma and BAL urea concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=4)</th>
<th>RAO (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AA mmol/l</td>
<td>11.2 ± 0.7</td>
<td>8.0 ± 2.6</td>
<td>0.002</td>
</tr>
<tr>
<td>BAL AA (umol/l)</td>
<td>13.1 ± 4.0</td>
<td>2.1 ± 1.4</td>
<td>0.013</td>
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<tr>
<td>ELF AA (mmol/l)</td>
<td>3.0 ± 1.9</td>
<td>0.2 ± 0.1</td>
<td>0.06</td>
</tr>
</tbody>
</table>

To the best of our knowledge there are no reports of concentrations of AA in BAL of healthy horses or in plasma, BAL or ELF of RAO affected horses. The concentrations of AA in ELF measured in the control horses are 20 to 30 times greater than those of reduced glutathione previously reported in horses. We conclude that AA is the major anti-oxidant in equine lung lining fluid and that AA is reduced in plasma, BAL and ELF of RAO affected horses.
A CLINICAL TRIAL TO ASSESS THE EFFICACY OF TWO NUTRACEUTICALS FOR THE AMELIORATION OF LAMENESS IN HORSES

Dyson, S.J., Park, N.R., Harris, P., Preston, S.

The value of many horses is linked to their ability to be ridden and to move effectively without lameness in a pain-free manner. In recent years there has been a marked increase in the number and variety of nutraceutical products aimed at the management of lameness in horses. Most of these products are based on glucosamine or chondroitin sulphate, or a combination of the two. Although there have been many anecdotal reports about the efficacy of these products there has been a lack of published clinical trials.

The purpose of this study was to determine, using a double blind cross-over design, whether any clinical response could be seen in a group of lame horses following administration of 2 different joint support products.

Twenty-seven horses initially identified as showing lameness in one or more limbs were randomly assigned to two groups (A or B). Prior to the commencement of the study, lameness (including flexion tests) was evaluated and scored by an experienced clinician. Each horse received a dietary supplement of 10g of either supplement A or B for a period of 6 weeks after which lameness was re-evaluated. Following a 6 week wash-out period the horses were re-examined before commencing a 6 week period of the other product. This final cross-over phase was followed by a further examination. All horses remained in normal work throughout the study and the same experienced technician handled the horses at each examination.

Lameness scores as recorded by the clinician were converted into a simple numerical scale to facilitate statistical analysis, using methods outlined by Jones and Kenward (1989) for analysing two-period cross-over trials. Non-parametric statistical methods (Mann-Whitney) were used due to the nature of the data.

Eighteen horses completed the study and there was no significant change in lameness between the initial examination and after the end of the wash-out period, which means that any observed effect after each treatment period was likely to be due to the treatment. Twelve horses showed some improvement, with 9 out of the 18 judged by the clinician to be significantly improved following treatment with supplement A, B or both. Statistically there was a significant effect (p<0.05) on the degree of lameness following treatment with supplement B. Although supplement A did not have a statistically significant effect, the differences between supplements A and B were not marked.

This trial was designed to mimic the situation in real life where owners often feed nutraceuticals to horses which are not considered lame enough to warrant veterinary treatment. The products in this study contained glucosamine and chondroitin sulphate, together with a number of other possible active components only some of which have been identified. Such components are of particular interest as they may be preferable to the long term use of steroidal or non-steroidal anti-inflammatory drugs, in that they have fewer and less severe adverse effects. Despite the fact that clinical studies of this nature contain some limitations it does appear that in this study there was a significant clinical benefit in a large proportion of the horses from feeding these particular products.
We examined the effect of voluntary exercise, (24 hour or 1 week access to running wheels), on antioxidant enzyme activities catalase (cat), glutathione peroxidase (gpx), superoxide dismutase (total-sod)), in skeletal muscle (hind & fore limb) and heart in short-tailed field voles (Microtus agrestis). DNA oxidative damage was determined in lymphocytes and hepatocytes using the comet assay and site-specific enzymes (endonuclease III & formamidopyrimidine glycosylase). Individuals (@ 6 weeks old) were exposed to a 16:8 light regime (lights on 0500hrs) and chose to run primarily during darkness. Antioxidant enzyme activities were measured in 6 groups; control (-wheel), 24 hour (+ wheel) and 1 week (+ wheel), with individuals sacrificed at 0500hrs (no rest) or 1300hrs (8 hours rest). DNA damage was determined at 1300hrs in 3 groups; control (-wheel), 24 hour (+ wheel) and 1 week (+ wheel). Daily energy expenditure (DEE, measured by doubly-labelled water) was 40% higher in runners compared to controls and mean (±se) distance run per night was 7.8±1.2km. Skeletal muscle cat, gpx or total-sod and heart cat and gpx activity did not differ significantly between groups, but heart total sod activity did (F5,54 = 2.58, p= 0.037), with the lowest levels observed in 24 hour and 1 week runners at 0500hrs. Groups did not differ in either lymphocyte or hepatocyte DNA oxidative damage. In this model, voluntary wheel running did not increase either skeletal muscle antioxidant enzyme activities or DNA oxidative damage, despite elevating DEE by over 40%.
THE FERRET AS AN ANIMAL MODEL TO STUDY VITAMIN A METABOLISM IN CARNIVORES
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The ferret (Mustela putorius furo) has become a popular pet but also suggest as animal model to study carotenoid absorption and metabolism. In contrast to humans, however, ferrets do transport vitamin A in fasting blood not only as retinol but predominantly as retinyl esters as observed in dogs and other carnivores. To evaluate the ferret as a model for non-specific vitamin A transport in plasma and urinary excretion of vitamin A we conducted three experiments in 12 mature female ferrets (2-5 y; 850-1250 g body weight). The investigations focussed on the effects of different concentrations of vitamin A in the diet on the levels of retinol and retinyl esters in plasma and organs as well as the excretion of vitamin A in the urine as has been reported for dogs and other canines (Raila et al., 2000).

In experiment 1, two ferrets were dosed daily for 4 weeks with 2 g $-carotene/kg diet together with 10 g taurocholic acid/kg diet. Compared to control animals, the feeding of the $-carotene diet resulted in higher $-carotene concentration in the plasma as well as in a $-carotene accumulation in liver and kidneys. These results confirm that ferrets absorb $-carotene across the intestinal mucosa as observed in dogs and cats (Chew et al. 2000a, b). Furthermore, we found that $-carotene supplemented ferrets were able to excrete $-carotene in the urine. This may be an indication that the kidneys possess a major role in $-carotene metabolism in ferrets.

Experiment 2 was designed to study the response of oral 25,000 IU vitamin A supplementation/d for three consecutive days on the excretion of vitamin A in the urine. Control samples were taken at day 1. After the first vitamin A supplementation (day 2), urinary vitamin A (predominantly retinyl palmitate) increased from 9 ± 6 ng/ml to 439 ± 233 ng/ml. Further vitamin A supplementations on day 2 and 3 had no increasing effect on the excretion of vitamin A in urine. The results show that ferrets have the ability to excrete vitamin A with the urine. The response in the urine seems to be dependent on the oral vitamin A supplementation, which is in good accordance with results in healthy dogs (Schweigert and Bok, 2000).

In experiment 3, the effect of different vitamin A concentrations in the diet on the concentrations of retinol and retinyl esters in plasma, urine, liver and kidneys were investigated. Four ferrets were fed a basal diet and were supplemented orally 10,000 IU retinyl palmitate (VA +) every second day for four weeks. Four control animals (VA-) were fed the basal diet only. In the VA+ group, the vitamin A concentrations in plasma, urine as were significantly higher. However, no significant differences were found between the vitamin A levels in liver and kidney specimens of the VA+ and VA- group. This supports the hypothesis of differences in the vitamin A metabolism between carnivorous species (Raila et al., 2000). In conclusion, all experiments performed show that the ferret can be a appropriate model for studying vitamin A as well as carotenoid metabolism in carnivores.

References:
PLASMA CONCENTRATIONS OF LEPTIN MIRROR CHANGES IN BODY WEIGHT BUT DO NOT INFLUENCE THE PATTERN OF THE PRE-OVULATORY LUTEINIZING HORMONE (LH) SURGE IN MINK (MUSTELA VISON)

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The discovery of leptin, the protein product of the obesity gene (Zhang et al., 1994), has generated a vast amount of research into leptin’s different roles in metabolism. One area of special interest is reproduction: from empirical research it is well-known that nutritional status is important for the reproductive processes, but still there is no complete understanding of how nutrition regulates reproduction. Leptin has been suggested to play an important role in the nutrition–reproduction interaction, possibly by acting as a metabolic gate (Ahima et al., 1997, Chehab et al., 1997, Cheung et al., 1997). Nutrient supply is reflected in LH secretion: when insufficient LH pulse frequency is suppressed, but rapidly restored in response to refeeding. The objective of this study was to evaluate whether plane of nutrient supply prior to breeding did influence plasma leptin and other metabolic hormones and LH during the pre-ovulatory surge in a reflex ovulator, the mink.

A total of 30 one-year-old female mink were used. They were fed to remain in energy balance (CON, n=10), according to a flush feeding model comprising a 2-week period of restricted feeding (50% of the supply to CON) followed by a 2-week period of ad libitum refeeding (FLUSH, n=10) or to be in negative energy balance throughout the experiment (NEG, n=10; 60% of the CON food supply). Animals were weighed and blood samples were taken at the start of the experiment, when restriction started and ended and after refeeding in the FLUSH group. By the end of the feeding period animals were mated. Blood samples were taken before, immediately after, 4, 8, 12, 24 and 48 h after mating for determining the pattern of the pre-ovulatory LH surge. Plasma concentrations of leptin (multi-species assay), insulin, IGF-1 and thyroid hormones (thyroxine (T4) and tri-iodo-thyronine (T3)) and LH were analysed. All assays were validated for mink plasma.

Animal live weights clearly reflected food supply. Plasma concentrations of leptin were in clear reflectance of body weight changes. They were significantly affected by day of sampling and interaction between sampling day and treatment group: in CON animals concentrations remained stable whereas they decreased from 1.5 ng/ml to 1.1 ng/ml (P=0.01) during restriction and then increased to 1.7 ng/ml (P<0.001) during refeeding in the FLUSH group. In NEG animals plasma concentrations decreased from 1.5 ng/ml to 1.1 ng/ml (P=0.03). Similar patterns of changes in hormone concentrations were recorded for insulin, T3, T4 and IGF-1. The pattern of the LH surge was not significantly different between treatment groups, but there was a tendency for a more sluggish release among NEG animals. One female with a very low body weight had no detectable LH surge (Tauson et al., 2000). Plasma concentrations of leptin on the day of mating were not significantly correlated to basal, maximum or mean LH concentrations. Similarly, the area under the LH curve was not significantly related to plasma leptin concentrations or changes in leptin concentrations during the experimental period.

The present data suggest that plasma leptin concentrations in mink are very responsive to changes in food supply and body weight, results in agreement with Tauson and Forsberg (2001), but that within the range of body weight of the animals in this investigation, it could not be shown that plasma leptin concentrations were reflected in plasma LH concentrations during the LH surge.
SUBSTRATE OXIDATION AND ENERGY EXPENDITURE IN MALE BLUE FOXES (ALOPEX LAGOPUS) DURING FEEDING, FASTING AND REALIMENTATION.

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When living in the wild the blue fox may experience extended periods without access to food during the winter. As a survival strategy it accretes large amounts of fat during the autumn, a body reserve that has dual purposes: it can serve as insulation of the body during periods of extreme cold, and as energy reserve during periods of food scarcity. The objective of this study was to evaluate how the fox economises with its body reserves during a period of fasting, and how reserves are restored during realimentation.

Eight male blue foxes, about 6 months old were kept in metabolism cages in the laboratory and fed a diet providing 19.6 MJ metabolisable energy (ME)/kg dry matter, out of which 20% was derived from protein, 50% from fat and 30% from carbohydrate. Two animals were controls and fed throughout the study whereas the remaining 6, after the initial balance period, were fasted for 8 days, and then given a one-week realimentation period. Water was freely available at all times. The animals were measured in balance and respiration (indirect calorimetry in an open-air circulation system) experiments during feeding, then days 2, 4 and 8 of fasting and on days 2 and 6 during realimentation. Repeated measures statistical analyses were performed by procedure MIXED in SAS.

All animals remained healthy during the experiment. The main results appear from Table 1.

| Table 1. Animal live weights (LW), heat production (HE), oxidation of protein (OXP), fat (OXF) and carbohydrate (OXCHO), and excretion of urinary nitrogen (UN). |
|---------------------------------|------|------|------|-----------------|-----------------|
|                                 | Feeding | Fasting | Realimentation | P-value, effect of Period (p) | Day in p |
| LW, kg                         | 10.58 | 9.68   | 9.61           | 0.08                          | <0.001       |
| HE, kJ/kg0.75                  | 450   | 407    | 437            | 0.53                          | 0.05         |
| OXP, kJ/kg0.75                 | 112   | 43     | 104            | <0.001                        | <0.001       |
| OXF, kJ/kg0.75                 | 157   | 361    | 160            | 0.001                         | 0.03         |
| OXCHO, kJ/kg0.75               | 178   | 3      | 176            | <0.001                        | 0.01         |
| UN, g/kg0.75                   | 1.08  | 0.37   | 0.90           | <0.001                        | 0.02         |
| OXP, % of HE                   | 25.4  | 10.7   | 24.1           | <0.001                        | <0.001       |
| OXF, % of HE                   | 34.7  | 88.2   | 35.5           | <0.001                        | 0.01         |
| OXCHO, % of HE                 | 39.6  | 1.0    | 40.1           | <0.001                        | 0.02         |

Heat production decreased steadily during fasting from 438 kJ/kg0.75 day 2 to only 373 kJ/kg0.75 on day 8 (P=0.04), although the differences between periods were non-significant. The substrate utilization changed from a mixed contribution of carbohydrate, fat and protein during feeding to predominantly fat and practically no carbohydrate oxidation during fasting. The high rate of fat oxidation and the non-significant carbohydrate oxidation were reached already on day 2 of fasting, whereas the rate of protein oxidation declined slightly from day 2 to day 8, which also was reflected in values for UN. These findings suggest that the fox is able to adapt to food scarcity by use its body fat stores as metabolic fuel, but that a 8 days fasting period does not imply any particular mobilisation of muscle mass.
Several insect species are commercially raised as food for captive reptiles and amphibians. Studies to date suggest a diet consisting solely of insects is likely to be deficient in calcium, Vitamin A and several other nutrients. Strategies to enhance the nutritional composition of these insects have centered on dietary manipulation, but systematic experiments are limited. This study was designed to develop a series of regression equations, which allows for the development of diets to enhance the nutritional value of selected insect species.

Adult house crickets (HCA; weight 408 mg) and house cricket nymphs (HCN; weight 68 mg) were fed dry powdered diets containing graded levels of calcium (n=5 or 6; 1.03 – 8.58%) or Vitamin A (n=5 or 6; 18900 – 109600 IU/kg). Mealworm larva (MWL; weight 135 mg) were fed dry wheat bran diets containing graded levels of calcium (n=6; 0.11 – 8.15%) or Vitamin A (n=5; 0 – 85900 IU/kg). Silkworm larva (SWL; weight 1251 mg) were fed moist artificial diets containing graded levels of calcium (n=4; 0.24 – 1.63%). Insects were fed the diets for 48 hours after which they were frozen and analyzed. Insects of each species were also fasted to empty the gastrointestinal tract, frozen and analyzed. Diets were analyzed in triplicate, insects in duplicate. After subtracting values for calcium or Vitamin A from fasted individuals, insect calcium or Vitamin A composition was plotted as a function of dietary concentration.

For HCA, HCN and SWL calcium and Vitamin A composition (HCA and HCN only) was a linear function of dietary calcium or Vitamin A concentration (Table 1). For MWL a curvilinear response was observed over the range of concentrations tested for both calcium and Vitamin A composition was plotted as a function of dietary concentration.

From the slope of the regression equations the residual food in the insect’s gut was calculated as follows (HCA – 5.1%; HCN – 6.1%; SWL – 15.4%; MWL – 4.4%). Using these data, information concerning the dietary requirements of the prey species and the composition of the various insect species, nutritionists can develop “gut loading” diets to enhance the nutritional value of insects commonly used as food for captive reptiles and amphibians.

| Table 1. Body composition of HCA, HCN and SWL as a function of dietary composition. |
|--------------------------------------|-----------------|-----------------|-----------------|
| Treatment                           | Intercept       | Slope           | ANOVA           |
| HCA – Calcium                       | -0.021 + 0.008  | 0.044 + 0.002   | 844.1 0.0001    |
| HCA – Vitamin A                     | -369 + 416      | 0.056 + 0.006   | 100.8 0.0021    |
| HCN – Calcium                       | 0.012 + 0.021   | 0.047 + 0.004   | 152.8 0.0002    |
| HCN – Vitamin A                     | -302 + 418      | 0.075 + 0.006   | 157.1 0.0002    |
| SWL – Calcium                       | - 0.002 + 0.001 | 0.154 + 0.003   | 153.2 0.0001    |

| Table 2. Body composition of MWL as a function of dietary composition. |
|-------------------------------|-----------------|-----------------|-----------------|
| Treatment                     | Intercept       | Diet            | F-Ratio         |
| MWL – Calcium                 | 0.000 + 0.004   | 0.043 + 0.003   | 905.3 0.0001    |
| MWL – Vitamin A               | -17 + 66        | 0.045 + 0.004   | 550.9 0.0018    |