

Proceedings
of the
Joint Nutrition Symposium

August 21-25, 2002
Antwerp, Belgium



Foreword

*“Just a perfect day
feed animals in the zoo”
(Lou Reed)*

This book results from the will of a very diverse range of scientific societies – read: people – to work together:

- ESVCN: the European Society of Veterinary and Comparative Nutrition,
- ECVCN: the European College of Veterinary and Comparative Nutrition,
- AAVN: the American Academy of Veterinary Nutrition,
- ACVN: the American College of Veterinary Nutrition,
- EZNRG: the European Zoo Nutrition Research Group,
- CNS: the Comparative Nutrition Society.

Packed with original research, interesting case studies and reviews, this book is like a good pellet: a wide range of components that are stuck together by a sweet molasses, called nutrition. It is tasty, highly digestible, and stimulating.

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Joint Nutrition Symposium PROGRAMME

Posters are printed in italics

Wednesday, AUGUST 21

IMMUNONUTRITION & HEALTH PROMOTING NUTRITION (Hill's session)

- 9:00 [Nutrition and immune function in the aged: clinical implications and molecular mechanisms - Simin Nikbin Meydani](#)
- 9:45 [Nutrition and Immunity: A key focus in production and health - Korinn E. Saker : book](#)
- 10:30 BREAK & POSTER SESSION
- [Parenteral nutrition with or without early enteral nutrition in young dogs with parvovirus - Jürgen Zentek², Kerstin Will, Ingo Nolte](#)*
- [Effects of dietary antioxidant supplementation before and after oral acetaminophen challenge in cats - A. S. Hill¹, S. L. O'Neill, M. M. Christopher, Q. R. Rogers](#)*
- [The effect of trial length on canine fecal microflora response to chicory ingestion - G.L. Czarnecki-Maulden, A.R. Patil](#)*
- [Influence of organic feeding and housing on "health" parameters in organic pig fattening - S. Millet, M. Hesta, E. Cox, E. Ongena, S. De Smet, G.P.J. Janssens](#)*
- [Effects of lactulose as feed additive on chyme composition and intestinal microflora of weaned piglets - R. Tabeling, O. Stuke, S. Bollmann, G. Amtsberg, J. Kamphues](#)*
- 10:45 [The effect of sprouted barley-corn with antioxidant properties on the humoral immune response of turkey poults - E. Carlin, Ch. Iben, E. Wagner, C. Neubauer](#)
- 11:00 [Effect of full-length and truncated IgY on the nutritional cost of infection in mallard ducks \(Anas platyrhynchos\) - B.D. Humphrey, C.C. Calvert, K.C. Klasing](#)
- 11:15 [Systemic and pulmonary antioxidant status in equine recurrent airway obstruction \(RAO\) - D.J. Marlin, C.M. Deaton, N.C. Smith, P.A. Harris, R.C. Schroter & F.J. Kelly](#)
- 11:30 [Antioxidant Supplementation in Horses Affected by Recurrent Airway Obstruction - C.M. Deaton, D.J. Marlin, N. Smith, P.A. Harris, F. J. Kelly and R.C. Schroter](#)
- 11:45 [Effects of Tasco \(a brown seaweed\) and heat stress on immune function and antioxidant activity of wether lambs - K.E. Saker, J.H. Fike, H.P. Viet, D.L. Ward](#)
- 12:00 [Influence of lactulose as feed additive on chyme composition and intestinal microflora in pigs infected artificially with E. coli O139:K82 and Salmonella Derby - S. Bollmann, O. Stuke, R. Tabeling, J. Kamphues, G. Amtsberg](#)
- 12:15 [Dietary effects on bifidobacteria and Clostridium perfringens in canine faeces - Jürgen Zentek, Bettina Marquart, Tanja Pietrzak, Olivier Ballèvre, Florence Rochat](#)
- 12:30 LUNCH & POSTER SESSION
- 14:00 [Dietary resistant starch supplementation restores the integrity of rat inflamed colonic mucosa - N. Moreau, L. Martin, C. Toquet, C. Laboisse, B. Siliart, P. Nguyen, M. Champ and H. Dumon](#)
- 14:15 [Effect of oligosaccharide supplementation on an ETEC infection - S. Vancaeneghem, Y. Van der Stede, T. Verfaillie, F. Verdonck, S. Arnouts, P. Deprez, E. Cox, B.M. Goddeeris](#)
- 14:30 [The impact of prebiotics during salmonellosis on nutrient retention and Salmonella typhimurium var. Copenhagen excretion in adult pigeons \(Columba livia domestica\) - G.P.J. Janssens, S. Millet, F. Van Immerseel, M. Hesta, R. Ducatelle: book](#)
- 14:45 [In vitro studies on the effects of short-chain fatty acids on the invasiveness of Salmonella - F. Van Immerseel, J. De Buck, F. Pasmans, P. Velge, E. Bottreau, F. Haesebrouck and R. Ducatelle](#)
- 15:00 [Influence of PUFA on immune cell and tissue MAPK in the feline - K.E. Saker, W.R. Huckle, B.E. Cowing, J.H. Herbein](#)
- 15:15 [Efficacy of a hypoallergenic diet containing soy isolate hydrolysate for the diagnosis and management of food hypersensitivity in dogs : a multicentric field study - D. Van Pottelberge, V. Biourge, P. Marniquet and R. Sergheraert](#)

OBESITY & ENERGY BALANCE

15:30 BREAK & POSTER SESSION

- [Evolution of blood parameters during weight loss in experimental obese Beagle dogs - M. Diez, C. Michaux², Jeusette, C. Cuvelier, C. Tonglet, L. Istasse, V. Biourge](#)
[High fat/high energy diets and body weight regulation in cats - V. Riou, C. Jean, A. Patil, E. Rowe](#)
[Effect of ovariectomy on daily energy requirement in beagle dogs - I. Jeusette, J. Detilleux, C. Cuvelier, L. Istasse, M. Diez](#)
[Home made diets for cats and dogs: do calculated nutrients approximate analysed nutrients? - M. Hesta, J. Debraekeleer, S. Millet and G.P.J. Janssens](#)
- 16:00 [Substrate oxidation in pigs during feeding, fasting and refeeding - A. Chwalibog, A-H. Tauson, G. Thorbek](#)
- 16:15 [Leptin, food intake and energy balance in pregnant and lactating mink \(Mustela vison\) - A-H. Tauson, M. Forsberg, A. Chwalibog](#)
- 16:30 [Modulation of uncoupling protein \(UCPs\) expression in adipose tissue, skeletal muscle, and liver in obese insulin resistant dogs - Véronique Leray, Constance Gayet, Edwige Bailhache, Lucile Martin, Henri Dumon, Brigitte Siliart and Patrick Nguyen](#)
- 16:45 [Hypersecretion of TNF \$\alpha\$ and IGF1 in the development of insulin resistance - Constance Gayet, Edwige Bailhache, Lucile Martin, Henri Dumon, Brigitte Siliart and Patrick Nguyen](#)
- 17:00 [Hepatic lipidosis in cats: new concepts on pathogenesis - Géraldine Blanchard, Bernard M Paragon, Colette Sérougne, Jacqueline Férézou, Fabien Milliat, Claude Lutton](#)
- 17:15 [Early effects of neutering on the energy intake and expenditure of domestic cats - \(Felis catus\) - R.C. Backus, M. L. Kanchuk, J.G. Morris, Q.R. Rogers](#)
- 17:30 [Effect of ad libitum feeding on body weight and blood metabolites in spayed female beagle dogs - I. Jeusette, J. Detilleux, C. Cuvelier, L. Istasse, M. Diez](#)
- 17:45 [Evaluation of several home made diets for food allergy in dogs or obesity in cats - M.Hesta, J. Debraekeleer, S. Millet, L. Wilmaerts and G.P.J. Janssens](#)

Thursday, AUGUST 22

MINERALS & BONE METABOLISM

- 8:30 [Estimation of the mineral content of grass, hay and grass-silage - Brigitta Wichert, Angela Glocker and Ellen Kienzle : book](#)
- 8:45 [Nutrition and developmental orthopedic disease in horses : results of a survey on 76 yearlings from 14 breeding farms in Basse Normandie \(France\) - B.-M. Paragon, J.-P. Valette, Géraldine Blanchard, and J.-M. Denoix](#)
- 9:00 [Growth rates and the incidence of osteochondrotic lesions in Hanoverian Warmblood foals. - Preliminary data - I. Vervuert, M. Coenen, A. Borchers, M. Granel, S. Winkelsett, L. Christmann, O. Distl, E. Bruns, B. Hertsch](#)
- 9:15 [Ultra trace element intake of Cercopithecinae in comparison with humans - W. Arnhold; G. Krische; M. Anke; M. Seifert; M. Jaritz; K. Eulenberger; A. Bernhard: book](#)

UNWANTED DIETARY COMPOUNDS & UNWANTED NUTRITIONAL BEHAVIOUR

- 9:30 [Undesirable substances in feed – an overview - Josef Leibetseder](#)
- 9:45 [Feeding practices in the German BSE epidemic: a preliminary survey of the first 65 Bavarian cases - M. Clauss, E. Kienzle](#)
- 10:00 [Crib-biting in foals is associated with gastric ulceration and mucosal inflammation - A.J. Waters, C.J. Nicol, A.D. Wilson, P.A. Harris and H.B. Davidson](#)
- 10:15 BREAK & POSTER SESSION
posters on MINERALS & BONE METABOLISM
[Case report: runting, diarrhoea and cachexia in fallow deer responsive to micronutrient supplementation - D. Ranz, C.A. Schmittinger, M. Clauss, Brigitta Wichert, U. Wehr, M. Müller, O. Geisel, Ellen Kienzle](#)
[Apparent digestibility of calcium and phosphorus in growing beagles influenced by mineral supply - Dobenecker, B.](#)
[Effects of commercial dry diets in two persian cats with struvite urolithiasis - Cristina Fanchi](#)
[Recent developments in phytase research / Methods to improve thermostability of phytases - M. Van paemel, S. Millet, M. Hesta, G.P.J. Janssens](#)

[Long-term consumption of an acidifying diet affects acid-base status and bone density in cats - G.L. Czarnecki-Maulden, D.G. Chausow, C.J. Cupp](#)

[Nutritional disorders of skeleton in emus and rheas - Petra Wolf, Norbert Kummerfeld and Josef Kamphues: book](#)

[Diurnal variation in concentrations of various markers of bone metabolism in goat and sheep - A. Liesegang, M-L. Sassi, J. Risteli, M. Wanner](#)

[Urinary acidifying effect of dietary calcium chloride in fattening pigs - G.P.J. Janssens, V. Debal, S. Millet, M. Hesta and R.O.M. De Wilde](#)

[Effect of exercise on apparent digestibility in horses - S. Van Wevenberg, M. Hesta, S. Millet, G.P.J. Janssens](#)

[The effect of beefhide strips on digestibility and dental calculus in dogs - C. J. Cupp, G.L. Czarnecki-Maulden](#)

[Feeding and nutrition practice in 82 Hanoverian breeding farms during breeding season - S. Winkelsett, M. Granel, M. Coenen, I. Vervuert, A. Borchers, O. Distl, E. Bruns, B. Hertsch, L. Christmann](#)

posters on UNWANTED DIETARY COMPOUNDS & UNWANTED NUTRITIONAL BEHAVIOUR

[Deaths in horses caused by ingestion of a hay contaminated by bracken fern \(*Pteridium aquilinum*\) - Petra Wolf, T. Janetzko, S. Aboling and J. Kamphues](#)

[The influence of the ratio of structured/unstructured feed on oral disturbances in captive giraffids - J. Hummel, M. Clauss, E. Baxter, E.J. Flach, K. Johansen, L. Kolter: book](#)

[Digestive tolerance of sorbitol in cats - Géraldine Blanchard, Marie-Hélène Saniez, Daniel Wils, Bernard-Marie Paragon](#)

[Cats are more sensitive to the toxic effects of oral lipoic acid than humans, dogs, or rats - Ana S. Hill, Jonathan A. Werner, Quinton R. Rogers, Dennis A. Wilson, Sharron L. O'Neill, and Mary M. Christopher.](#)

[Effects of dietary clenbuterol and cimaterol on performances, muscle composition and endocrine response in broiler chickens - Schiavone A., Pagliasso S., Tarantola M., Perona G., Badino P. and Lussiana C.](#)

10:45 [Zinc intoxication in budgerigars \(*Melopsittacus undulatus*\) - Petra Wolf, Cristiane Haupt and J. Kamphues: book](#)

11:00 [Distinct weight loss and elevated hepatic enzymes in horses caused by straw contaminated with Deoxynivalenol \(case report\) - Annette Zeyner, U. Fischer, Andrea Lindner](#)

LIPID METABOLISM

11:15 [Interaction of dietary unsaturation level with linolenic acid and a-tocopherol deposition in poultry meat - Cortinas L., Barroeta A.C., Villaverde C., Baucells M.D. and Jensen S.K.](#)

11:30 [Relationship between dietary PUFA level and apparent absorption of vitamin E in poultry - C. Villaverde, M.D. Baucells, L. Cortinas, S.M. Martín-Orúe and A.C. Barroeta](#)

11:45 [Influence of dietary lipid source and genotype on fatty acid composition of Muscovy duck meat - Schiavone A., Romboli I., Chiarini R., Marzoni M.](#)

12:00 [Dietary supplementation of polyunsaturated fatty acids in cats - U. Wehr, Sonja Wilhelm, D. Ranz, W.A. Rambeck](#)

12:15 [Reverse cholesterol transport in dogs - Edwige Bailhache, Khadija Ouguerram, Thierry Magot, Michel Krempf, Brigitte Siliart and Patrick Nguyen](#)

AMINO ACIDS & PROTEIN

12:30 LUNCH & POSTER SESSION

[Rumen bypass of free methionine and lysine for double-muscle Belgian Blue bull - E. Froidmont, P. Rondia, Y. Beckers, A. Thewis](#)

[Nitrogen requirements for maintenance of captive *Aceros*- and *Buceros* Hornbills - SG Foeken, M de Vries, EG Hudson, CD Sheppard, ES Dierenfeld: book](#)

[Blood serum branched chain amino acids and tryptophan modifications in horses competing in long distance rides of different length - Assenza A, Bergero D., Tarantola M, Piccione G., Caola G.](#)

14:00 [Six months taurine or methionine supplementation in Newfoundland Dogs suffering from low whole blood taurine - R Willis, G. Desprez, J. Dukes-McEwan, V. Biourge, R.C. Backus, Q.R Rogers](#)

14:15 [A model for the development of taurine deficiency in dogs by the use of cholestyramine - C.L. Tôrres, A.J. Fascetti and Q.R. Rogers](#)

14:30 [The minimal dietary lysine requirement of kittens for maximal growth - J.G. Morris and Q. R. Rogers](#)

14:45 [Hair pigmentation can be affected by diet in dogs - V. Biourge, R. Sergheraert](#)

- 15:00 [Feeding type \(conventional versus organic\) and lysine/energy ratio influence meat and carcass characteristics of organic fattening pigs - S. Millet, M. Hesta, M. Seynaeve, E. Ongenae, S. De Smet, G.P.J. Janssens](#)
- 15:15 [BioProtein as protein source to growing mink kits and slaughter chickens - energy expenditure and nitrogen metabolism - A-H. Tauson, A.L. Frydendahl Hellwing, A. Chwalibog](#)

DIGESTIVE KINETICS

- 15:30 BREAK & POSTER SESSION
- [Fluid and particle passage rate in captive black rhinoceros \(*Diceros bicornis*\) - M. Clauss, T. Froeschle, M. Lechner-Doll, E.S. Dierenfeld, J.-M. Hatt: book](#)
- [Morphological correlates of accelerated passage rates in very large herbivores - B. Kiefer, W. Loehlein, M. Clauss: book](#)
- [Nutrition, physiological adaptation and re-introduction; a case study of the knot \(*Calidris canutus*\) - A. Brans, J. van Gils, B.B.H. van Wijk, H.J. Kuipers: book](#)
- [The influence of L-carnitine on nutrient retention in pigeons \(*Columba livia domestica*\) fed corn or peas - G.P.J. Janssens, A. M. Abd-Ellah, M. Hesta, S. Millet, R.O.M. De Wilde: book](#)
- [Changes in gastrointestinal transit time and pH in piglets around weaning - V. Snoeck, N. Huyghebaert, E. Cox, A. Vermeire, J. Saunders, J.P. Remon, F. Verschooten, and B.M. Goddeeris](#)
- [Seasonal variation of grazing available forage for domestics and wild ungulates in different alpine areas - P.Aceto, A.Cavallero, P.P.Mussa, C.Abba, R.Viterbi, B.Bassano](#)
- [Starvation of rumen bacteria and their shift-up growth under different *in vitro* conditions - A. Moharrery](#)
- 16:00 [Influences of rabbit breed on relative size of the intestinal tract and the composition of its content - Petra Wolf, Birgit Zumbrock, R. Tabeling and J. Kamphues](#)
- 16:15 [Relationship of genotype, body composition and sexual maturity in females of different rabbit breeds - Kinga Fodor, L. Zöldág, S. Fekete, A. Bersényi, Emese Andrásófszky, Margit Kulcsár and R. Glávits](#)
- 16:30 [Influence of body size on fermentative activity and faecal consistency in dogs - David Hernot, Mickael Weber, Lucile Martin, Henri Dumon, Brigitte Siliart, Vincent Biourge and Patrick Nguyen](#)
- 16:45 [Age-related changes in digestibility and gastric emptying in growing dogs - Mickael Weber, Fouzia Stambouli, David Hernot, Lucile Martin, Henri Dumon, Vincent Biourge and Patrick Nguyen](#)
- 17:00 [Seasonal changes in digestive tract function in marmots - Ian D. Hume](#)
- 17:15 [Effect of different fibre types on the digestibility of nutrients in cat - S. Fekete, I. Hullár und Emese Andrásófszky, F. Kelemen](#)
- 17:30 [Digesta kinetics in feral pigeons \(*Columba livia*\) - J.-M. Hatt: book](#)
- 17:45 [Dietary management of dental disease in the dog and cat - DM Morgan, AJ Lepine, ER Cox](#)

Friday, AUGUST 23

FEED INTAKE

- 8:30 [Diet selection by the White-naped Pheasant Pigeon *Otidiphaps nobilis aruensis* at the Barcelona Zoo - Helena Marqués, Maria D. Baucells, Elena Albanell: book](#)
- 8:45 [Energy and nutrient intake, feeding behaviour and activity budget of Douc langurs \(*Pygathrix nemaeus nemaeus*\) in captivity - K. Klumpe, C. Schwitzer, W. Kaumanns: book](#)
- 9:00 [Epidemiologic study of hospitalized dogs voluntary food intake - Nathalie Priymenko, Isabelle Lesponne, Claire Besson, Patrick Verwaerde](#)
- 9:15 [Some Preliminary Observations on Herbivorous Insect Composition: Nutrient Advantages from a Green Leaf Diet? - Ellen S. Dierenfeld:](#)
- 9:30 [The role of insects in primate nutrition: how is chitin utilized? - Charlotte O'Sullivan, Mauvis Gore, Sophie Foley, Kathy Velandar:](#)
- 9:45 [the EAZA Atlantic Rainforest Campaign – Kristin Leus](#)
- 10:00 BREAK & POSTER SESSION
- [Open trial to determine voluntary food intake in hospitalized cats - Nathalie Priymenko, Claire Besson, Isabelle Lesponne, Patrick Verwaerde](#)
- [Feed choice in pigeons with or without L-carnitine supplementation - G.P.J. Janssens, A.M. Abd-Ellah, M. Hesta, S. Millet and R.O.M. De Wilde](#)
- [The use of n-alkanes to estimate diet composition, intake and digestibility in sheep fed mixed diets - O. Valiente, A. de Vega, J. A. Guada, C. Castrillo](#)
- [Food intake and body weight development of captive roe deer \(*Capreolus capreolus*\) fawns fed diets of different tannin content - M. Clauss, M. Lechner-Doll, K. Lason: book](#)

Investigations on ingestion, amounts and composition of casting and digestibility of organic matter in different birds of prey (kestrel falcon, common buzzard, and eagle owl) - P. Wolf, M. Lüdtke und J. Kamphues: book

session contributed to the ATLANTIC RAINFOREST CAMPAIGN

- 10:30 *Nutrition of marsupials in captivity - Ian D. Hume: book*
 11:30 *HACCP based bacteriological study of the feed of fruit-eating birds in the Rotterdam Zoo - A.E.P. Knipscheer, J. Nijboer and L.J.A. Lipman: newsletter*
 11:45 *Evaluation of the use of organic formulated bird foods for large psittacines - Debra McDonald: newsletter*
 12:00 *Global wildlife nutrition database: Howler monkey (Alouatta pigra) prototype - António G. Vidigal, Ellen S. Dierenfeld, Suzanne I. Boardman: newsletter*
 12:15 *A glutenfree diet for callitrichids and marmosets in Emmen-zoo, the Netherlands - C. Berndt, R. Spijker and G. Wind: newsletter*
 12:30 LUNCH & POSTER SESSION
Handfeeding of young parrots – techniques, diets and recommendations - Petra Wolf: newsletter
Experimental data on feeding extruded diets in parrots - Petra Wolf, Sabine Graubohm and Josef Kamphues book
Feed composition and digestive capacity in parrots - Josef Kamphues, Petra Wolf, Karen Heisler and Markus Frömbling: book
An overview of captive Aceros- and Buceros hornbill diets in some Dutch- and US facilities - SG. Foeken, M.de Vries, T.R. Huisman: book

Saturday, AUGUST 24

RUMINANTS

- 8:30 *Allometry and ecology of feeding behavior and digestive capacity of herbivores: a review revisited - P.J. Van Soest: newsletter*
 9:30 *Body size limitations in ruminants, or why buffalo defecate pies not pellets - M. Clauss, M. Lechner-Doll, W.J. Streich, R. Frey, G.E. Rössner: book*
 9:55 *Browse silage: the solution for browsers in wintertime? - J. Nijboer, M. Clauss, J.J.L. Nobel: newsletter*
 10:20 BREAK & POSTER SESSION
Blood parameters of captive roe deer (Capreolus capreolus) fawns on diets of different tannin content - M. Clauss, M. Lechner-Doll, K. Lason, T. Grune: book
Ruminants: why browsers are non-grazers - M. Clauss, M. Lechner-Doll, W.J. Streich: book
The attribution of a feeding type to a ruminant species based on morphological parameters: the example of the okapi (Okapia johnstoni) - M. Clauss, J. Hummel, J. Völlm: book
Faecal Dry matter content in captive wild ruminants: implications for the browser/grazer-dichotomy - M. Clauss, M. Lechner-Doll, W.J. Streich: book
Reaction of a group of captive giraffe (giraffa camelopardalis) to the introduction of a tannin-containing pelleted diet - M. Clauss, E.J. Flach, M. Lechner-Doll, J.-M. Hatt: newsletter
Tannin-binding salivary proteins in three captive rhinoceros species - J. Gehrke, J. Fickel, M. Lechner-Doll, R. Hermes, E.J. Flach, J.-M. Hatt, M. Clauss: book
Induction of salivary tannin-binding proteins in captive black rhinoceros (Diceros bicornis) by dietary tannins - M. Clauss, J. Gehrke, J. Fickel, M. Lechner-Doll, E.J. Flach, E.S. Dierenfeld, J.-M. Hatt: book
Salivary tannin-binding proteins are not affected by mid-term feeding history in captive roe deer (Capreolus capreolus) - J. Gehrke, J. Fickel, M. Lechner-Doll, K. Lason, M. Clauss: book
 10:50 *Irregular Patterns for low amounts of dietary tannins in captive roe deer (Capreolus capreolus): Implications for the validity of preference Trials - M. Clauss, K. Lason, M. Lechner-Doll, J. Fickel, J. Gehrke: book*
 11:15 *Grazing, ruminating and resting in Bos taurus, when herbage availability is limiting - E.J. Finegan, J.L. Atkinson, J.G. Buchanan-Smith, J.P. Cant*
 11:40 *Considerations on feeds used for zoo ruminants - J. Hummel, M. Hovenjürgen, E. Niess, K. Johansen, J.Nijboer, W.Zimmermann : newsletter*
 12:05 *The effect of dietary sugar content on glucosuria in a female okapi (Okapia johnstoni) - F. Verammen, R. De Deken, J. Brandt: book*

GENERAL NUTRITION

12:30 LUNCH & POSTER SESSION

- [Recent activities of the United States National Research Council Committee on Animal Nutrition - Jamie S. Jonker; Christopher Rogers; Gary L. Cromwell; Charlotte Kirk Baer](#)
[Nutritional aspects of the dry-season diet of mountain gorillas in Bwindi Impenetrable National Park, Uganda: preliminary results - Jessica M. Rothman, Alice N. Pell, Ellen S. Dierenfeld, Colleen M. McCann, Eloy Rodriguez](#)
 14:00 [Eggs, endothermy and milk: A novel scenario for the evolution of lactation - O.T. Oftedal](#)
 14:25 [The protein-to-fiber ratio does not predict feeding selectivity among primate frugivores - N.L. Conklin-Brittain, A.J. Marshall, C.D. Knott, R.W. Wrangham, and M. Leighton](#)
 14:50 [Australian marsupials eat Acacia ! - N.A. Irlbeck, I.D. Hume](#)
 15:15 [Brookfield Zoo Databank Overview - Kimberly D. Ange and Susan D. Crissey : newsletter](#)

VITAMINS

15:40 BREAK & POSTER SESSION

- [Effect of supplemental ascorbic acid on T3-induced heart failure syndrome and metabolic parameters of broiler chickens - Hassanzadeh, M; Buyse, J & Decuypere, E.](#)
[The influence of an increased cobalt supply on microbial vitamin B₁₂ synthesis in the rumen of dairy cows - Kirsten Stemme, P. Lebzien, G. Flachowsky, H. Scholz](#)
[The effect of cobalt supply to pregnant cows on the vitamin B₁₂ status of their calves - Kirsten Stemme, U. Meyer, G. Flachowsky, H. Scholz](#)
 16:00 [Vitamin A nutrition of cockatiels - E.A. Koutsos* and K.C. Klasing: book](#)
 16:25 [Determination of 25-Hydroxy Vitamin D in seed fed grey parrots - M.D. Stanford: book](#)
 16:50 [Differential absorption of natural versus synthetic alpha-tocopherol in Asian and African elephants - J.E. Swanson, R.A. Pulver, R.S. Parker, E.S. Dierenfeld: book](#)
 17:15 [Investigations on the influence of dietary cobalt supply on the vitamin B₁₂ status of dairy cows - Kirsten Stemme, U. Meyer, G. Flachowsky, H. Scholz](#)

Sunday, AUGUST 25

LIPIDS

- 8:30 [Individual variation in pre-hibernation polyunsaturated fatty acid intake and its effect on over-winter survival by golden-mantled ground squirrels, Spermophilus lateralis - Wendy R. Hood and Craig L. Frank](#)
 8:55 [Effect of a sub-maintenance, low-fat diet on body composition in Steller sea lions - D.A.S. Rosen, A.W. Trites](#)
 9:20 [Do cats need arachidonic acid in the diet for reproduction? - James G. Morris](#)
 9:45 [Milk fat synthesis and draw down on body fat in the muskox \(Ovibos moschatus\) - R. G. White, W.E. Hauer, R. Kedrowski](#)
 10:10 [Measuring nutrient intakes of free-ranging animals - Stuart A. Altmann: book](#)

10:35 BREAK & POSTER SESSION

posters on LIPIDS

- [Different dietary fat sources on broiler performance - A. Haghazadeh, G. Rahimi](#)
[Fatty acid composition of plasma and red cells in a group of captive asian \(Elephas maximus\) and African \(Loxodonta africana\) elephants - M. Clauss, Y. Wang, K. Ghebremeskel, W.J. Streich, C. Lend: book](#)
[Effects of extruded linseed supplementation on the milk fatty acids pattern of dairy ewes - P. Rondia, Y. Larondelle, Ch. Delmotte, F. Dehareng, J. Fabry, J. Laloux, X. Derycke, N. Bartiaux-Thill](#)

posters on MINERALS

- [Dietary considerations for iron storage disease in birds and implications for high vitamin A contents of formulated bird foods - Debra McDonald: book](#)
[Observations regarding the capacity of selenium to intensify the activity of vitamin E in chickens encephalomalacia - Adriana Orasanu, Jenica Bucur, N. Alexandru, St. Nicolae](#)
[Diet and diet related diseases in captive Short-tailed Leaf-Nosed bats \(Carollia perspicillata\) - A. de Boer, S. van Hall, J. Govers, P. Veenliet and T.R. Huisman: book](#)

MINERALS

- 11:00 [Black tea consumption, iron status and risk for cardiovascular disease - David J. Baer, Joseph T. Judd, and Michael Davies](#)
- 11:25 [The role of ferritin in iron storage in birds - G.M. Dorrestein, Y.R.A. van Zeeland, A.B. Vaandrager, P.C.J. Dorrestein : book](#)
- 11:50 [Biotin is the first limiting nutrient for the growth of salmonella in chickens and iron is second limiting - Kirk C. Klasing](#)
- 12:15 [A process-based model to estimate air emissions from animal feeding operations - Jamie S. Jonker; Christopher Rogers; Perry R. Hagenstein; Robert G. Flocchini; Charlotte Kirk Baer](#)

immunonutrition
&
health promoting nutrition

(Hill's session)

Chairpersons : Jacques Debraekeleer (Belgium-UK) and Jürgen Zentek (Austria)

KEY-NOTE LECTURE

Nutrition and immune function in the aged: clinical implications and molecular mechanisms

Simin Nikbin Meydani, D.V.M., Ph.D.

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The influence of nutrition on immune function has been recognized since the 1800's. The incidence of neoplastic and infectious diseases is increased in the aged, as is the resulting morbidity and mortality. The well-documented age-associated dysregulation of immune function is an important contributor to the increased incidence of mortality from these diseases. The underlying mechanisms for this age-associated immune dysregulation is not well understood; however, since marginal deficiency of several nutrients is prevalent in the aged, and because nutrients play a key role in regulation of the immune response, these deteriorations have been partly attributed to the poor nutritional status of the aged. In the last twenty years, we have investigated the role of nutrients in age-associated dysregulation of the immune function, developed strategies to reverse/reduce these changes and their clinical manifestations, and elucidated some of the cellular and molecular mechanisms involved in these interactions. The results from these studies and their implications for maintaining optimal nutrient intake and health status of the aged will be discussed.

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KEY-NOTE LECTURE

Nutrition and immunity: a key focus in production and health

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Prevention and treatment of disease are primary concerns of those involved in the production of domestic food animals and the maintenance of companion animals. Maintaining an abundant supply of food and animal products is vital to both human and animal well-being from the perspective of both consumers and producers. There are many potential causes for immunosuppression including inadequate nutrition. The relationship between specific nutrients such as protein, fatty acids, vitamin E, selenium, copper and zinc have been well-documented in altering growth, feed efficiency, reproduction, stress response to handling and disease challenge, carcass and milk quality and yield, drug usage, and environmental contamination. Many areas across the world have marginal or deficient nutrient stores in soils and plants, making optimal animal production and health a challenge. Over the years, studies have elucidated the physiological demands that production and disease place on nutrient utilization, and identified various cellular and molecular mechanisms involved in these interactions. Work in these areas continues to advance our knowledge base and develop creative methods of improving animal health and production through nutrition. A summary of specific nutrient-related immune challenge studies in food and companion animals will be discussed.

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The effect of sprouted barley-corn with antioxidant properties on the humoral immune response of turkey poults

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Objectives: The aim of this study was to evaluate the acceptance of a growing and fattening feed for turkeys containing 28 % of starch of sprouted barley-corn only and a diet containing 28 % of starch of sprouted barley-corn and 10 % bruised barley-sprouts with antioxidant properties (tocopherol 9.93 mg/kg; tocotrienol 41.27 mg/kg) and their effect on the humoral immune response of turkey poults.

Methods: Two experiments were conducted in the current study. Three groups of 19 one-day-old male poults were placed each in a floor-pen in experiment 1. The first feeding-phase lasted until the end of week 3 and the second phase until the end of the trial (week 8). Group 1 was fed commercial turkey feed as a control diet, group 2 was fed a diet containing 28 % of starch of sprouted barley-corn and group 3 was fed a diet containing 28 % of starch of sprouted barley-corn and 10 % bruised barley-sprouts, (additional feed-ingredients: extracted soybean meal, minerals, vitamin and traceelement supplementation, rapeseed oil, wheat, corn, methionin, lysin). The poults were vaccinated against Newcastle disease virus (NDV) at the age of two weeks. Anti-NDV antibody titers were measured on the day of vaccination, on day 35 and at the end of the trial, on day 56. In the second experiment two groups of 19 one-day-old female poults were placed each in a floor-pen. Group 1 was fed commercial turkey feed as a control diet and group 2 was fed a diet containing 10 % bruised barley-sprouts. Housing, feeding and vaccination against Newcastle disease virus and measurements of anti-NDV antibody titers were arranged as in experiment 1.

Statistical evaluation

All variables were evaluated using the Kolmogorov-Smirnov method in order to assess their normal distribution. The statistical evaluation of the body weight gains was performed using analysis of variance. The anti-NDV antibody titers were compared using the Mann-Whitney-U-Test.

Results: Feed intake, body weight gains and feed conversion (kg feed/kg weight gain) were in the normal range in both experiments, (average body weight gains of male turkeys in experiment 1: 4050.53 g, SD 496.06 g; average body weight gains of female turkeys in experiment 2: 3203.95 g, SD 411.2 g). The acceptance of sprouted barley-corn and starch of sprouted barley-corn and its use as fattening feed was shown by the results of feed consumption and feed conversion. In experiment 1 anti-NDV antibody titers of poults fed the diet containing sprouted barley seemed to show a better antibody response than poults fed the control diet and experiment 2 showed a significant difference between both groups.

Conclusion: Diets containing 28 % of starch of sprouted barley-corn and 10 % bruised barley-sprouts can be used as feed. The fattening yield was in the normal range in both experiments. In both experiments no significant difference in fattening yield and feed conversion could be observed. In experiment 1 the immune response in group 3 (starch and bruised barley-sprouts) was significantly higher on day 35 and no antibody response could be observed in group 2 (starch) on day 56. In experiment 2 the anti-NDV antibody-titers were significantly higher in group 1 (control diet) on day 14, equal in both groups on day 35 and significantly higher in group 2 (bruised barley- sprouts) on day 56 than in group 1.

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Effect of full-length and truncated IgY on the nutritional cost of infection in mallard ducks (*Anas platyrhynchos*).

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During the initial stages of infection, a series of metabolic and behavioral changes occur within an animal that are collectively referred to as the acute phase response (Suffredini *et al.*, 1999). These changes include anorexia, lethargy, somnolence, and repartitioning of nutrients away from growth and other physiological processes and toward immunological processes (Klasing, 1988). Collectively, the acute phase response results in a detrimental impact on growth and nutrition-related physiology by altering body conditioning and energy metabolism. Ducks (*Anas platyrhynchos*) produce two isoforms of the immunoglobulin ν -chain; a full-length isoform (IgY), and a truncated isoform (IgY Δ Fc) lacking a Fc region responsible for mediating most effector functions (Warr *et al.*, 1995). The lack of a Fc region would presumably weaken the ability of the IgY(Δ Fc) isoform to stimulate the acute phase response, and consequently minimize nutritional losses associated with fighting infection. To examine this relationship, fixed *Escherichia coli*-antibody complexes with various ratios of IgY:IgY(Δ Fc) (100%:0; 50%:50%; 0:100%; 0:0 [-control]) were injected intravenously into naïve mallard ducks and the acute phase protein hemopexin and IL-1 β mRNA levels were determined. *E. coli*-antibody complexes were injected intravenously into the femoral vein and serum was collected at 0 and 24 hours for hemopexin analysis, while liver and spleen samples were collected at 2-h post-injection for determination of IL-1 β mRNA expression. Hemopexin and liver IL-1 β mRNA levels were significantly effected by treatment with ratios of IgY:IgY(Δ Fc) at 100:0 and 0:100 resulting in their highest production ($P < 0.05$). Spleen IL-1 β mRNA decreased directly with increasing IgY(Δ Fc) ($P < 0.05$), and IgY:IgY(Δ Fc) ratio of 0:100 did not differ from no immunoglobulin (- control). These results indicate that the absolute ratio of IgY:IgY(Δ Fc) results in differential regulation of the acute phase response across organs, and that equimolar ratios of the two isoforms serve to down-regulate the acute phase response and nutritional costs of infection. The predominance of IgY(Δ Fc) upon repeated exposure to antigen represents a unique immunological niche in mallard ducks not found in mammalian or chicken systems that can serve as a model to explore the impact of mounting an immune response on the nutritional status of an animal.

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Systemic and pulmonary antioxidant status in equine recurrent airway obstruction (RAO)

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Recurrent airway obstruction (RAO, formerly known as equine chronic obstructive pulmonary disease, COPD) is a condition with many similarities to human asthma and has been estimated to be the most common medical condition of horses in Northern Europe. Recently we have reported that ascorbic acid (AA) is the major non-enzymatic antioxidant in equine epithelial lining fluid (ELF) and is markedly reduced in RAO-affected horses in crisis (i.e. with marked airway inflammation). Here we present systemic and pulmonary ascorbic acid and glutathione measurements in RAO-affected horses in both crisis and remission. Remission being defined as bronchoalveolar lavage [BAL] neutrophil numbers less than 20 cells/ μ l).

	Non-RAO (controls) n=8	RAO (Crisis) n=6	RAO (Remission) n=6
Plasma AA (μ mol/l)	19.5 \pm 4.6 ^a	7.2 \pm 1.0 ^b	8.6 \pm 2.1 ^b
Plasma ARR (%)	0.8 \pm 1.7 ^a	8.2 \pm 7.3 ^b	10.9 \pm 3.5 ^b
ELF AA (mmol/l)	2.0 \pm 0.7 ^a	0.3 \pm 0.4 ^b	1.1 \pm 0.7 ^{ab}
ELF DHA (mmol/l)	0.1 \pm 0.2 ^a	0.4 \pm 0.5 ^a	0.1 \pm 0.1 ^a
ELF tAA (mmol/l)	2.1 \pm 0.7 ^a	0.7 \pm 0.4 ^b	1.2 \pm 0.6 ^b
ELF ARR (%)	6.7 \pm 9.2 ^a	57.4 \pm 46.3 ^b	11.9 \pm 15.2 ^a
RBC GSH (mmol/l)	1.26 \pm 0.25 ^a	1.39 \pm 0.38 ^a	1.29 \pm 0.40 ^a
RBC GRR (%)	3.2 \pm 1.5 ^a	5.0 \pm 0.6 ^a	4.0 \pm 0.7 ^a
ELF GSH (μ mol/l)	52 \pm 13 ^a	39 \pm 52 ^a	132 \pm 63 ^b
ELF GSSG (μ mol/l)	5 \pm 4 ^a	41 \pm 40 ^b	8 \pm 4 ^{ab}
ELF TGSH (μ mol/l)	57 \pm 12 ^a	80 \pm 42 ^{ab}	140 \pm 64 ^b
ELF GRR (%)	9.1 \pm 8.4 ^a	60.6 \pm 42.5 ^b	6.6 \pm 3.4 ^a
BAL neutrophils (%)	3 \pm 2 ^a	24 \pm 22 ^b	2 \pm 2 ^a
BAL neutrophils (/ μ l)	8 \pm 5 ^a	28 \pm 11 ^b	3 \pm 2 ^a

AA ascorbic acid; DHA dehydroascorbic acid; tAA = AA+DHA; ARR ascorbate redox ratio (DHA/tAA*100); GSH reduced glutathione; GSSG oxidised glutathione; TGSH (GSH+GSSG); GRR glutathione redox ratio (GSSG/TGSH*100). Differences between groups significant at least at P<0.05 (ANOVA). Cells in same row with different superscripts differ by at least P<0.05 (Newman-Keuls). RAO affected horses in crisis have significantly increased numbers of neutrophils in BAL and show evidence of both systemic and pulmonary oxidative stress based on plasma ARR and ELF ARR and GRR compared with non-RAO affected controls. In remission, when the BAL neutrophil numbers are no longer elevated, ELF ARR and GRR are not different from non-RAO affected controls. However, plasma AA and ELF tAA are still lower and plasma ARR, ELF GSH and TGSH are increased compared with the non-RAO affected controls. In RAO-affected horses in remission, systemic oxidative stress persists and may no longer be related to pulmonary events. However, despite an absence of pulmonary oxidative stress, there is decreased AA status, which is only partially compensated for by a small increase in GSH.

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Antioxidant Supplementation in Horses Affected by Recurrent Airway Obstruction

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Recurrent airway obstruction (RAO, formerly known as equine chronic obstructive pulmonary disease, COPD) is an asthma-like condition of the horse characterised by periods of acute airway inflammation, pulmonary oxidative stress and bronchoconstriction (crisis), and periods of remission. We have previously demonstrated that ascorbic acid is quantitatively the major non-enzymatic antioxidant in equine pulmonary epithelial lining fluid (ELF). In addition, the concentration of ascorbic acid in plasma and ELF is significantly reduced in RAO-affected horses in both crisis and remission compared to healthy controls. We therefore studied the effects of a dietary supplement containing a mixture of natural antioxidants including vitamin E, ascorbic acid and selenium (WINERGY® VENTILATE™). Using a cross-over design study with a four week washout period, the supplement was provided for four weeks, during which we examined its effects on the systemic and pulmonary antioxidant status in five RAO-affected horses in clinical remission. The horses were housed in stables and performed regular exercise. Remission was defined as bronchoalveolar lavage neutrophil numbers less than 20 cells/μl.

Antioxidant supplementation significantly increased the plasma ascorbic acid concentration ($21 \pm 4 \mu\text{mol/l}$) compared to a placebo ($11 \pm 3 \mu\text{mol/l}$, $p < 0.05$), but had no effect on the ELF concentration of ascorbic acid ($2.9 \pm 3.0 \text{ mmol/l}$ and $1.5 \pm 0.9 \text{ mmol/l}$, respectively) or the plasma and ELF concentrations of dehydroascorbate (the oxidised form of ascorbic acid). The concentration of glutathione in red blood cell haemolysate and ELF was also unaffected by antioxidant supplementation, however the concentration of oxidised glutathione (GSSG; $14 \pm 24 \mu\text{mol/l}$) in ELF was significantly reduced compared to the placebo ($39 \pm 54 \mu\text{mol/l}$, $p < 0.05$). In summary, antioxidant supplementation increased the concentration of plasma ascorbic acid in RAO-affected horses in clinical remission, such that the concentration was similar to that of healthy horses, but had no significant effect on the concentration of the major non-enzymatic antioxidants in ELF over the time period studied. However the supplement did reduce the concentration of GSSG in the ELF, suggesting that the antioxidant supplement decreased the degree of pulmonary oxidative stress.

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Effects of Tasco (a brown seaweed) and heat stress on immune function and antioxidant activity of wether lambs

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Aim: Over the years investigators have attempted to overcome the endophyte fungus-associated production and health consequences (fescue toxicosis) to livestock managed on tall fescue (*Festuca arundinacia*, Schreb.) with minimal success. Recent work indicates a reversal of adverse clinical signs and diminished immunocompetence in livestock endophyte-infected (EI) tall fescue treated with a brown seaweed extract, Tasco. Grazing and feedlot-finishing study data demonstrates improved animal health and carcass quality, most likely via an antioxidant related mechanism.^{3, 4} To date, the effects of Tasco supplementation to post-harvest forage have not been reported for livestock production systems. The purpose of our study was to evaluate select innate immunity and oxidative stress in response heat stress in a Tasco-fescue hay system.

Methods: EI tall fescue-based pasture received 0 or 3kg/ha of Tasco-Forage (an extract of the brown seaweed *Ascophyllum nodosum*) prior to harvest and was compared to direct-feeding Tasco-EX (an extract of *A. nodosum* in liquid form). Twenty-eight wether lambs (initial avg BW 40 kg), blocked by weight, were randomly assigned to one of three diets. Diets were 1) control hay, 2) treated hay, and 3) #1 + Tasco-EX fed at an additional 1% of the as-fed diet. Heat stress was applied for 10 d following a 27-d diet adjustment period with measurements obtained at d4 and 10.

Results: Tasco-EX application increased monocyte phagocytic activity (P=0.01) in lambs compared with pre-harvest (Tasco-Forage) and control diets. Heat stress influenced cell activity across all treatments (P<0.0001). Phagocytic cells obtained from Tasco-EX treatment lambs exhibited increased (P<0.05) capacity for oxidative burst as compared to Tasco-Forage and control lambs. Phagocytes obtained from the Tasco-EX lambs maintained their capacity for oxidative burst throughout the 10 d heat stress period (P<0.01). Cell function decreased in response to heat in control and Tasco-Forage lambs. A heat x diet interaction was apparent (P=0.10). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) was consistently higher in leukocytes from lambs on both Tasco-treatments vs control during heat stress periods. Minimal (P<0.001) cell peroxidation occurred during heat stress due to Tasco-EX treatment.

Discussion: Pre-harvest treatment with Tasco-Forage to tall fescue appeared to provide residual effects on animal antioxidant availability in short-duration (4-d) heat stress. Post-harvest Tasco-EX supplementation to tall fescue hay enhanced immune function in wether lambs and protected them against prolonged heat-induced oxidative stress. Hay treatment with Tasco has the potential to provide substantial health benefits to ruminants in sub-optimal production scenarios.

References:

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⁴ Allen, et al., 2001. J Anim Sci 79(E. Suppl.):E21-31.

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Influence of lactulose as feed additive on chyme composition and intestinal microflora in pigs infected artificially with *E. coli* O139:K82 and *Salmonella* Derby

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Objectives: By addition of lactulose as prebiotic to the diet benefits are expected for growth and increase of bifidobacteria and lactobacilli, while clostridia, *E. coli* and salmonella should be inhibited. In a study with weaned piglets Sutton and Patterson (1996) observed a reduced concentration of *E. coli* (K88⁺) on days 6-10 following to weaning when fed 1% lactulose. Also in a field study with salmonella infected fattening pigs (Wiemer 1999) lactulose (0.7 g/kg BW) caused a significant reduction of salmonella excreting pigs in herds. Aim of the study was to investigate the potential of lactulose to prevent the incidence of edema disease in weaning piglets and to reduce the excretion and translocation (from the intestine to other tissues) of *Salmonella* Derby in fattening pigs.

Methods: The pigs were offered a starter diet ad libitum containing 5 % lactulose preparation (2.51 % lactulose) for treatment groups and corn starch in exchange for the control groups (per kg DM: 15.2 MJ ME; 170 g crude protein; 33 g crude fiber). Five days post weaning 20 piglets (two groups each of 5 treatment and 5 control piglets; Ø 7.6 kg BW) were orally infected with *E. coli* O139:K82 (1. group: $3.7 \cdot 10^9$ cfu; 2. group $2.3 \cdot 10^{10}$ cfu). For the study on *Salmonella* Derby fattening pigs (Ø 60.6 kg BW) received an infective dose of $2.6 \cdot 10^9$ cfu per os. Feed intake, animal health as well as the dry matter content and pH-value of faeces were controlled daily. Also counts of *E. coli* O139:K82 and qualitative analysis of *Salmonella* Derby in faeces were measured (culture method) on several days. Pigs without clinical signs of edema disease were killed 8 days post *E. coli* infection. Observation of *Salmonella* Derby infected animals lasted up to 42 days. The removed intestinal tract was divided into stomach, small intestine (part 1-3), caecum, colon ascendens and rectum. In the chyme dry matter content and pH-value, concentrations of short chain fatty acids, ammonia, l-lactat were estimated. Also counts of bifidobacteria and *E. coli*/*Salmonella* Derby were estimated. Additionally for fattening pigs the translocation of *Salmonella* Derby into *ln. jejunalis*, *ln. ileocolici* and the tonsils were investigated by enrichment method.

Results: No differences were determined for feed intake, dry matter content and fecal pH, but in several parts of the intestinal tract pH-values and the concentration of ammonia were significantly lower in the treated groups. In both of *E. coli* infected groups the counts of *E. coli* in faeces and number of piglets showing clinical signs of edema disease were similar. A higher number of *E. coli* O139:K82 was observed in colonic chyme of piglets with apparent edema disease than in healthy controls. In *Salmonella* Derby infected fattening pigs excretion and the frequency of translocation were similar for treated and control pigs. More pigs of the treatment group than controls showed *Salmonella* Derby in samples of small intestinal (part 3) and colon mucosa and chyme. The counts of bifidobacteria in intestinal chyme (small intestine part 3, colon) were similar in both groups.

Conclusion: These experiments with artificial infection failed to demonstrate positive effects of lactulose addition to the diet on the incidence of edema disease in weaned piglets and the excretion and translocation of *Salmonella* Derby in fattening pigs. Preventive effects of lactulose on colonisation by these pathogenic bacteria were not observed.

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Dietary effects on bifidobacteria and *Clostridium perfringens* in canine faeces

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Dietary effects on the intestinal microflora have gained increasing interest due to the increasing evidence that a balanced microecology in the gut is important for health and well being. Bifidobacteria are considered as representing the ‘beneficial’ bacteria whereas *Clostridium perfringens* may have negative impacts on the digestive system. The composition of the diet is one of the key factors for the control and the balance of this intestinal ecosystem. The aim of the present study was to evaluate the effect of two different diets on faecal Bifidobacteria and *Cl. perfringens*. The diets fed differed in the level and quality of protein, as well as in the presence or absence of a prebiotic fibre source.

Material and methods: Two extruded, dry diets, one supplemented with 1.5% non digestible oligosaccharides (NDO) and the other with 3 % glucose (Glu) were compared to a protein rich wet diet (PR+) based on low quality animal derived protein sources. Nutrient compositions are given in table 1. Nine adult beagles or beagle-mongrels (age 8.7±2.0 years;

Table 1: Protein, fat and fibre concentrations in the experimental diets (g/kg, dry matter basis)

	Diet		
	PR+	NDO	Glu
Crude protein	726	265	250
Crude fat	124	106	120
Crude fibre	36.9	23.3	24.0

body weight 11.7±1.9 kg) were randomly assigned to 2 groups (A and B). The feeding schedule was designed as a consecutive cross over trial with each feeding period lasting 3 weeks. All dogs started with diet PR+, after which group A dogs received Glu and B dogs received NDO. After an intermediate wash-out period with diet PR+ for 3 weeks the A

dogs were switched to NDO and B dogs were switched to Glu. In the final period all dogs were fed with diet PR+. In all experimental periods faecal consistency was scored using a 5 point visual scale scoring system (1= hard, crumbly faeces, 3 = ideal, 5= watery diarrhoea). Additionally fecal samples were collected during each period for dry matter and pH measurements. Fecal bifidobacteria and *Cl. perfringens* were quantified at the end of each feeding period. **Results:** Consumption of dry diets increased faecal dry matter and reduced fecal pH from 6.9-7.4 with the high protein diet to 5.9-6.5 with the dry diets. The dry diets induced a firmer faecal consistency, with no significant difference between NDO or Glu. *Cl. perfringens* was found in all faecal specimens after feeding PR+ with bacterial counts of log 8.2 - log 8.8 cfu/ g faeces. Both dry diets reduced the counts of *Cl. perfringens* significantly (log 3.3-4.0 cfu/g faeces). Switching from the dry diets to the high protein wet diet induced an increase of *Cl. perfringens* within 1 day, independent of the previous diet. In dogs fed PR+, bifidobacteria were detected in only four faecal samples and exclusively in the initial feeding period. During the remainder of the experiment the counts fell below the detection limit (log 3.3 cfu/g feces). The faecal concentrations of bifidobacteria increased with both dry diets. Slightly higher concentrations (log 9.6 - log 9.7cfu/ g faeces) were obtained from dogs fed the dry diet containing NDO compared to the diet containing glucose (log 9.3 - log 9.4cfu/ g faeces). The small increase may be related to the low level of NDO added to the diet as well as to the rapid switch between diet. In **conclusion**, the study demonstrates a clear antagonistic pattern of bifidobacteria and *Cl. perfringens* in dogs. A low protein diet favoured the growth of bifidobacteria and reduced the faecal levels of *Cl. perfringens* significantly. These results suggest that the higher carbohydrate level favors a higher rate of fermentation with a decrease in fecal pH, which stimulates the growth of bifidobacteria.

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Dietary resistant starch supplementation restores the integrity of rat inflamed colonic mucosa

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Introduction : End-products of the cæco-colonic microbial fermentation of dietary fibres, acetate, propionate and butyrate represent the main short-chain fatty acids (SCFAs). Among them, butyrate presents prophylactic and therapeutic interest in the pathogenesis of inflammatory bowel diseases (IBD). Some dietary substrates such as resistant starch (RS) (type 3, Novelose 330[®]) are known to yield high levels of colonic butyrate and would be a promising alternative in IBD treatment. The aim of the present study was to evaluate the effect of RS on the cæco-colonic mucosa of rats with induced colitis. Involved in fibre effect, SCFAs were also investigated.

Materials and methods : A chronic active colitis was induced in 24 rats with a Dextran Sulfate Sodium (DSS) treatment [5% for 7 days then 3% DSS for 7 or 14 days]. During the chronic phase of colitis (3% DSS), rats were fed *ad libitum* a fibre-free basal diet (BD) for 7 days (BD-7) or 14 days (BD-14) or the same diet supplemented with 6% RS for 7 days (RS-7) or 14 days (RS-14). Cæco-colonic inflammation was assessed macroscopically, histologically and by measurement of myeloperoxidase (MPO) activity, an index of neutrophil infiltration into the distal inflamed tissue. Intestinal permeability was assessed by urinary recovery of iodixanol measured by high performance liquid chromatography (the greater the amount recovered, the more increased the permeability is). SCFAs were quantified in cæco-colonic contents by gas chromatography.

Results : The RS enriched diet restored part of the integrity of the inflamed mucosa. Indeed, we observed a significant decrease in macroscopic and histological scores, in MPO activity and in iodixanol permeability in RS groups compared to BD groups :

	BD-7	RS-7	<i>P</i> <i>value</i>	BD-14	RS-14	<i>P</i> <i>value</i>
Macroscopic score	6.6±1.1	6.8±1.2	<i>NS</i>	6.7±0.9	2.7±0.6	<i>0.0022</i>
Histological score	17.0±1.2	10.9±0.6	<i>0.0035</i>	15.5±0.5	11.6±1.1	<i>0.0042</i>
MPO activity (U ⁻¹ .g tissue)	123.3±27.2	55.5±11.6	<i>0.0019</i>	93.3±20.6	56.6±8.9	<i>0.0419</i>
Urinary Iodixanol (mg)	154.2±47.7	75.3±17.1	<i>0.0255</i>	92.5±29.7	29.8±5.8	<i>0.0036</i>

SCFAs and particularly butyrate (in micromoles) measured in cæco-colonic contents were significantly increased in the cæcum of RS-7 and RS-14 rats compared to BD-7 and BD-14 rats respectively.

Conclusion : A diet enriched with resistant starch (RS3) improves digestive inflammatory injuries induced by DSS in the rat's colon. This RS effect could be partially explained by a higher concentration of SCFAs, particularly butyrate, in the digestive contents. This confirms the interest of butyrogenic fibres in IBD treatment.

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Effect of oligosaccharide supplementation on an ETEC infection in piglets

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Diarrhoea caused by enterotoxigenic *Escherichia coli* (ETEC) is one of the major problems just after weaning. Antibiotics are the treatment of choice, since no vaccine is available. However, due to the risk for development of resistant bacteria, there is a search for alternatives. Oligosaccharides are known immunostimulants that activate the innate immune system, thereby enhancing protection against infections (Raa, 1996). In the present study 6 particulate oligosaccharides, differing in origin and/or extraction procedure, were tested for their protective effects against an ETEC infection. After weaning, six groups of piglets (n = 4 to 5) were fed different oligosaccharide food supplements during 14 days and whereas no supplement was given to the control group (n = 4). Three days after treatment, all pigs were inoculated with the F4⁺ ETEC strain GIS26. All piglets excreted F4⁺ ETEC in the faeces, but there was a statistically insignificant reduction in the ETEC excretion in the oligosaccharide-supplemented pigs. The two best oligosaccharides were from yeast (oligo 1) and fungi origin (oligo 6). An effect of both oligosaccharides was seen on the antibody response in serum and on the number of F4-specific antibody secreting cells (ASC) in spleen and Peyer's patches. The F4-specific IgA and IgM serum antibody response was lower in oligosaccharide fed pigs in comparison with the control pigs. The number of F4-specific ASC per 10⁷ mononuclear cells in the spleen was highest in the control pig (302 IgM ASC-14 IgA ASC) followed by the oligo1-supplemented pig (126 IgM ASC-2 IgA ASC) and the oligo6-supplemented pig (22 IgM ASC-0 IgA ASC). In the Peyer's patches, a lower number of IgM and IgA ASC were found in the oligo6- supplemented pig in comparison with the control pig. The mRNA expression of cytokines IL-6, TGFβ and IL-10 in the spleen and Peyer's patches was influenced by the fungal glucan. Results suggest a protective effect of both oligosaccharides.

The financial support of the Encouragement of Scientific research in Industry and Agriculture, Brussels, Belgium and INVE Technologies is greatly acknowledged.

Reference:

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The impact of prebiotics during salmonellosis on nutrient retention and *Salmonella typhimurium* var. Copenhagen excretion in adult pigeons (*Columba livia domestica*)

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Salmonella is a prominent intestinal pathogen for pigeons. Lactose has been shown the ability to act against Salmonella in chickens (Ziprin et al., 1991). Fructo-oligosaccharides are widely used as prebiotics and these carbohydrates have also been tested with success against Salmonella in chickens (Fukata et al., 1999).

Four groups of eight male adult pigeons (*Columba livia domestica*) were individually housed in metabolism cages and received a drinking water supplement of 2% fructo-oligosaccharides (FOS; Fibrulose® F97, Socodé, B), 2% lactose (LAC) or no supplement (CON and ZERO) for seven weeks. All pigeons were fed an amount of the same commercial feed (Quartes, Belgium) that was below normal intakes, i.e. 20 g/d. At d21 the FOS, LAC and CON pigeons were intubated with 10⁹ *Salmonella typhimurium* var. Copenhagen (STC). Three digestion trials were performed (d8-12; d15-19; d43-47) in which total excreta were collected per pigeon, excreta consistency was scored daily and water and feed intake was measured. All pigeons were weighed at the start of the experiment and at the start and the end of each collection period. Nutrient retention were calculated from proximate analyses of feed and excreta. Two pigeons (FOS, LAC) did not survive the STC challenge. At the end of the trial (d47) all remaining pigeons were killed and dissected for STC counts in crop, spleen, small and large intestine and liver. Caeca were not looked at as they are rudimental in pigeons compared to chickens. Excreta samples from d16, 18, 22, 25, 28, 36 and 43 were analysed for STC.

The pigeons lost about 77±27 g over the first week due to restricted feeding. During the first digestion trial (healthy animals), the FOS animals drank more and had less consistent droppings with higher moisture contents. Nutrient retentions did not differ between groups. During salmonellosis, feed intake and excreta consistency dropped whereas water intake increased significantly compared to the first digestion trial and to the ZERO group.

In the last digestion trial, all surviving pigeons had recovered and showed normal eating, drinking and excreta consistency, including the FOS pigeons. STC excretion was not significantly different between FOS, LAC and CON, but fibre digestion was higher with FOS and LAC compared to CON, although only significant for LAC. The ZERO group did not show any STC excretion.

The effect of LAC and FOS was rather limited, maybe because of a limited adaptation period of the intestinal flora to FOS and LAC.

References

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In vitro* studies on the effects of short-chain fatty acids on the invasiveness of *Salmonella

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Introduction. Increasing the concentration of short-chain fatty acids (SCFA) in the intestinal environment is a well-known nutritional strategy to control pathogenic bacteria in poultry. Addition of prebiotics to the feed, such as fructo-oligosaccharides, can increase butyrate concentration in the caeca. Beads coated or impregnated with formate and propionate are used as feed additives. By doing so, high concentrations of SCFA can be reached in the chicken caecum, which is the predominant site for *Salmonella* colonization. Therefore a study was carried out to evaluate the effects of SCFA on invasion of *Salmonella* Enteritidis in intestinal epithelial cells *in vitro*.

Materials and Methods. Bacteria were incubated for 4 hours in growth media, supplemented with different concentrations of the individual SCFA formate, acetate, propionate and butyrate and with mixtures of SCFA which mimic the *in vivo* situation as determined by gas chromatographical analysis of caecal contents of chickens. Thereafter, equal numbers of bacteria were incubated for 1 hour at 37°C with cells of a chicken intestinal epithelial cell line. After rinsing, gentamycin was added for 1 hour to kill extracellular bacteria, whilst letting intracellular bacteria multiply. Then cells were lysed and the number of colony forming units was determined by bacterial titration on brilliant green agar.

Results. *Salmonella* bacteria grown in propionate and butyrate supplemented medium had a significantly lower invasion of the chicken intestinal epithelial cells, compared with bacteria grown in acetate and formate supplemented medium and controls. Bacteria grown in ‘*in-vivo* like’ mixtures of SCFA had significantly higher numbers of invaded bacteria compared with butyrate exposed bacteria. Adding 5 times more butyrate to the mixtures did not change the invasion properties of the bacteria as compared to those in the original SCFA mixture.

Discussion. *In vitro* data suggest that acetate, present in high concentrations in the chicken caecum, increases invasion of *Salmonella* spp. in chicken intestinal epithelial cells, compared with propionate and butyrate. As a consequence, systemic spread of the bacteria can be enhanced. Propionate and butyrate have an inhibiting effect on epithelial cell invasion when bacteria are exposed to the individual SCFA. In contrary, increasing the butyrate concentration in *in-vivo* like caecal mixtures had no effect on epithelial cell invasion compared with SCFA mixtures without additional butyrate supplementation. This indicates that altering the butyrate levels in the caecum of chickens *in vivo*, as can be done by for example fructo-oligosaccharide addition in the feed, will not decrease invasion of *Salmonella* in intestinal epithelial cells. The effects on fecal shedding however cannot be evaluated in this model. Moreover, the high concentrations of acetate in the chicken caecum may add to the high susceptibility of newly hatched chicks to *Salmonella* infection.

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Influence of PUFA on immune cell and tissue MAPK in the feline

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Aim: Epidermal growth factor/mitogen-activated protein kinase (EGFR/MAPK) pathway is a cell-surface associated signal transduction cascade, suggested to be involved in breast cancer cell development. MAPK and its effectors are associated with tumor cell growth that may likely be modulated via the action of cell membrane-incorporated polyunsaturated fatty acids (PUFA) directly or indirectly altering the action of the EGFR/MAPK pathway ¹. The ability to identify alterations in peripheral leukocytes and mammary adipose MAPK based on dietary PUFA concentrations would be beneficial as a cancer prognosticator and anti-tumorigenic agent for the feline cancer patient. We investigated if MAPK could be detected in white blood cell (WBC) populations and mammary adipose, using a feline model, and if its activity was influenced by dietary (n-6):(n-3) PUFA ratio.

Methods: Twenty-eight domestic felines were fed one of 4 diets varying in (n-6):(n-3) PUFA ratios for 24 weeks (7cats/diet). The highest (n-6):(n-3) ratio was provided by a commercially available diet. The remaining 3 dietary treatments provided PUFA in successively decreasing (n-6):(n-3) ratios. WBC were isolated and stimulated with 13-phorbol-15-myristateacetate and analyzed for MAPK activity via flow cytometry and immunoblotting, using phospho- and total MAPK monoclonal antibodies. Subcutaneous mammary/inguinal adipose tissue was obtained from all subjects and immediately stored in liquid nitrogen. Tissue was processed and analyzed for n-6 and n-3 PUFA and active MAPK, respectively.

Results: MAPK was detectable in WBC and adipose tissue from all 28 cats. Across all time periods (P=0.02) and within each time period, the percent of immune cells staining positively for activity of MAPK was altered. Increased dietary (n-6):(n-3) PUFA was associated with increased MAPK activity in WBC. Active MAPK in WBC subpopulations appeared to be influenced by cat age and time on diet. Granulocyte populations responded (P=0.0004) to changes in dietary (n-6):(n-3) PUFA by week 12; and the mononuclear cell population responded (P=0.0069) by week 24. Dietary n-6:n-3 PUFA reflected adipose PUFA concentration across all dietary treatments. pMAPK tended to decrease from baseline through week 24 for all (n-6):(n-3) treatment groups, with the lowest (n-6):(n-3) treatment exhibiting the most profound depression (P=0.03) of pMAPK. Subjects with developing tumors exhibited a significant increase in immune cell MAPK over time that mirrored mammary adipose tissue changes.

Discussion: Our data suggests that dietary PUFA ratio and age of cat influenced MAPK activity in both immune cells and tissues in a similar manner. This implies that immune cell and tissue MAPK can be influenced by dietary PUFA; and that peripheral blood MAPK activity may be a useful indicator of MAPK activity within tumor tissues. This preliminary data suggests a mechanism by which tumor development and growth in a feline cancer model may be modified by dietary PUFA. Further investigation in this area is currently ongoing in our research lab with respect to feline cancer directly and using the feline as a model to evaluate dietary interventions for human breast cancer.

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Efficacy of a hypoallergenic diet containing soy isolate hydrolysate for the diagnosis and management of food hypersensitivity in dogs : a multicentric field study.

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Food hypersensitivity is a nonseasonal, pruritic skin disorder of dogs that is associated with the ingestion of a substance found in the dog's diet. In dogs, food hypersensitivity is the third most common hypersensitivity skin disease after flea bite allergy and atopy. Until recently, the definitive diagnosis of food hypersensitivity can only be made on the basis of elimination diets (i.e. diets made of novel ingredients for the dog). Because they are easily customized and free of food additives homemade diets have often been recommended by veterinary dermatologists for the diagnosis and the management of food hypersensitivity. However, owners are often reluctant to feed homemade diets because they are time consuming and expensive. Moreover, they are difficult to balance. Lately, elimination diets based on protein hydrolysates have become available to veterinarians. Protein hydrolysates have been used with success to prevent and treat milk allergies in babies.

The purpose of this study was therefore to assess the efficacy of a diet containing soy isolate hydrolysate for the diagnosis and the treatment of food hypersensitivity in a multicentric field study.

Materials and methods : 109 dogs from 76 veterinary clinics across Europe and presenting the clinical signs of food hypersensitivity (nonseasonal pruritic skin disease) were included in the study following thorough clinical examination and exclusion of parasitic skin diseases. Based on severity of clinical signs, oral prednisone and/or antibiotherapy were allowed at initiation of the diet for 1 and 2 weeks respectively.

Owners of those dogs were then instructed to feed exclusively and for 2 months a diet with soy isolate hydrolysate as the main source of protein (Hypoallergenic Programme, Royal Canin) as only treatment. After 30 and 60 days on the diet, dogs were re-evaluated by the veterinarian. Pruritus was scored (0= absent, 1=mild, 2=moderate, 3=severe) and skin lesions (erythema, excoriation, alopecia, ...) recorded at each visit. Owners were also asked questions to assess compliance, palatability and digestive tolerance to the diet. Dogs were considered as cured if the pruritus score was 0 and complete resolution of the skin lesions was observed.

Results : 78/109 (72 %) completed the study. Among the dogs that did not complete the study 50 % (16 dogs) were dropouts and 50 % were excluded because of poor compliance or drug treatment. In the dogs that completed the study, 25 % showed complete recovery (No pruritus and absence of skin lesion) , 41 % showed marked improvement (decrease in pruritus by at least 2 scores, minor skin lesions left) and 34 % little (pruritus improved by 1 score , major skin lesions left) or no improvement. Pruritus scores improved from grade 3-2-1 to 0 in 39 % of the dogs, from grade 3-2 to 1 in 38 % and grade 3 to 2 in 8 %. No improvement or worsening was observed in 15 %. Palatability and digestive tolerance were considered as very good by owners.

Discussion : Those results indicate that food hypersensitivity might be more prevalent in the population of dogs presented to European veterinarians than previously reported. A diet based on soy isolate hydrolysate might be beneficial for the diagnosis and treatment of food hypersensitivity in dogs.

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Parenteral nutrition with or without early enteral nutrition in young dogs with parvovirus

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Aim of this study was to investigate the effect of parenteral nutrition and early enteral nutrition in dogs suffering from canine parvovirus with regards to the clinical outcome, the xylose absorption capacity, haematology and serum biochemistry.

Material and methods: Dogs (n = 19, age: 2 – 12 months) with parvovirus (hemorrhagic gastroenteritis, age ≤ 12 months, leucopenia < 6 x 10⁹/l) were randomised either to receive parenteral nutrition for 4 days (group P, n = 9) or parenteral nutrition and additionally a hydrolysed enteral nutrition (group PEN, n = 10) from the second day from admission to the clinic. The parenteral formula contained amino acids (Aminosteril® KE 10 % without glucose: 49.8 ml/kg BW^{0.75} x day), lipids (Lipovenös® 20 %: 12.5 ml/kg BW^{0.75} x day), carbohydrates and electrolytes (Glucosteril® 50 %: 35 ml/kg BW^{0.75} x day and Jonosteril® D5: 21 ml/kg KM^{0.75} x day; Fresenius, Bad Homburg, Germany) and provided 5 g amino acids and 0.5 MJ ME/kg BW^{0.75} x day. The total infusion was administered within 10 hours. Group PEN received additionally 74 kJ/kg BW^{0.75} enterally from the second day and 148 kJ/kg BW^{0.75} for remaining days of the study. The enteral formula was hydrolysed by the addition of pancreatin and was used to hydrolyse t had the following composition; 60 % cottage cheese, 10 % sunflower-oil, 12.3 % glucose, 2.5 % egg yolk, 1.7 % mineral/vitamin supplement, 2.4 % sodium bicarbonate, 1 % pancreatin and 10 % water. Blood was collected to measure haematology and clinical chemistry. Concentrations of glucose, triglycerides, electrolytes and blood gases were measured before starting the infusion and after 6 and 10 hours. Fibrinogen, total protein, albumin, liver enzymes, total bilirubin, cholesterol, urea and creatinin were controlled daily before starting parenteral nutrition and 6 hours later. Plasma concentrations of immunoglobulin A and G were measured on day 1 and 3 of the infusion interval. At day 5 a xylose absorption test was performed in order to measure the intestinal capacity to absorb carbohydrates.

Results: In group PEN all 10 dogs survived, 2 of the 9 dogs of group PN died. The parenteral infusions were tolerated without clinical signs of distress. Early enteral feeding was tolerated by most dogs, but vomiting was observed mainly on the first day of application. Haematology and clinical chemistry showed anaemia, hyperfibrinogenaemia, hypoproteinaemia, hypoalbuminaemia, acidosis, hyperglycaemia and low serum potassium and sodium levels. In few cases bilirubinaemia, elevated concentrations of the alkaline phosphatase and azotaemia appeared. Immunoglobulin A and G plasma concentrations were low in all patients compared to aged matched controls. The absorption of xylose was decreased in both groups compared to healthy controls, when tested on the fifth day of the treatment. There was no obvious influence of early enteral nutrition compared to parenteral nutrition alone.

In **conclusion** patients suffering from canine parvovirus tolerate parenteral nutrition with comparably short infusion intervals of 10 hours. Early enteral nutrition with hydrolysed enteral liquid diet did not show clear advantages compared to parenteral nutrition, but small number of patients requires more extensive clinical work up.

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Effects of dietary antioxidant supplementation before and after oral acetaminophen challenge in cats

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Introduction: Since lipoic acid (LA), vitamin E (E), and cysteine (cys) are used to treat or prevent oxidative damage in diabetic polyneuropathy and acute hepatocellular damage in humans, antioxidants could benefit the nutritional management of similar diseases in cats. This study investigates the effects of these antioxidants on markers of oxidant damage and immune activity in cats before and after a challenge with oral acetaminophen (Ac).

Methods: Twenty-four intact adult cats were divided into 4 groups (3 male and 3 female per group, 3 of same sex per cage) and housed in a controlled environment. For 25 weeks, group A was fed a basal dry diet and groups B, C, and D received this diet supplemented with, respectively, E (2 g/kg DM) + cys (9 g/kg DM), LA (150 mg/kg DM), or all 3 antioxidants. Cats were fed daily, weighed twice weekly, and had venous blood drawn every 5 weeks. Assays done were: CBC, serum chemistry analysis, lymphocyte blastogenesis, malondialdehyde (MDA), protein carbonyl (PC), 8-OH d-guanosine (8OH-dG), reduced glutathione (GSH), free amino acids, LA and dihydrolipoic acid (DHLA). At 15 weeks, all cats received one 90 mg/kg dose of oral Ac. Clinical effects were observed and methemoglobin (MetHgb) concentrations measured before and after dosing.

Results: Health, weight, CBC and serum chemistry measures remained normal throughout the study. No notable differences arose between groups for any oxidant marker before Ac challenge except that lymphocyte blastogenesis increased significantly at wk 5 for groups C and D. For all groups, MetHgb increased significantly within 4 hours of dosing with Ac: C > D > A and B, and returned to baseline by 24 hours. Clinically, cats showed mild or no facial edema and cyanosis, which both resolved by 24 hours. Day 1 after Ac dosing, group C cats had significantly less GSH and greater 8OH-dG than controls, while group B cats had the lowest increase in PC. These measures reached baseline by the next sampling point. Plasma LA in C and D cats plateaued by wk 10, while DHLA peaked once at wk 10.

Discussion: LA, E, and cys, fed alone or in combination, produced no consistent changes in markers of oxidant damage or immune activity. After Ac dosing, LA supplementation resulted in the greatest MetHgb increase, while E + cys exerted a protective effect from LA.

Conclusion: LA does not act as an antioxidant in cats at 150 mg/kg DM, but E + cys may provide some beneficial effects.

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The effect of trial length on canine fecal microflora response to chicory ingestion

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Consumption of chicory, a rich source of the soluble fiber inulin, results in increased fecal bifidobacteria in dogs (Czarnecki-Maulden and Russell, 2000). Bifidobacteria are beneficial bacteria that help improve the intestinal environment. The length of time required for dietary changes to cause a change in fecal microflora has not previously been published. Therefore, a study was conducted to determine optimal trial length for dog fecal microflora trials. Eight Beagle dogs (4 males, 4 females) from the Nestle Purina kennel were used in the trial. Dogs ranged in age from 6 to 14 years of age. All dogs were fed a nutritionally complete extruded dry diet that did not contain chicory during the initial 4 weeks of the trial (Phase 1). This phase served as a washout to normalize fecal microflora levels. Fecal quality scoring, digestibility, and fecal microflora analysis were done for baseline comparison. All dogs were then fed the same diet containing 2% chicory for 4 weeks (Phase 2). Fecal scoring and digestibility were done on days 8-13 of each phase. Fecal samples were collected weekly for microflora analysis. Fecal microflora analysis was done by differential plating on selective media. Fecal moisture was significantly higher when the dogs were fed 2% chicory. However, this slight increase in fecal moisture did not result in decreased fecal consistency. Fecal quality was excellent in all dogs. Likewise, fecal quantity and frequency of defecation were not affected by chicory ingestion. Dry matter digestibility was significantly higher when dogs consumed chicory. This was reflected in a slight but non-significant increase in nitrogen digestibility. Fecal bifidobacteria levels were stable throughout the control phase. Fecal bifidobacteria were significantly higher than the control on days 14, 21 and 28 of chicory consumption. The average response to chicory ingestion increased linearly between days 7 and 21 ($r^2 = 0.958$, $y = 0.326x + 0.105$). Thus, while chicory consumption resulted in a significant increase in fecal bifidobacteria by day 14, the average response was greater on day 21. An increase or decrease in a bacterial population of 0.75 log cfu/g feces is typically considered by microbiologists to be physiologically significant. We therefore considered a 0.75 log increase in bifidobacteria (vs control phase) to represent a significant response to chicory ingestion. Based on this criterion, fecal bifidobacteria increased within 7 days of chicory ingestion in some dogs. Other dogs took 21 days to respond. The average response was slightly over 1 log (10 fold). However, bifidobacteria increased as much as 2 logs in some dogs. The variability in response could not be correlated with age or previous dietary history. In conclusion, chicory ingestion resulted in a significant increase in fecal bifidobacteria and digestibility. Soluble fibers such as chicory should be fed to dogs for at least 21 days in order to determine potential effects on fecal microflora.

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Influence of organic feeding and housing on “health” parameters in organic pig fattening

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Introduction

Environment, production of safe feeds and animal welfare are major points of concern in organic production systems.

Interest in organic production is growing over the last years. However, organic agriculture makes only a small percentage of agriculture and scientific knowledge on this production form is limited.

The influence of housing and feeding type, either an organic or a conventional one, on a set of “welfare” parameters was monitored in the present study.

Material and methods

A group of 32 pigs grown in an organic way and housed in an organic stable was compared to a group of 32 conventionally grown pigs that were housed in a conventional stable. In each stable 8 groups of 4 pigs were formed (each time 2 barrows and 2 gilts), equally divided over two diets, either an organic or a conventional one, thus implying 4 replicates per diet and housing combination.

The pigs were injected with bovine thyroglobuline at onset and three weeks later to obtain a specific immune response. Serum samples were taken at onset, at 3, 6, 9 weeks after onset and in the slaughterhouse. Specific antibodies titers against bovine thyroglobuline were measured in this serum samples.

In the slaughterhouse, lactate level was measured on whole blood with a lactate analyzer (accusport; Boeringer Mannheim, Castle Hill, Australia). Cortisol level at slaughter was measured in plasma of blood samples taken in the slaughterhouse.

Results

There was no clear effect of feed on the measured parameters. The organic housed pigs showed a more rapid response on the bovine thyroglobuline injection than the conventional ones. The lactate level of the organic housed pigs mounted to a significantly higher amount in the pigs out of the conventional stable than those grown in the organic stable. The cortisol level at slaughter was not influenced by housing type.

Discussion

Influences of an organic feed on “health” characteristics could not be concluded out of the findings of the present study. The difference in immune response can be due to the housing type itself but also to the difference in breeding. As housing type did not influence the cortisol level at slaughter, the higher lactate level in the conventional pigs will probably be due to a ‘training’ effect rather than to a stress effect.

This study was supported by the Belgian Ministry of Small Enterprises and Agriculture.

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Effects of lactulose as feed additive on chyme composition and intestinal microflora of weaned piglets

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Objectives: Due to its highly fermentable character lactulose is used by intestinal microflora. Effects of microbial lactulose fermentation in the large intestine proved from studies for human therapeutic and preventive use are laxation and reduced ammonia absorption. Also growth and increase of bifidobacteria and lactobacilli, and reduction of clostrides, E. coli and salmonella in intestine are described effects. In this study effects of lactulose in the diet of weaned piglets on their chyme composition and intestinal microflora were investigated.

Methods: 10 piglets (age 21-28 days, Ø 7.35 kg BW) were offered a nonpelleted starter diet (per kg DM: 15.2 MJ ME, 170 g crude protein, 33 g crude fiber) ad libitum. The experimental diet (lac.) contained 5 % of a lactulose preparation (25.1 g lactulose/kg diet) in exchange for corn starch (con.). Feed intake, dry matter content and pH in faeces were determined daily. 11-15 days post weaning piglets were sacrificed 6 h ppr. (feed removed 2 h before killing). The digestive tract was divided into stomach, small intestine (part 1-3), caecum, colon ascendens and rectum. Analyses made in chyme of different digestive parts were: fresh matter and dry matter content; pH-value; concentration of lactulose, short chain fatty acids, l-lactate, ammonia, and lipopolysaccharides and counts of aerobes and anaerobes, E. coli, enterococci/streptococci, Gram negative anaerobic bacteria, lactobacilli and Clostridium perfringens.

Results: Similar values were observed for feed intake, dry matter content and pH of faeces and intestinal chyme. The lactulose content was higher in ileal chyme compared to stomach. In caecal and colonic chyme lactulose was not detectable (n.d.). Ammonia concentration in the 1th part of small intestine (mmol/l FM con. 6.15 ± 1.63 ; lac. 3.37 ± 0.35) and caecum (mmol/l FM con. 13.5 ± 4.54 ; lac. 5.90 ± 1.66) was markedly reduced when lactulose was added to the diet. The amount and pattern of short chain fatty acids and the l-lactate content was not influenced in intestinal chyme estimated from stomach, 3rd part of small intestine and colon. The content of lipopolysaccharides showed a distinct increase in the chyme of colon ascendens. Counts of E. coli tended to be lower in small intestine 3 and were markedly reduced in colon ascendens, whereas other counts of bacteria were unaffected.

part of intestine	lactulose (g/kg DM)		pH-value				NH ₃ (mmol/l FM)				LPS (µg/g FM)				
	con.	lac.	con.		lac.		con.		Lac.		con.		lac.		
		g	±s	g	±s	g	±s	g	±s	g	±s	g	±s	g	±s
stomach		5.98	5.96	1.67	0.39	1.82	0.47	5.04	1.68	3.48	0.92	1.16	1.17	1.30	1.08
small int. 3	n.d.	25.5	16.8	6.30	0.39	6.66	0.39	5.14	2.23	3.58	1.40	7.95	7.14	2.94	1.76
colon asc.		n.d.		5.93	0.42	5.78	0.30	18.9	6.69	11.0	5.64	202 ^a	108	365 ^b	110

Conclusions: Lactulose was digested almost completely in the small intestinal tract, and did not cause a laxative effect in weaned piglets. Although lactulose in large intestine was not detectable any more the intestinal microbial ecosystem was influenced in reduction of E. coli counts in colon but lactobacilli and enterococci/streptococci were not favoured markedly. Also the capacity for fixation of ammonia was observed in several parts of intestine when lactulose was added.

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obesity & energy balance

Chairperson : Patrick Nguyen (France)

Substrate oxidation in pigs during feeding, fasting and refeeding

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The aim of the present investigation was to evaluate oxidation of protein, carbohydrate and fat during feeding, fasting and refeeding.

Materials and methods

Twelve castrated male pigs of Danish Landrace were used, with six pigs being measured in the live weight range 20-25 kg (Group A) and six pigs in the range 30-40 kg (Group B). The animals were fed with an identical diet containing 154 g/kg protein and 16.5 MJ/kg gross energy. The feeding period was carried out with the pigs being fed *ad libitum* in six days with daily collection of feed residuals, faeces and urine and measurements of gas exchange (indirect calorimetry) during the last three days. The fasting period, consisting of four days, followed immediately after the feeding period and the gas exchange was measured each day together with daily collection of faeces and urine. In the following refeeding period the pigs were fed *ad libitum* in four days with daily measurements of gas exchange and collection of feed residuals, faeces and urine. There was free access to water during the whole experiment. The ambient temperature was 22 °C and a relative humidity 60 %.

Heat production (HE) and oxidation of protein (OXF), carbohydrate (OXCHO) and fat (OXF) were calculated from O₂-consumption, CO₂-production and nitrogen excretion in urine (UN) in accordance with Chwalibog et al. (1992).

Results

The pattern of HE was identical in both groups with steep fall after one day of fasting followed by a moderate fall in the following days. The HE values increased during the refeeding period, being close to the feeding values by the end of the refeeding period. The lowest HE was observed at the fourth day of fasting and there was no significant difference ($P > 0.05$) between Group A and B in relation to metabolic live weight, with the mean value 592 (SEM 21.1) kJ/kg^{0.75}. Nitrogen excretion in urine was lowest on the last day of fasting and with the mean value of 226 (SEM 11.0) mg/kg^{0.75}.

Oxidation of protein, carbohydrate and fat in relation to the total HE showed an identical pattern for both groups. The contribution of OXP to HE was low with values between 9-11 % in the feeding and refeeding periods, decreasing to about 4 % during fasting. It is remarkable that no OXF occurred in the feeding period and during the last three days of refeeding and HE was covered entirely by OXP and OXCHO. However, there was a shift between OXCHO and OXF, with decreased OXCHO and increased OXF, from the last day of feeding to the first day of fasting and from the last day of fasting to the first day of refeeding. During the following three days of fasting no OXCHO occurred and OXF was a major substrate for HE.

Assuming no influence from previous feeding on the last day of fasting energy requirement must be covered by utilization of body substrates. The values of OXF and OXP demonstrate that 140 and 200 g fat and 14 and 20 g protein were mobilized from the body reserves on the fourth day of fasting in Group A and B respectively. The 4 days refeeding period was sufficiently long to induce a complete recovery of metabolism.

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Leptin, food intake and energy balance in pregnant and lactating mink (*Mustela vison*)

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Leptin plays an important role in the regulation of food intake and energy balance. In pregnant animals hyperleptinemia may occur which, however, neither seems to be related to body fat content nor causes a depression of food intake. The mink exhibits a low food intake and negative energy balance during the late part of their 30 days true gestation. The objective of this study was therefore to evaluate if this was connected to a gestational hyperleptinemia.

Material and methods

A total of nine 2-year-old mink dams of the standard brown colour type were measured in consecutive one-week balance periods, each including a 22 h respiration experiment by means of indirect calorimetry in an open-air circulation system, throughout gestation and during the four first weeks of lactation. Blood sampling by venipuncture was carried out at weekly intervals, except for the period around expected implantation. The separated plasma was analysed for leptin (multispecies assay), insulin and thyroid hormones (total triiodothyronine; TT3, total and free thyroxine; TT4 and FT4). Statistical analyses were performed according to the MIXED procedure in SAS. Results are reported in relation to the following average stages of gestation/lactation: Before implantation (BEFIMP; 41 d before parturition), around implantation (IMP; 30 d before parturition), and the first, second and third parts of the true gestation (GEST1/3; 22 d before, GEST2/3; 13 d before and GEST3/3; 4 days before parturition), and the first to fourth weeks of lactation (W1; 4 d, W2; 11 d, W3; 18 d and W4; 27 d *post partum*).

Results

All dams entering the experiment gave birth. The average number of kits per litter during the suckling period was 5.7. Kits gained weight at a normal rate (average weight 31 days *post partum* 192.1 g). The intake of metabolisable energy (ME) was high during BEFIMP, IMP and GEST1/3 (778, 828 and 768 kJ/kg^{0.75}, respectively) and then declining during GEST2/3 (643 kJ/kg^{0.75}) to reach a very low level during GEST3/3 (435 kJ/kg^{0.75}). Meanwhile, heat production (HE) remained stable, ranging from 626 (GEST1/3) to 711 (GEST3/3) kJ/kg^{0.75}. Hence, dams were in positive energy balance until GEST2/3 when it started to turn negative and during GEST3/3 it was clearly negative (-262 kJ/kg^{0.75}). During lactation ME intake and HE increased as lactation progressed. Plasma leptin concentrations were approximately doubled from GEST1/3 to GEST2/3, and they remained on this high level during GEST3/3 (4.1, 9.5 and 9.8 ng/ml, respectively). Immediately after parturition they declined to 4.4 ng/ml and by W4 5.3 ng/ml was recorded. Plasma insulin concentrations declined from BEFIMP to GEST3/3 and they remained low during lactation. The thyroid hormone concentrations declined during gestation, but TT4 and FT4 increased throughout W1 to W4 whereas TT3 remained stable.

Conclusions

The mink exhibited a marked gestational hyperleptinemia. Concomitantly, ME intake was low as well as insulin and thyroid hormone concentrations decreased, the latter findings being somewhat unexpected in relation to data from other species.

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Modulation of uncoupling protein (UCPs) expression in adipose tissue, skeletal muscle, and liver in obese insulin resistant dogs

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Objective: Uncoupling proteins (UCPs), including UCP1, UCP2 and UCP3, are mitochondrial proteins which are able to regulate energy balance, fatty acid metabolism and pancreatic function. Their expression can be altered by nutritional status, clinical obesity and diabetes. The tissular expression of UCPs and its modulation by long-term adaptation to hyperlipidic hypercaloric diet are still unknown in dogs. The aim of this study was to examine the UCPs mRNA expression in adipose tissue, skeletal muscle and liver in obese insulin resistant dogs.

Materials and methods: Four healthy male beagle dogs, 3-9-year old, weighing 8.80-15 kg were studied. 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. Insulin sensitivity was assessed by euglycemic hyperinsulinemic clamp technique. Adipose tissue, skeletal muscle and liver samples were taken at the beginning of the experiment and after 7 month on a hypercaloric diet. Total mRNA was extracted from samples and the mRNA expression of UCP1, UCP2, and UCP3 was measured by semi-quantitative RT-PCR.

Results: In the samples of liver and adipose tissue, only UCP1 and UCP2 were expressed. The high fat diet decreased the UCP1 mRNA level and increased the UCP2 mRNA level. Only skeletal muscle expressed the three UCPs. The mRNA expression of UCP1 and UCP3 was increased after overfeeding, whereas the UCP2 mRNA level was decreased in obese dogs.

Conclusion: It is suggested that a modulation of uncoupling protein expression in obese overfed dogs. These results could, at least in part, explain the alteration of metabolic efficiency and the insulin resistance observed in these dogs.

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Hypersecretion of TNF α and IGF1 in the development of insulin resistance

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Objective: Obesity causes or exacerbates a lot of health problems, independently as well as in association with other diseases. Among metabolic changes that are related to this pathological status, insulin resistance (IR) has retained particular attention. The main objective of this study was to identify markers that would be easy-to-use tools to follow evolution of whole-body insulin sensitivity.

Materials and methods: Seven beagle dogs were used. They were given a high-fat diet, at twice the NRC recommendation. Animals were weighed once a week. Insulin sensitivity was assessed using the euglycemic hyperinsulinemic clamp technique, before and during the period of high-fat hyperenergetic diet feeding. Blood was collected every two weeks for markers assays. The changes in plasma hormone and cytokine levels: leptin, insulin, cortisol, tetraiodothyronin (T4), prolactin, insulin-like growth factor 1 (IGF1), tumor necrosis factor alpha (TNF α), were then evaluated as well as changes in non-esterified fatty acid (NEFA) concentration..

Results: Dogs put on weight (46.8 ± 7.8 % of their initial body weight; mean \pm SE) in 21 ± 3 weeks. NEFA concentration significantly increased from 974 ± 94 μ mol/L when lean up to $1,590 \pm 127$ μ mol/L when the dogs were obese. There were no significant changes in the plasma concentrations of T4, cortisol, leptin, prolactin concentrations while three parameters exhibited significant changes. Postprandial insulin tended to exceed 40 μ U/ml from the 10th week. In a first step, IGF1 and TNF α increased regularly (from 0 to 265 ± 29.5 ng/ml and 134 ± 38.5 pg/ml respectively), and decreased from 27th week on.

Conclusion: Feeding a high-fat diet to beagle dogs for about 21 weeks induced a weight gain of more than 45% . It has been shown that such a gain was mainly fat tissue. This dramatic increase in adipose tissue could be responsible for the hypersecretion of TNF α . At the same time, the increase of nonesterified fatty acids (NEFA) could have resulted from elevated rate of lipolysis), and may have been responsible for a decrease in insulin sensitivity, which over time, may lead to failure of the pancreas β -cells and insulin deficiency. After the 27th week, the decrease in plasma IGF1 level could have resulted from cytokines action (especially TNF α). Moreover, it has been shown that TNF α may inhibit preadipocyte differentiation. These negative effects on the adipocytes might correlate with suppression of other adipocyte genes such as those encoding cellular components that mediate the metabolic effects of insulin and/or control its own secretion.

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Early effects of neutering on the energy intake and expenditure of domestic cats (*Felis catus*)

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Undesired body weight gain and increased risk for obesity result from the neutering of domestic cats. The disturbance of energy balance that drives the weight gain is unclear. In the current study, energy intake and expenditure were determined in 10 adult (2 y) male cats from food intake and 12-day double-label water washout measurements, respectively. The cats were kept in individual cages (0.67 m²) in a light and temperature (23±2°C) controlled room and continuously provided a commercial dry-type diet (ME=16 kJ/g) expected to produce an RQ of 0.85. After baseline measurements were conducted in all cats (12 d), half of the cats were neutered. A second 12 day double-label water washout procedure was conducted 17 days later. During the trial, food intake, body weight, and body composition were determined daily, weekly, and once during each washout period, respectively. The baseline observations on energy expenditure (296±11 kJ/kg), food intake (74±4 g/d), body weight (4.7±1 kg), lean mass (4.3±1 kg), and fat mass (0.3±0.0 kg) were not significantly different between the two groups. Energy intake (food intake x diet energy density) of neutered cats increased (p<0.05) an average of 37±6 % above pre-neuter levels and was greater than energy intake of intact cats, beginning the second post-neuter day and continuing through the second washout period. In contrast, from days 17-29 post neutering, energy expenditure in the neutered cats (332±32 kJ/kg) was not significantly different from pre-neuter levels (296±16 kJ/kg) nor levels found in intact cats during the first (296±16 kJ/kg) and second (353±13 kJ/kg) washout periods. During the trial, body weight slightly increased (6.7±2.5 %, p<0.06) in neutered cats but was unchanged in intact cats. Although rodent studies show that reduction in metabolic rate contributes to gonadectomy-induced weight gain, the present research shows that weight gain caused by the neutering of cats is driven by an immediate rise of food intake after neutering, without substantial change in energy expenditure. The mechanism of the effect of neutering on food intake should be determined.

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Effect of ad libitum feeding on body weight and blood metabolites in spayed female beagle dogs

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Objectives: Ovariohysterectomy can result in significant weight gain in bitches fed *ad libitum* (Houpt, 1979). The aim of this study was to determine the effect of *ad libitum* feeding on weight gain and blood metabolites in spayed female Beagle dogs.

Materials and Methods: Four young adult 2 year-old female beagle dogs were ovariectomized. First, the amounts fed of a commercial maintenance diet was adjusted during 26 weeks to maintain stable body weight (BW) (period I). Then, a high-energy commercial dog food (Royal Canin Energy Croc, crude protein 30 %, fat 20 %, ME 3890 kcal/kg as fed) was fed in large amount during 16 weeks (period II). The amount offered was twice the amount that maintained BW. Food left uneaten after 1 hour was collected and weighted. Cholesterol, triglycerides, glucose and insulin were measured every 2 weeks during period II.

Results: During period I, dogs received (mean \pm SEM) 120.9 ± 2.5 kcal/ kg BW^{0.75} to maintain BW. Mean BW at the end of this period was 13.3 ± 0.4 kg. When a high energy food was allowed in double amounts, dogs spontaneously ate significantly ($p < 0.05$) more food with food amounts corresponding to 181.9 ± 5.4 kcal/ kg BW^{0.75}. At the end of the second period, mean BW was 16.7 ± 0.8 kg, which represents a significant increase ($p < 0.05$) of BW of 21.9 %. Period II can be divided in 4 subperiods of 4 weeks. Each subperiod was compared with period I for statistical analysis. The first four weeks, mean energy consumption was 211.5 ± 9.4 kcal/ kg BW^{0.75} ($p < 0.01$) and dogs consumed all the proposed food. Then, the ingested amounts decreased spontaneously. Mean energy consumption was 180.6 ± 12.5 ($p < 0.01$), 176.1 ± 9.9 ($p < 0.01$), 159.6 ± 7.2 ($p < 0.05$) kcal/ kg BW^{0.75}, for each subperiod respectively. No significant effect was seen on blood metabolites during the second period.

Conclusion: *Ad libitum* feeding induced overconsumption and subsequent weight gain in spayed female beagle dogs. This overconsumption was observed during 16 weeks (length of the study) but was more important during the first 4 weeks. It seems that *ad libitum* feeding induced no significant effect on blood metabolites during the study.

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Evaluation of several home made diets for food allergy in dogs or obesity in cats

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Although a lot of commercial therapeutic diets are available, a homemade diet can be indicated in certain cases. Only 15% of the Belgian veterinarians never prescribe a homemade diet (De Smet and Poels, 1995). Even though many recipes for home made diets can be found in the literature, Roudebush and Cowell (1992) found 90% of the home made elimination diets prescribed by 116 veterinarians not adequate for adult maintenance of dogs and cats. If the recipe meets the requirements, problems are still possible if the owner does not follow the guidelines of the veterinarian. Substitutions are frequently made and on a long-term basis vitamin and mineral supplements are often omitted.

Eight recipes from the literature were prepared for two indications: food allergy in dogs (3), obesity in cats (4)^{1,3,4,8}. A low residue diet for dogs was also prepared². Two other formulations (food allergy in dog and obesity in cat) were prepared by the owners 4 months and several years after prescription in practice. The homemade diets were analysed for weende components and Ca, P, Na and K were analysed by ion chromatography.

Ingredients and proportions: One diet for cats had no carbohydrate source with consequently a high protein content. Two diets for cats had no supplemented fat source; one of them having a low analysed fat content. All diets contained animal protein sources. All recipes contained a supplemental calcium source but two recipes did not mention the addition of a multi vitamin/mineral supplement.

The analysed diets were compared with the recommendations for adult maintenance (AAFCO, 2002; Hand et al., 2000) One diet for cats contained only 16% protein on DM basis and another contained 78%. Even if the lower energy intake is taken into account the protein content of the high protein diet is still high and the low protein diet is below the NRC (1986) minimum. One diet for cats had a low fat content (4.0%DM), which could predispose to essential fatty acid deficiency. All other diets were between the recommended 7-14% fat for obesity treatment in cats (Hand et al. 2000). None of the obesity diets had high fibre contents. One diet for cats and one for dogs had a low calcium content (0.25 and 0.49%DM). On the other hand one diet for dogs and three diets for cats had rather high Ca contents (1.12 to 1.92%DM) although below the maximum of AAFCO. One diet for cats had a rather low P (0.4%DM) content and three diets for cats and two for dogs had a high P concentration (1.0-2.1%DM) although part of the P may not have been available in one diet since bran (phytic acid) was added. Three of the cat diets had a too low Ca/P ratio (0.52/1 to 0.69/1) and one dog diet had a higher Ca/P ratio (2.2).

Provided an adaptation of Ca and/or P supplementation in some diets and the addition of a multivitamin/mineral supplement in one diet, all diets for dogs are useful. On the contrary 2 diets for cats are not adequate at all (too low or too high protein content). One is useful if Ca, P and fat are supplemented. In two other diets a lower Ca and/or P supplementation is justified.

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Evolution of blood parameters during weight loss in experimental obese Beagle dogs

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The objectives of this study was to measure the effects of weight loss on blood parameters in 8 adult experimental obese Beagles (4 neutered males and 4 intact females, 5.5 (range 4-7) years) fed either a high protein, low starch and high fibre dry expanded diet –DP- (crude protein 44.1 %, fat 8.7 %, crude fibre 10.0 % -as fed) or a commercial moderate protein, high starch and high fibre dry diet -HF- (crude protein 21.6 %, fat 7.7 %, crude fibre 21.0 % -as fed). The dogs were allotted into 2 groups matched for sex, body scores and body weight (BW) at baseline and were fed either the DP or control HF diet for 12 to 26 weeks, until they reach their optimal BW (Diez et al., 2002). Dogs underwent hormonal and biochemical evaluation monthly (carnitine, creatinine, urea, free T4 (FT4), total T4 (TT4), plasma alkaline phosphatases (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), potassium, total proteins) or bimonthly (cholesterol, triglycerides, non-esterified fatty acids (NEFA), IGF1, glucose, insulin) over the whole study. Blood parameters were assayed by standard procedures and results were analysed by SAS Mixed Procedure for longitudinal data with treatment (diet) and sex as fixed effects.

Results. Dogs reached their optimal BW within 12 to 24 weeks for the HF group and 21 to 26 weeks for the DP group (Diez et al., 2002).

Before weight loss, plasma triglycerides and cholesterol concentrations were respectively (mean \pm SEM) 0.75 ± 0.02 and 2.49 ± 0.02 g/l for the obese dogs. The 2 diets decreased the plasma concentrations of these 2 metabolites but the difference was only significant for the DP diet. The basal plasma mean NEFA concentration was 0.40 ± 0.03 mM/l and increased regularly over the period with the HF diet but the difference between the 2 diets was not significant.

Over the weight loss, mean plasma carnitine concentration ranged between 21 ± 2.08 and 28.3 ± 4.9 μ M/L with no difference between the diets. Mean urea and creatinine plasma concentrations ranged respectively between 0.185 ± 0.01 and 0.358 ± 0.04 g/l and between 7.4 ± 0.4 and 9.9 ± 0.7 mg/l without difference between the 2 diets. No effect of diet was observed on overnight fasted plasma ALP, AST, ALT, potassium, TT4, FT4, IGF1, glucose and insulin which remained constant over the weight loss period. Blood urea and IGF1 concentrations were significantly higher in females than in males over the study ($P < 0.05$). Weight loss induced a decrease in FT4 plasma concentrations : 12.5 ± 0.7 ng/l in obese dogs versus 7.7 ± 0.6 ng/l at the end of the weight loss ($P < 0.001$). Mean plasma concentration of ALP was higher in males than in females ($P < 0.05$).

In conclusion, there was only an effect of diet on plasma cholesterol and triglycerides levels. Weight loss induced significant decreases in plasma cholesterol, triglycerides and FT4, whatever the diet offered. Based on blood parameters, at the tested energy intake, both diets assured a safe weight loss.

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High fat/high energy diets and body weight regulation in cats

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Most animal species overeat when given unlimited access to a highly palatable, high-energy foods, and deposit the excess calorie intakes as body fat stores. It is unclear whether cats can regulate their energy intake to maintain body weight when given foods differing in energy content. This study investigated the effect of ad libitum feeding of extruded dry cat foods differing in energy content on body weight regulation. Thirty-seven cats 2-13 yrs old were allocated to two groups matched for body weight and food intake following a stabilisation phase (3 weeks) on dry cat food. Group one (n=18) was fed a low fat dry cat food (32% protein, 10% fat, and 3.2 kcal/gram) and group two (n=19) was given a high fat dry cat food (32% protein, 20% fat, and 4.0 kcal/gram) for a period of 11 weeks. Food intakes were recorded daily, body weights twice weekly, and body condition was scored every three weeks by double blind veterinary examination on a 1-4 point scale from very thin (1) to obese (4). Girth and limb measurements were done at 11 weeks to predict body fat content. Cats fed the high fat diet gained significantly ($p<0.01$) more (9.4%) body weight than cats fed the low fat diet (2.8%). Food intakes did not differ significantly between the groups with cats in group two eating slightly more calories (248 kcal vs 237 kcal/day) and slightly lower food volume (63 vs 71 grams/day). The profile of individual body weight changes in cats fed the high fat diet showed that 3 cats had excessive weight gain ($> 15\%$) and 7 cats gained 10-15% of their starting weights. Only 25% cats maintained their body weights ($\pm 5\%$). These changes in weight gain were reflected in increases in subjective body condition with 52% of cats consuming the high fat diet rated overweight or obese. The high fat fed group also had significantly higher ($p<0.05$) levels of body fat at the end of the trial (29.8%) than the control group (26.4 %) as assessed from girth and limb measures. Cats receiving the high fat food were then switched to the low fat diet; intakes and bodyweight changes were measured for an additional 9 weeks. These cats lost an average of 207 g body weight over this period primarily associated with a reduction in energy intake (248 kcal to 196 kcal/day). Therefore, consumption of a high fat, high calorie dry cat food can override calorie regulating mechanisms in most adult cats and can lead to weight gain and increased fat deposition.

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Effect of ovariectomy on daily energy requirement in beagle dogs

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Objectives: Ovariohysterectomy can result in significant weight gain in bitches fed ad libitum (Houpt, 1979), but when fed a restricted amount of food and exercised regularly ovariectomized bitches may not gain weight (Leroux, 1983). The aim of this study was to determine the daily energy requirement (DER) in adult bitches to maintain optimal body weight (BW) after ovariectomy.

Materials and Methods: Four young adult 2 y -old female beagle dogs were ovariectomized. The bitches were housed together in their usual kennel. BW at the day of the sterilisation was (mean \pm SEM) 13.7 ± 0.8 kg, and was considered their optimal BW, based on the body condition score using a 9-points scale. Food consumption and BW were checked weekly during 32 weeks, starting 6 weeks before surgery (period I) until 26 weeks after surgery (period II). After surgery, the amount fed was reassessed and adjusted if necessary each week to maintain optimal BW by a reduction or an increase of 5% of the amounts offered. Dogs received a food for adult maintenance (Royal Canin Premium Croc, crude protein 24.0 %, fat 16.1 %, and 3730 kcal/kg as fed) during the whole study. Statistics were proceeded by analysis of repeated measurements with an autoregressive AR (1) structure (Proc Mixed, SAS).

Results: Mean BW during period I was 14.0 ± 0.3 kg. During period II, mean BW was 13.7 ± 0.1 kg which corresponds to optimal BW. Before ovariectomy, dogs received 171.6 ± 3.1 kcal/ kg BW^{0.75}. After surgery, energy offered had to be significantly ($p < 0.01$) decreased to maintain ideal BW. During period 2, dogs received 120.9 ± 2.5 kcal/ kg BW^{0.75}, which corresponds in a 30% decrease.

Conclusion: These results suggest that ovariectomy can induce a significant decrease of DER in female Beagle dogs. The underlying mechanism is unknown. A control of the food intake seems necessary to maintain ideal BW after gonadectomy.

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Home made diets for cats and dogs: do calculated nutrients approximate analysed nutrients?

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When homemade diets are used, two kinds of problems are prominent: the diet and the owner. Roudebush and Cowell (1992) noted that 90% of the hypoallergenic home made diets prescribed by 116 veterinarians in the US were not adequate for maintenance of adult dogs and cats. The second problem is the accuracy and precision by which the owner follows the guidelines of the veterinarian. Several recipes are available in literature, sometimes with calculated nutrient contents but few recipes are analysed or tested in vivo.

The aim of the present experiment was to compare the calculated nutrient content with the analysed nutrient content of several recipes^{1 3 4 5 8} indicated for food allergy in dogs and obesity in cats. For each indication, the pet owners also prepared diets that were formulated at the lab. Nutrient contents were calculated using tables^{2 7}. Proximate analysis of the diets was performed. Ca, P, Na and K were analysed by ion chromatography. The differences between the calculated and analysed nutrient content were evaluated statistically.

	Mean Paired diff.±SD	Anal./ Calc.	Anal.- calc.	Median Anal./Calc.	Range Anal./Calc.	p Paired t test	R ²	p Corr.	n
Moisture	4.3 ± 12.5	1.14	9.05	1.01	0.9-1.9	NS	0.766	0.010	10
Protein% DM	-0.14 ± 6.2	1.02	4.46	1.01	0.7-1.7	NS	0.934	<0.001	10
Fat % DM	-1.8 ± 4.9	0.91	3.63	0.79	0.5-1.6	NS	0.640	0.046	10
NFE % DM	-1.4 ± 7.5	0.98	5.5	0.96	0.8-1.3	NS	0.919	<0.001	10
Fibre % DM	2.5 ± 1.4	8.26	2.47	2.41	1.2-24	<0.001	-0.117	NS	10
Ash % DM	0.64 ± 2.6	1.14	2.14	1.18	0.5-2.4	NS	0.708	0.033	9
Ca % DM	-0.04 ± 0.27	1.64	0.22	1.08	0.6-7.1	NS	0.941	<0.001	9
P % DM	0.20 ± 0.4	1.27	0.32	1.22	0.5-2	NS	0.814	0.004	10
K % DM	0.01 ± 0.34	1.27	0.29	1.03	0.4-4.1	NS	0.795	0.006	10
Na % DM	-0.05 ± 0.55	1.69	0.42	0.83	0.12-7.7	NS	-0.266	NS	10
ME*	-262 ± 159	0.86	262.3	0.86	0.7-0.95	0.001	0.666	0.035	10

$$* \text{ME (kJ/100g DM)} = 15 * \text{protein\%} + 36 * \text{Fat\%} + 15 * \text{NFE\%}$$

The correlation of moisture content was rather limited because of moisture absorption during the cooking process. Therefore all other nutrients were expressed on dry matter (DM) content. The nutrients that were best estimated by calculation were Ca, protein, NFE, and P. Nutrients that could not be estimated by calculation were fibre and Na. In some recipes a pinch of salt had to be added. This amount is difficult to estimate, explaining the low correlation. The analysed fibre content was significantly higher compared to the calculated. The opposite was true when comparing metabolisable energy (ME) derived from analyses versus ME derived from calculated values, although digestion trials probably would have revealed higher ME because of higher digestibility of high quality ingredients.

If homemade diets are prescribed as a dietary treatment of diseases with very narrow allowances for certain nutrients, an analysis of the diet is recommended before long term prescription. In these cases a strict follow-up of the guidelines by the owner is also crucial.

¹ Brown et al. 1995, Comp. Cont. Ed. Pract. Vet.17, 637-658

² Debraekeleer et al. 2000, Small animal clinical nutrition p1123-1133

³ Kronfeld 1986, tijdschrift voor diergeneeskunde111, 137S-141S

⁴ Meyer 1990, Ernährung des Hundes p227

⁵ Remillard et al. 2000, Small Animal Clinical Nutrition p163-181

⁶ Roudebush and Cowell 1992, Veterinary Dermatology 3, 3-28

⁷ Souci et al. 1986-1987 Food composition and nutrition tables

⁸ Strombeck 1999 Home prepared dog and cat diets: the healthfull alternative p217-236

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minerals & bone metabolism

Chairperson : Ellen Kienzle (Germany)

Estimation of the mineral content of grass, hay and grass-silage

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The mineral content in grass, hay and grass-silage presents a problem for the ration calculation for horses. Mineral analysis may be considered too expensive and time consuming by the horse owners. Therefore in our investigation we reviewed the literature for the quantitative effects of well-known factors such as soil, fertilisation, plant age, plant type, and climate influencing the mineral content of plants. Our goal was to identify factors which affect mineral content in a predictable way and quantify their effect for each mineral, to be able to estimate the mineral content of fodder from parameters which may be known by the horse owner. For instance, most horse people know whether their hay is a first or second cut, and if it is a first cut, whether it is a late cut. Only effects relevant for ration calculation were considered. For the estimation of calcium and magnesium content the most important factors were the stage of vegetation (time of cutting, calcium: figure 1) and the plant type (grass, or legumes, or herbs). For the prediction of phosphorus content the effects of soil-pH, P-fertilisation and stage of vegetation were most relevant. To estimate the potassium content, potassium-content of soil, potassium-fertilization and stage of vegetation were most important. Sodium is unlikely to be predictable from parameters known to horse owners. However, in any fodder produced in Germany it is most likely that the sodium content is only a fraction of the requirements, and therefore is not relevant to the amount which has to be supplemented.

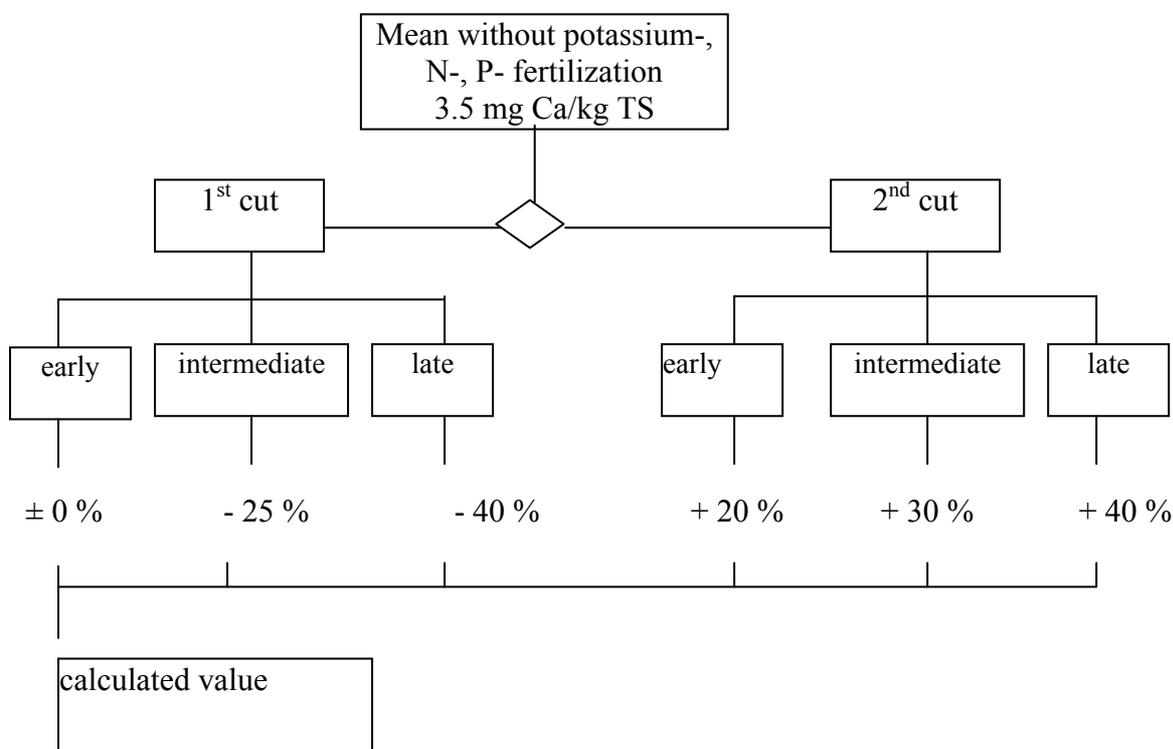


Figure 1: Influence of harvest time and plant age on the calcium content of grass-silage or hay

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Nutrition and developmental orthopedic disease in horses : results of a survey on 76 yearlings from 14 breeding farms in Basse Normandie (France)

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Developmental Orthopaedic Disease (DOD) in horses is a particularly common problem in breeding farms. The disease and its manifestations occur primarily in fast-growing light horse breeds. Lesions are reported to be present in 20 to 25% of Thoroughbreds. The major factors predisposing the growing animal to any of the DOD are (1) rapid growth, (2) trauma to the bone growth plates or articular cartilage, (3) genetic predisposition and (4) nutritional imbalances.

The aim of this survey is to identify the main nutritional imbalances in mare and foal diets which can be linked to a poor radiological score in yearlings in some French breeding farms.

During a three years survey (1997-1999) of 28 horse breeding farms of Western France (Basse Normandie), the diet of the 286 mares and their foals, and the growth of 439 foals from birth up to 2 years old have been followed. From this pool, X-ray scoring has been done on 79 yearlings in order to estimate the influence of the diet on DOD.

The energy requirement of the mares was correctly covered. The nitrogen and calcium requirements were generously covered while the copper and zinc requirements were hardly satisfied. This situation is a good reflection of the field quality in this part of the country. The breeding farms presenting the least DOD (mean X-ray score between 0 and 1) are those with the highest nitrogen ($p < 0.01$) and calcium ($p < 0.05$) supply of the mares. The energy and nitrogen requirements of 6 to 10 months old foals were met, while their mineral supply was generous however with a large variability. The breeding farms presenting the least DOD include foals who received a moderate energy and nitrogen supply, a generous calcium supply and a Ca/P ratio higher than 2:1. A copper and/or zinc supplementation, beyond the foals requirements, does not constitute a significant preventing factor against DOD.

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Growth rates and the incidence of osteochondrotic lesions in Hanoverian Warmblood foals. - Preliminary data -

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Rapid growth in foals is associated with a higher risk of osteochondrotic lesions. To date, there is limited information about the optimal growth rate in Warmblood foals. The available data encompass only a small number of foals, which may not reflect growth rates under normal conditions and the occurrence of osteochondrotic lesions. The presented study was performed to obtain information on growth rates and the incidence of osteochondrotic lesions in Hanoverian Warmblood foals under typical field conditions. This investigation was part of a larger project in which feeding, housing, and genetics were evaluated with special reference to the development of osteochondrosis in foals.

Material and methods: 629 Hanoverian Warmblood foals born between December 26, 2000 and July 01, 2001 (308 males, 321 females) from 82 farms in Germany were included in this study. Over a period of six months foals were weighed monthly on a portable electronic scale. The height of the withers at the highest point was measured using a standard measuring stick. Evaluation of osteochondrosis (OC) was performed between the fifth and tenth month after birth using x-ray. Radiology diagnosis showed 198 foals with signs of osteochondrotic lesions (OC lesions in foals: 64 tibiotarsal joints only, 112 fetlock joints only, and 22 both types of joints). The effects of sex, birth month, and growth rates on the incidence of OC were tested by analysis of variance. All results are presented as means \pm SD.

Results: Birth weights of the foals were 56.8 ± 8.0 kg (N=147). The patterns of growth rate are shown in the table below. Sex, birth month, the development of body weights, and withers heights were not statistically different between OC affected and unaffected horses.

Age (days)	body weight (kg)				withers height (cm)			
	without OC		with OC		without OC		with OC	
	male	female	male	female	male	female	male	female
0 - 30	80 \pm 17	82 \pm 19	85 \pm 16	81 \pm 15	108 \pm 4.9	108 \pm 5.0	109 \pm 4.2	109 \pm 5.0
31 - 60	120 \pm 18	119 \pm 20	122 \pm 21	119 \pm 18	116 \pm 8.9	116 \pm 4.4	117 \pm 4.7	117 \pm 4.4
61 - 90	155 \pm 19	154 \pm 21	157 \pm 22	151 \pm 21	123 \pm 4.0	122 \pm 9.0	124 \pm 4.7	123 \pm 4.0
91 - 120	189 \pm 21	188 \pm 23	189 \pm 25	183 \pm 22	129 \pm 5.5	128 \pm 4.3	128 \pm 4.6	128 \pm 3.6
121 - 150	217 \pm 22	216 \pm 25	218 \pm 29	209 \pm 25	132 \pm 3.7	132 \pm 4.2	133 \pm 4.6	132 \pm 4.4
151 - 180	243 \pm 25	241 \pm 26	237 \pm 35	234 \pm 27	135 \pm 3.9	135 \pm 3.9	135 \pm 4.7	135 \pm 4.3
181 - 210	262 \pm 26	264 \pm 25	250 \pm 33	256 \pm 27	137 \pm 3.9	138 \pm 3.7	138 \pm 3.9	137 \pm 3.5

Discussion: The weights used by the Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen (GEH, 1994) to calculate nutrient requirements for foals seem to be appropriate. The present study did not indicate significant differences in body weights and withers heights between OC affected or unaffected Warmblood foals. These findings were confirmed by Jelani et al. (1996) in Thoroughbred horses (N= 798).

Jelani Z.A. et al., 1996. *Pferdeheilkunde* 3, 291-295.

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Ultra trace element intake of Cercopithecinae in comparison with humans

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The essentiality of ultra trace elements was determined for various animal species. Very low concentrations of these elements in semisynthetic diets cause deficiency symptoms, although no biological functions are known in animal and humans, yet. Their normal diets contain enough amounts of ultra trace elements to meet their requirements. However, the environmental overload of some of these elements are more important for the organism in practice than a marginal intake. Beside toxic primary effects the ultra trace elements interact with essential nutritional components and can cause secondary deficiency symptoms of minerals and trace elements. This aspect becomes important for the feeding of animals in zoos. That is why the vanadium (V), chromium (Cr), strontium (Sr), barium (Ba) and cadmium (Cd) intake of Cercopithecinae kept in captivity was investigated and compared with the ultra trace elements intake of humans.

The trace element intake was determined in 24 clinically healthy individuals of 5 species of Cercopithecinae kept in 8 groups at the Leipzig Zoo. The animals were given 4 meals every day. Following the duplicate method, quantitatively and qualitatively identical samples of the offered feed, as well as feed residues, were registered (n = 7 per group) on 7 successive days. Thus, it was possible to determine the consumption of the different kinds of feed and the trace element intake of these species. After dry ashing at 450 °C the ultra trace elements were determined with ICP-OES and AAS with graphite furnace.

The daily dry matter intake of monkeys and humans produced considerable effect on the ultra trace element consumption. Related to the metabolic body mass, Cercopithecinae consumed 1.5 to 23 times more ultra trace elements than humans.

Mean dry matter and ultra trace element intake in Cercopithecinae in comparison to humans

Species	Mean dry matter intake in g/kg ^{0.75} body mass	Mean intake in µg/kg ^{0.75} body mass				
		V	Cr	Sr	Ba	Cd
Cercopithecinae						
DeBrazza Guenon	38	5.2	14	673	149	2.1
Hamlyn's Guenon	33	8.7	17	1065	168	3.2
Diana Monkey	29	3.9	10	499	112	1.9
Campbell's Guenon	42	6.3	14	780	220	3.2
Lion tailed Macaque	38	6.2	16	677	189	2.1
Human						
Mixed diet (men)	14	1.2	3.2	84	23	0.33
(women)	13	0.47	2.6	42	22	0.31
Vegetarian diet(men)	20	1.6	4.1	142	42	0.71
(women)	19	2.4	4.1	132	46	0.54
Fp Cercopithecinae	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
Fp together	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Cercop. : Humans	1.5 – 3.2	1.6–18.5	2.5 – 6.5	5.0 - 23	2.4 - 10	2.7 – 10

The monkeys' diet contained less V, Cr, Sr, Ba and Cd than the toxic levels in animals and humans. However, the monkeys took in more V and Cr than the recommended amounts for humans, and more Cd than the concentration in semisynthetic diet that caused Cd deficiency symptoms. Furthermore, the contribution of the various feed components, including beverages, to the daily ultra trace element intake were discussed and compared with the ultra trace element intake of people with mixed diets and vegetarians.

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Runting, diarrhoea and cachexia in fallow deer responsive to micronutrient supplementation

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A variety of different symptoms, (runting, reduced growth, cachexia and diarrhoea of unknown cause), were sporadically observed in a fallow deer (*Cervus dama*) enclosure in Upper Bavaria (district of South Germany). The enclosure is three hectares in size, located at 850m above sea level and houses a seasonally-varying stocking density between 35 to 40 animals. Two young animals perished and three other affected animals were shot down. A pathological examination of one perished and two shot animals showed no uniform cause of illness that related to the symptoms mentioned above. As a slight anaemia was discovered in the autopsied animals, the copper (Cu) content in the liver of some deceased and killed animals was investigated due to a provisional diagnosis of Cu deficiency. The mean Cu content in the liver (n = 3) was 11.55 mg/kg dry matter (range: 9.40 – 14.92 mg/kg DM) which was far below the lower ruminant reference range (35 mg/kg DM) and also below the Cu concentrations measured by Geisel et al. (1996) in fallow deer livers. Free-ranging fallow deer have even higher liver Cu concentrations (Anke et al., 1980).

The nutritional case history showed that the basic feed components consisted of hay, aftermath, grass and oats, but no mineral supplement was offered to the animals. The analysis of the basic feed components demonstrated a high hygienic food quality and Cu concentrations within the normal reference range. For practical reasons, the owner was not interested whether the disease is due to primary or secondary Cu deficiency and cobalt (Co) deficiency, but in solving his problem. Therefore, we did no further trace element analysis and focused on supplementing micronutrients to the dams diet.

In order to increase the Cu status of the herd, a special mineral supplement specifically including Cu-sulphate was provided, so that the calculated Cu content of the ingested diet was 15 mg/kg DM. This is nearly twice as high as the recommended concentration of 8mg Cu/kg DM for nonlactating ruminants. As a Co deficiency can also cause anaemia, vitamin B₁₂ was also added to the supplement, so that the ingested diet was enriched with 18µg vitamin B₁₂/kg DM. The mineral supplement was incorporated into a pelleted feed on a crushed oats-basis.

After feeding the new supplement to the pregnant dams during the following winter, no weak offspring were born, and all calves showed normal growth development.

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Apparent digestibility of calcium and phosphorus in growing beagles influenced by mineral supply

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The importance of correctly supplying calcium (Ca) and phosphorus (P) for the healthy development of the skeleton, is commonly known by now. The negative effects of a lack in Ca supply was already shown by Marek and Wellmann (1931) as well as the consequences of an oversupply with Ca for skeletal health (Marek a. Wellmann 1931, also: Hedhammar et al. 1974, Hazewinkel et al. 1991). A general inability of young growing dogs to decrease the digestibility of Ca was assumed from investigations of Hazewinkel et al. (1985 and follow-up studies) where Great Danes were raised on a threefold Ca oversupply. Limited information is available about the digestibility of Ca and P in puppies with an insufficient supply with those minerals.

A feeding trial with 20 Beagles was carried out from weaning until 6 months of age to determine and quantify the ability of growing dogs with malnutrition of Ca and P to modify the apparent digestibility (aD) corresponding to over- and undersupply at different ages during growth in order to regulate the uptake of these minerals. Four groups were formed to carry out 3 trials of approximately 6 weeks duration each. The Ca-supply was adjusted to 15, 50, 150 and 300% of the requirement while the P-supply met the requirements. A diet based on tripe, rice and cellulose was supplemented individually with minerals and vitamins. All but the nutrients in question were supplied after the requirement figures of Meyer and Zentek (1996), corresponding to 11g Ca and 8g P/kg dm in the control diet). Five dogs were raised as a control group, i.e. all nutrients met the requirements.

As intended no impairment of bone health was clinically evident. The weight curve was not influenced systematically by the feeding regime. At the age of 6.5 to 13.5 weeks of age (first trial) no statistically significant differences in the aD of Ca were seen among the groups independent of the feeding regime. In the second trial (13.5-19.5 weeks of age), a difference between the groups could be measured. Here, in the 15% group, a significant higher digestibility of Ca compared to the 300% group could be measured. In relation to the control group (100%) a distinct tendency to an elevated capacity to use the Ca in the food was visible. This was not true for the group which was fed with 50% of the Ca requirements. No down-regulation of the aD of Ca was detectable in the oversupplied group (300%). In contrast to the aD of Ca, the aD of P was influenced significantly in the youngest dogs.

The results suggest that Beagles are not able to completely adjust the Ca uptake in relation to the -intake before the age of 4 to 5 months. The lack of capability in young growing dogs to down- or upregulate the aD of Ca depending on the supply, strongly indicates the need to supply them with the correct amounts of Ca.

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A case study on commercial dry diets in two persian cats with struvite urolithiasis

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Two persian cats (cat 1: four-year-old intact male, BW= 4 kg, cat 2: six-year-old neutered male, BW= 4.5 kg) were presented for acute pollakiuria, dysuria and hematuria, inappetence, poor coat condition. As they refused moist foods, their usual diet was only dry. After having anaesthetized the animals and removed urine by urethral catheterization, a bladder lavage was performed. The urinalysis revealed a pH > 7, leucocytes, blood and struvite crystals. A therapy with enrofloxacin (at dose of 5 mg/kg orally) for 10 days and a calculolithic diet, in appropriated quantity, for a period of 2 months was administered. During the treatment, both cats had two micturitions per day, and the first cat had increased urinary volume (30%).

Then the animals were fed a struvite prevention diet (diet A) with 92% of dry matter (DM), containing 1.09% DM calcium, 0.76% DM phosphorus, 0.49% DM sodium and 0.065% DM magnesium.

The first cat showed a normal condition and his urinary pH was 6.3 steadily. Six months later, it was fed diet A mixed with a maintenance acidifying diet (92.5 % of DM) in 50 % for one month. During this test, one micturition every 2 days was noted.

It was recommended, therefore, to provide the cat only with a struvite prevention diet.

The second cat began to suffer from dysuria 2 months after feeding diet A. It produced concentrated urine with acid pH and struvite crystalluria. The calculolithic diet was administered again. After one week, dysuria disappeared and 3 months later urinary volume was about 60-70 ml/day.

The animal was fed a new struvite prevention diet (diet B) (92% of DM) rich in phosphorus (0.98% DM), potassium (0.92% DM) and magnesium (0.1% DM). Since first days of feeding this diet, it drank more water, consequently producing an increased volume of urine. Nevertheless, one month later, a reappearance of dysuria and pollakiuria were reported.

A further struvite prevention diet (diet C) didn't improve the state of health. It was suggested to carry on feeding a calculolithic diet for 10-12 months at least with regular serum biochemical examinations (urea, creatinine, K, Cl, Na).

Recently, the animal was in good health.

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Recent developments in phytase research / Methods to improve thermostability of phytases

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Phytases, *myo*-inositol-hexakisphosphate phosphohydrolases (EC 3.1.3.8 and EC 3.1.3.26), catalyse phosphomonoester cleavage of phytic acid, thereby releasing inorganic phosphate. Since phytic acid phosphorus is largely unavailable to monogastric animals, supplementation of the feed with microbial phytase increases the dietary availability of phosphorus. It was demonstrated in several animal experiments that supplementation of the feed with commercially available *A. niger* phytase could replace inorganic phosphate addition completely (1, 2, 3). As poultry and pig feed is commonly pelleted, a commercially attractive phytase should be able to withstand the temperatures that are reached temporarily during the pelleting process (60°C-90°C). To withstand high temperatures implies either that the enzyme keeps its functional conformation during heating (intrinsic thermostability) or that it undergoes a reversible heat denaturation and refolds to its active conformation when the high temperatures are no longer imposed. Despite an intensive search to isolate new phytases in thermophilic micro-organisms such as *Thermomyces lanuginosus* and *Myceliophthora thermophila* (4, 5), all known phytases unfold at temperatures between 56 °C and 69 °C. The *A. fumigatus* phytase is the only phytase that possesses the capacity to refold to a native like functional conformation after heat denaturation (6). Recent research has focused on the creation of phytases with higher intrinsic thermostability. Lehmann et al. (8) used the sequences of 13 phytases from six different fungal species, (the parent phytases), to construct a synthetic gene (consensus gene), containing at each position of the protein the amino acid occurring most frequently at that position in the fungal phytase family. The unfolding temperature of the consensus protein was 15 °C to 22 °C higher than that of each of the parent phytases and the catalytic properties were not compromised (7, 8). To investigate the contribution of the glycosylation level of the enzymes to their intrinsic thermostability, the yeasts *Pichia pastoris* and *Saccharomyces cerevisiae*, known as a hyperglycosylating organism, were used as expression hosts for the *A. niger* phy A gene, the *A. fumigatus* phy A gene and the *E. coli* r-AppA gene (EcAP). The resulting enzymes were heavier glycosylated and showed superior thermostability compared to their wild type counterparts while conserving their catalytic efficiencies (9, 10, 11, 12). The efficiency of the EcAP enzyme in improving the bioavailability of phytate phosphorus in corn-soybean meal diets to young pigs has already been tested and the enzyme turned out to be as effective as *A. niger* phytase (13).

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Long-term consumption of an acidifying diet affects acid-base status and bone density in cats

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The use of dietary modifications to control the formation of struvite uroliths is common. While acidic urine pH can prevent struvite stone formation and is an integral component in management of diet-associated feline lower urinary tract disease, there are problems associated with overuse of acidifiers. Over acidification can result in chronic metabolic acidosis and lower calcium and potassium balance (Ching et al, 1989). Although moderate urinary acidification resulted in decreased calcium and phosphorus balance, there was no effect on indices of bone formation (Fettman et al, 1992). The effect of longer-term, sustained acidification on bone density or bone content has never been reported. The purpose of this trial was to determine whether cats can adapt to chronic consumption of an acidifying diet and whether such diets cause changes in acid-base status and bone density. Two groups of five adult cats each were fed an extruded dry diet which was designed to produce either a moderately acidic urine pH (control diet; pH approximately 6.2-6.4) or a more acidic urine pH (test diet; pH <5.9) for two years. The urine pH produced by the test diet was below typical recommendations for control of struvite production. Body composition and bone density were non-invasively determined by DEXA. Blood pH, ionized calcium, and bicarbonate were measured as indicators of metabolic acidosis. A total daily urine collection was done to determine urinary calcium excretion. Urine pH was lower in cats fed the test diet (5.7) than in cats fed the control diet (6.3). Cats fed the test diet showed no clinical signs of metabolic acidosis; however, blood bicarbonate and pH were significantly lower and blood ionized calcium was significantly higher in cats fed the more acidifying diet. Urinary calcium excretion was significantly greater in cats fed the test diet. Body fat and lean muscle mass were unaffected by acidification. However, bone mineral density, bone mineral content, and total bone calcium were significantly lower in cats fed the more acidifying diet. In conclusion, long-term consumption of an acidifying diet resulted in sub-clinical metabolic acidosis and adversely affected bone mineralization. Diets designed for urinary pH modification should be thoroughly evaluated for effects on metabolic acidosis. Long-term use of supplemental urinary acidifiers should be discouraged in cats consuming diets designed for control of struvite urolith formation.

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Nutritional disorders of skeleton in emus and rheas

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Keeping of emus (*Dromaius novaehollandiae*) and rheas (*Rhea americana*) in European environmental conditions has often been discussed. The local climate and areas available for grazing and activity do not favour birds which originated from South America (rhea) or Australia (emu). Furthermore there are risks for animals' well-being, due to a lack of suitable experience and species-specific complete diets not being continuously available. Home-mixed diets are fed, which are often unbalanced, occasionally resulting in nutritional disorders of the skeleton. The following case reports describe problems caused by faults in feeding, particularly relating to mineral supply.

Case 1:

Due to a supply shortage, an owner of a small zoo fed emus a home-mixed diet (ingredients: piglet pellet, rabbit pellet and a mineral product rich in protein, CaCO₃). The hatched emu chicks adapted very quickly to this diet and showed high growth rates in the first weeks of life. At the age of 3-4 weeks, 13 of 37 chicks showed a reluctance to rise or move. One week later these chicks showed ataxic moving activities, and slight thickening of the distal tibiotarsus and proximal tarsometatarsus which increased in the following 14 days. At the same time, the long bones developed rotational deformities (bones turned outwards and caused a 'paddling' movement).

Nutritional history: In a critical examination of mangers, a macroscopic difference between the offered feed (pelleted) and the refusals (white-grey, fines) could be observed. Crude fiber and starch of the refusals were lower, but crude ash and calcium contents were higher compared to offered ration. Blood chemistry data showed slightly lower mineral contents and X-rays confirmed the bad posture of the legs.

Assessment: Due to the different types of prepared feedstuffs in the home-mixed ration (pellets plus meal), the ingredients were not mixed well and the emus could select the preferred pelleted components and refused the mineral supplement. This selective ingesting behaviour, the high growth rate of emu chicks and the insufficient mineralization of skeleton encourages the occurrence of the observed clinical signs (perosis).

Case 2:

3 of 15 young rheas (3 months) showed unhealthy postures of the neck vertebral column. The 'diet' was based on corn, pelleted alfalfa, apples, lettuce and white bread in the first 8 weeks of life; afterwards a complete pelleted diet was fed.

Clinical history: The X-ray of vertebral column showed complete healed up fractures of individual vertebrae.

Nutritional history: Before feeding the complete diet the owner observed selective ingestion of corn and white bread, while the other components were refused. The preferred components are characterized by low mineral contents (especially calcium, copper).

Assessment: Due to insufficient mineralization of the skeleton and a high mechanical stress (rough handling to apply a deworming) fractures of the vertebral column occurred. Once fed the complete diet, these fractures healed due to the pelleted diet's improved mineral balance.

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Diurnal variation in concentrations of various markers of bone metabolism in goat and sheep

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It is known that goat and sheep differ in their metabolism of calcium during gestation and lactation. This study was performed to evaluate if goat and sheep differ in circadian rhythm of selected markers of bone metabolism. Twelve 1-year-old female goats and sheep were used in this study. Blood and urine samples were obtained in the morning before goats and sheep were fed and then at 2-hour intervals for 24 hours. Concentrations of osteocalcin (OC) and carboxyterminal telopeptide of type-I collagen (ICTP) were measured in serum using radioimmunoassay. Concentrations of bone-specific alkaline phosphatase (bAP) and degradation products of C-terminal telopeptid of type I collagen (CL) were analyzed with an enzymeimmunoassay in serum. All bone marker concentrations were significantly higher in goats than in sheep. OC concentrations in goat decreased during the day, increased thereafter and reached values similar to those obtained before feeding. The ICTP concentrations slowly decreased in goats until 4 pm, increased again, and returned to prefeeding values thereafter. The concentrations in sheep increased continuously, but not significantly, towards the morning sampling. The CL concentrations increased in both species during the night, but started to decrease to levels as at the beginning of the testing at 6am. The bAP concentrations decreased from noon to 12pm in goat. Changes in the concentrations of bone markers were mainly observed in goats in this study, although all animals were fed with the same diet according to the requirements. As documented for bone resorption and formation in other species, circadian rhythms were evident for concentrations of ICTP, CL, bAP and OC. From this study, it can be concluded that goat have physiologically a higher bone turnover than sheep, because the bone marker concentrations were always higher.

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Urinary acidifying effect of dietary calcium chloride in fattening pigs

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Environmental pollution from pig husbandry is a major concern in most countries with intensive pig production. Especially ammonia emission from animal husbandry has been named as one of the main threats of ecosystems throughout Europe.

Lowering the dietary electrolyte balance (dEB) can exert a faecal and urinary acidification. This should reduce the conversion of NH_4^+ into NH_3 in manure. The present trial tried to demonstrate the action of dietary calcium chloride (CaCl_2) supplementation on ammonia emission in fattening pigs.

In the first period a low dietary protein and a moderate dietary protein level were offered to two pigs. Urine was collected in three ways; 1) directly (“uncontaminated”=UC); 2) after having passed the slatted floor (“slightly contaminated”=SC); 3) faeces where added to the urine (“highly contaminated”= HC).

In the second and the third period, these collections were performed after dietary addition of 1% and 2% coated CaCl_2 , respectively. The dEB ($\text{Na}^+ + \text{K}^+ - \text{Cl}^-$, in meq/kg) shifted in these three periods from +250 over +100 to -50 meq/kg.

A pH drop was noted from 7.2 over 6.3 to 5.7. Slight faecal contamination of urine increased pH by 0.5. Further contamination with faeces had no supplementary effect on pH. Dietary protein level did not alter urinary pH.

Based on these preliminary data, CaCl_2 is a dietary additive that can likely to reduce ammonia emission from pig manure.

<i>pH</i>	<hr/>		
	<i>UC</i>	<i>SC</i>	<i>HC</i>
0% CaCl_2	7.2	7.31	7.30
1% CaCl_2	6.3	7.15	7.05
2% CaCl_2	5.7	6.36	6.36

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Effect of exercise on apparent digestibility in horses – a review

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Most digestibility studies in horses have been conducted with idle horses confined to metabolism stalls. The results of these studies are used for all horses, including the performance horse. Duren (1990) measured blood flow distribution during exercise in ponies. He proved that blood flow is shunted away from the gastrointestinal tract during exercise. Exercise also reduced mean retention time, so less opportunity for enzymatic digestion and microbial fermentation. (Orton et al., 1985) The question left was: what is the effect of exercise on nutrient digestibility?

It was Olson (1955) who first looked at this. He suggested that light work may improve apparent digestibility of dry matter, energy and crude protein and heavy work would have the opposite effect. Orton et al (1985) concluded that exercise increased the apparent digestibility. Their horses were trotted each morning for 1 h at 12 km/h. The same results were found by Pearson (1991) for donkeys. The animals walked for 4 hours at 3.6 km/h and all the digestibility coefficients of the diet increased in exercised donkeys. Pagan et al (1998) reported that apparent digestibility decreased when horses worked for 4 weeks 40 min/day at an average speed of 20 km/h. He also looked at bioavailability of minerals. The potassium (K) uptake as well as calcium (Ca) and magnesium (Mg) uptake decreased. Only the availability of sodium (Na) increased with exercise. The same results for Ca and Mg were found by Meyer et al (1992). However, his horses did light work (100 min-12 km/h) compared with those of Pagan.

On the basis of this review it can be concluded that light work and heavy work have opposing effects on apparent digestibility of dry matter, energy and crude protein. It is not clear what the effect of exercise is on mineral availability and whether this effect is the same for all minerals. It may depend on the horse's requirements; for example, horse sweat contains 3 times more Na than K (Snow et al., 1982). Logical questions which follow are:

- at what level of exercise intensity and duration would one expect to negatively influence nutrient digestibility?
- what would be the effect on mineral availability?
- what is the cause of all this? Level of feed? intake, mean retention time, water intake, blood flow, changing metabolic requirements?

Further research is necessary to answer these questions.

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The effect of beefhide strips on digestibility and dental calculus in dogs

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Control of calculus deposits on the teeth of pets may play a significant role in the prevention of periodontal disease. Studies have shown that the administration of beefhide to dogs on a daily basis has a beneficial effect on both calculus removal and calculus buildup (Lage et al, 1990; Goldstein et al, 1993 and 1994). The amount of rawhide offered to the dog on a regular basis is critical to dental efficacy, but could have nutritional implications. Two dental efficacy studies were conducted to assess buildup of calculus over different time periods (1 month, 3 months) in dogs consuming beefhide strips according to package directions (2 strips per day) along with standard dry dog food. A clean tooth model was used in both trials, and different groups of dogs were evaluated for the two trials. Results showed a 27% reduction in calculus for the dogs fed beefhide strips for one month, and a 44% reduction in calculus for the dogs fed beefhide for three months. In both studies, dogs in the control groups consumed standard dry dog food alone. All dogs maintained body weight over the course of the studies. Digestibility of beefhide was determined in a separate study. Zero, 10 or 20% ground rawhide was added to a nutritionally complete wet dog food. The diets were fed to six dogs each for 10 days. Fecal collection for digestibility determination was conducted on days 5 to 10 of the trial. The digestibility of the rawhide was calculated by regression analysis. Results of digestibility testing showed 77% dry matter digestibility, 87% nitrogen digestibility, 91% fat digestibility and 86% energy digestibility. Metabolizable energy of the beefhide was determined to be 3.97 kcal/gram. In conclusion, the administration of beefhide to dogs significantly slows the accumulation of dental calculus deposits when fed on a daily basis. Digestibility of beefhide is high and must be considered a contribution to caloric intake. Administration should not exceed the label recommendations of two strips per day (approximately 28 grams or 111 kcal per day).

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Feeding and nutrition practice in 82 Hanoverian horse breeding farms during breeding season

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There is little information about common feeding practice on German warmblood stud farms. Creep-feeding of mares and their foals during the winter in particular, varies enormously between the different farms. In order to produce healthy sport horses, it is necessary to begin feeding according to the nutrient requirements for a growing horse early in life. The purpose of this study was to evaluate feeding regimens in growing horse diets. This investigation was part of a larger project in which growth rates, feeding, housing, and genetics were evaluated with special reference to the development of osteochondrosis in foals.

Methods: From March to October 2001, monthly feeding practice of several breeding farms (n=82) with 629 mares and their foals, was recorded. Feed samples were taken and analyzed for crude nutrients and minerals. In addition, height and weight of the growing foals were estimated (refer to the presentation by Vervuert et al.).

Results: On almost all farms suckling foals were not fed separately from their dams. Forty farms fed traditional rations composed of varying combinations of oats, barley or pelleted feed, and hay or haylage. Forty breeding farms fed traditional rations as well, but fortified with feedstuffs such as coarse mix or a special concentrate mix for weanlings which were given to the mares as well. Most of the stud farms fed the horses twice daily with no difference between traditional and fortified feeding practice. Fifty-eight stud farms fed additional minerals. Only one stud farm fed hay or haylage to their mares and foals without any concentrate or minerals. In another breeding farm, mares and foals were fed exclusively with hay, sugar beet pulp, and minerals. All mares and foals had regularly access to pasture from May. During this period there was no additional feeding on thirty breeding farms. Mineral contents of the various feedstuffs are shown in Table 1 below.

Table 1. The mineral contents of the feedstuffs.

feedstuff	n	g/kg dry matter		mg/kg dry matter	
		Ca	P	Zn	Cu
grass	137	3.4±0.7 1.4-5.2	4.1±0.7 2.3-6.1	37.9±12.9 13.5-86.2	10.1±2.9 4.7-17.7
hay	71	4.9±1.3 2.5-7.4	3.0±0.8 1.8-5.8	33.3±33.7 9.5-277.7	7.0±2.4 1.3-14.7
haylage	52	5.1±1.4 2.5-8.2	3.7±0.7 2.4-5.1	37.2±17.5 13.9-131.1	8.3±2.8 1.8-14.9
pellets (ash:10.1±5.1%)	61	20.5±12.1 3.9-83.4	7.4±5.5 1.2-26.9	311.2±470.6 10.5-3289.0	63.5±15.7 5.0-422.5
coarse mix	25	13.2±7.7 1.0-30.6	4.9±1.7 2.8-11.2	179.1±125.2 12.8-651.9	30.6±15.7 1.8-61.5

Discussion: Traditional feedstuffs (grains and roughage), as well concentrates form common feeding practice on German stud farms. Suckling foals are not fed separately from their dams. During the winter months when horses are stabled adequate nutrient intake is expected in most cases. On pasture, without additional feeding, an inadequate supply of minerals is possible.

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unwanted dietary compounds & unwanted nutritional behaviour

Chairperson : Roland De Wilde (Belgium)

Zinc intoxication in budgerigars (*Melopsittacus undulatus*)

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Zinc intoxication in most domestic animals is well documented, while only a few reports in pet birds have been published (all from the USA). Furthermore, there is only one published experimental study, in adult cockatiels (*Nymphicus hollandicus*). Cases of zinc intoxication in psittacines in Europe were documented by Dorrestein et al. (2002). However, it seems that zinc intoxications are probably not uncommon in aviaries, especially in newly erected aviaries ('new wire disease'). The symptoms are not obvious and many birds may die before the real diagnosis is made.

Case report: The owner of a flock of 6 budgerigars bought a new aviary (200 x 100 x 200 cm) with a thermal galvanized wire mesh. Two months later one of the budgerigars showed health disorders in form of ataxia, tiredness and yellow-coloured feces. The budgie dropped repeatedly from the seat. Hepatomegaly can be seen by radiography. Three weeks later another budgerigar showed comparable symptoms. Ten weeks after being kept in the new cage a third bird was ill with similar symptoms of regurgitation of a colourless mucus and diarrhea. Two weeks later, another bird showed vomitus and a strong loss of plumage. Within three months all budgerigars were ill (increased body mass losses, vomitus, convulsions, ataxia, disorders of the central nervous system, feather losses, diarrhea, paralysis), and two of them died. However, after applications of Ca-EDTA and the moving the budgerigars to another cage no further health disorders occurred.

Nutritional history: The budgerigars were fed a commercial seed mixture, equivalent to conditions in the other cage without clinical signs.

Examination of the birds: No gross pathological signs could be observed at necropsy. No parasites were found. The microbiological investigation found no unusual organisms. The practitioner suspected a zinc intoxication. Therefore the livers of the two budgerigars that died were analyzed for zinc levels (Table 1) using atomic absorption spectrometry (after wet ashing procedure).

Table 1: Zinc levels in cases of an intoxication and physiological levels in liver of pet birds

Zn-level in liver tissue (mg/kg DM)		Kind	author(s)
physiological status	intoxication		
50.5 ± 12.7* (37.6 – 70.5) n=10	153 250	<i>budgerigar</i> (<i>Melopsittacus undulatus</i>)	* own results
57.9 ± 34.5 (28.1 – 156) n=14	179 ± 73.7 (n=7)	<i>monk parakeet</i> (<i>Myiopsitta monachus</i>)	Dorrestein et al. 2002
42.5 ± 8.90* (37.5 – 50.2) n=5	75 156	<i>lovebird</i> (<i>Agapornis roseicollis</i>)	Reece et al. 1986
38.9 ± 22.2 (12.0 – 115) n=77	150 ± 37.0 (n=3)	<i>macaw</i> (<i>Ara chloroptera</i> , <i>Ara macao</i>)	Dorrestein et al. 2002

Conclusion: The observed zinc levels in liver tissue were lower than comparable data in other animals (cattle, pig, horse, dog etc.) and possibly indicate a special sensitivity and a low limit of tolerance of pet birds towards zinc (specific for these bird species?).

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Distinct weight loss and elevated hepatic enzymes in horses caused by straw contaminated with Deoxynivalenol (case report)

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The possibility of an intoxication has to be regarded in all cases of health disorders with unknown etiology. For this, information about the nutrition should be as precise as possible. In many cases it may additionally be necessary to include special methods of feed analysis. **Case report:** In February 2002, about the half of 104 predominantly Warmblood-type riding horses in a stable in the middle of Germany suddenly lost weight. With some animals this was associated with poor performance. Individual horses fell ill with recurrent cough. In March nine of the affected horses (adults: 2 studs, 7 geldings) were subjected to a clinical and clinical-chemical examination which included haematological (haemoglobin; packed cell volume; erythrocytes: count, morphology and indices; white blood cells: total and differential count) and biochemical (in the sera: total protein, urea, creatinine, calcium, inorganic phosphorus, magnesium, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transferase [GGT], glutamic acid dehydrogenase [GDH], creatine kinase, lactate dehydrogenase) parameters. The clinical examination did not indicate any specific illness. Laboratory analysis revealed a marked elevation of liver enzyme activity (GDH 163 ± 108 U/l, GGT 151 ± 58.7 U/l). Total bilirubin was normal. Two horses particularly affected were treated with Amaryn (two times every 5 days). **Nutritional history:** All horses received whole oats and barley (1:1), a pelleted mixed feed and meadow hay. The horses had been fed this diet for several months before the health disorders were established. The bedding consisted of wheat straw. Only a few horses got wooden shavings. None of these horses fell ill. The provenance of all the straw used was uniform. After the wheat was harvested, the straw was stored as round bales (each about 300 kg) in two barns and the remainder on the field without any protection against the weather. Initially the horses got straw from a barn. Since this barn was destroyed in October 2001 straw from the field was given thereafter. Apparently no hygienic problems existed with the feed used except of the straw that was stored in the field. The surface of the bales was partly mouldy. All feedstuffs were subjected to ELISA to identify selected Fusarium mycotoxins (T-2-Toxin, Zearalenone, Fumonisin, Deoxynivalenol [DON]). The analysis revealed high DON concentrations (0.5-2.7 ppm) in the straw that was stapled on the field but not in the straw from the barn. Since this was discovered (April 2, 2002) only straw from the barn was used. Subsequently the horses slowly gained weight and the general condition became progressively better. Clinical-chemical controls occurred in April 16 and May 30, 2002. Accordingly GDH (2.8 ± 4.3 U/l) gradually dropped to physiological values (≤ 8 U/l). GGT was markedly decreased (50 ± 19 U/l) but the normal range (≤ 25 U/l) was obtained in only one horse. **Assessment and conclusion:** Changes in the horses weight and liver enzyme activities were obviously connected with the use and elimination, respectively, of the DON enriched straw. A recommendation for the DON limit in horse feed does not exist. Ration calculation showed that the DON concentration was clearly lower than the limits recommended for other farm animals (Anon, 2000). This may indicate a particularly high sensitivity of equines to DON. However, there may have been an additional aggravating mechanism, as yet unrecognised.

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Undesirable substances in feed – an overview

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Substances and products are called undesirable in animal nutrition if they are disadvantageous for the target animals, pose a risk for human health and are dangerous for the environment. Most of the individual European States established legal regulations of this matter. The European Communities entered into force the first Directive in 1974 (74/63/EEC) and the last one in 1999 (Council Directive 1999/29/EC). The following refers mainly to the latter directive, although it is currently under revision. The directive consists of 18 Articles and four Annexes. Annex I, divided in three parts, sets the maximum content of undesirable substances (Substances, Products, Botanical impurities) in feedingstuffs and feed materials. In Annex II feed materials containing undesirable substances are listed which can be used in animal nutrition only under certain conditions. Annexes III and IV have only administrative relevance. In addition to the definitions, the directive obliges the Member States (MS) to make the international directive national law. MS shall prescribe that feed materials may only be put into circulation within the Community if they are sound, genuine and of merchantable quality. MS shall prescribe that substances and products listed in Annex I shall be tolerated in feedingstuffs only under conditions set out therein and that feed materials listed in Annex II may be put into circulation only if their content of the undesirable substance or product mentioned in the Annex does not exceed the maximum level laid down in that Annex. The Directive also regulates the conditions feed material can be used in animal nutrition if the content of undesirable substances exceeds the maximum permissible level. They may restrict the use of such feed materials to certain manufacturers of compound feedingstuffs. Where a MS has detailed grounds for establishing that a substance constitutes a danger to animal or human health or the environment it may provisionally reduce the content or prohibit that substance in feed. It shall immediately inform the other MS and the Commission. MS shall take all necessary measures to control feedingstuffs officially. The Directive also regulates the measures to be taken. The Standing Committee on Feedingstuffs shall deliver its opinion about the Commission draft of measures. Annexes shall be reviewed and amended periodically, according to the scientific development.

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COUNCIL DIRECTIVE 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition

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Feeding practices in the German BSE epidemic: a preliminary survey of the first 65 Bavarian cases

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It is generally accepted that bovine spongiform encephalopathy (BSE) is transmitted by oral infection via the ingestion of contaminated material. Due to the nature of the potentially infectious agent and the physiological changes of the intestine during the ontogeny of cattle, the reported incubation periods, and the average age at which the disease is clinically observed, it is assumed that infection must take place in young calves, and calf feeding practices therefore have attracted particular attention.

We evaluated data from an epidemiological survey initiated by the Bavarian State Ministry of Health, Consumer Protection and Nutrition from the first 65 reported BSE cases in Bavaria. During the survey, the owners of the affected farms were asked a series of questions on the feeding practice, and asked to hand in documentation from which animal feed purchases could be traced back as far as possible, up to 5-6 years previously.

In 3 % of the cases it was claimed that the affected animal had not received a milk replacer (MRP). In 58 % of the cases a concentrate had been fed to calves that later became affected. 22 % of the affected animals had received MRP and no concentrates when calves. In one case it was claimed that neither MRP nor concentrates had been used. In more than half of the cases in which MRP only was used, and no additional mineral supplement given. Similarly, in half of the cases in which MRP was used exclusively, no other commercial feed for another animal species (e.g. pigs, poultry) had been purchased.

Unless the incubation period of BSE is overestimated at present, these results strongly suggest that milk replacer should be considered as a potential source of BSE infection, and should be further investigated.

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Crib-biting in foals is associated with gastric ulceration and mucosal inflammation

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Crib-biting is a stereotypic behaviour performed by approximately 5% of captive domestic horses. Risk factors for crib-biting, identified in recent epidemiological studies, include feeding high concentrate and/or low forage diets (Waters et al., 2002). Experiments have shown that such diets are likely to result in increased gastric acidity (Murray and Eichorn, 1996., Nadeau et al., 2000). We therefore propose that young horses initiate crib-biting in an attempt to produce alkaline saliva to buffer their stomachs when alternative opportunities for mastication are limited. The aim of this study was to determine whether there was an association between crib-biting behaviour and stomach condition in foals. Foals that had recently started to perform crib-biting were recruited into the study and compared with non-stereotypic foals. The stomachs of 15 crib-biting foals and 9 normal foals were examined using a video endoscope. Foals were then randomly allocated to a control or an antacid diet for a 3 month period. Behaviour was monitored by direct observation throughout the 3 month period, and foals re-endoscoped at the end. Videos were scored blind by an independent observer.

Crib-biting foals had significantly more inflamed, dry and ulcerated stomachs than normal foals on first examination (Mann-Whitney: $U = 36$; $N_1 = 15$; $N_2 = 9$; $p < 0.05$). Their stomachs also lacked normal folding on first examination, and were significantly smoother when re-examined (Mann-Whitney: $U = 11.5$; $N_1 = 12$; $N_2 = 7$; $p < 0.01$). Foals that received the antacid diet had fewer ulcers at the end of the trial (Mann-Whitney: $U = 27.5$; $N_1 = 13$; $N_2 = 9$). Most foals showed a reduction in crib-biting following treatment but the reduction was more pronounced in foals that received the antacid diet, and reduction in crib-biting correlated with reduction in ulceration (Rank correlation: $r = 58$; $N = 12$; $p = 0.05$). We suggest that the stomachs of crib-biting foals were exposed to more acid conditions or were more sensitive to normal acidity levels than the stomachs of normal foals. The results of this study support the hypothesis that the initiation of oral stereotypy can be a response to a disturbance of the normal digestive process.

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Deaths in horses caused by ingestion of a hay contaminated by bracken fern (*Pteridium aquilinum*)

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Poisoning due to bracken fern (*Pteridium aquilinum*) was reported many years ago (1). The intensification of grassland management and optimizing husbandry and feeding conditions means this toxication seems to be mainly in the past. However, ingestion of bracken fern is still possible, when hay is harvested from extensively cultivated areas, fallows near to forests or horses are kept on 'forest' pasture.

Case report

When offered a new batch of hay two of five horses in France showed acute colic symptoms as well as a strong diarrhea. One mare had a miscarriage. Both horses died within a few days after feeding on the hay. Two weeks later, a third horse showed heavy convulsions and colic symptoms and died, too.

Nutritional history:

The ration of the horses was based on hay, supplemented with concentrates and minerals. Because the last feedstuffs were unchanged, the roughage (hay, straw as bedding material) was suspected. These feedstuffs were characterized macroscopically as being of a good quality. The microbiological status of hay and straw was slightly reduced (especially content of moulds):

CFU/g	aerobe bacteria	moulds	Yeasts
Hay	4.5×10^5	4.5×10^4	$< 10^3$
Straw	4.5×10^4	1.0×10^5	n.d.

The predominant finding was a high contamination with bracken fern (*Pteridium aquilinum*): > 250 g/kg hay.

Assessment: Bracken fern contains the enzyme thiaminase, prussic acid containing glycosids and the saponine, pteridin. The highest level of these toxins can be found in fresh leaves, but also in older and dry leaves or in hay containing bracken fern. These toxic ingredients cause ataxia, motor disorders, bloody diarrhea as well as heavy bleeding from nose and mouth or convulsions, although clinical symptoms are different between cattle and horses. Whereas in horses, signs like ataxia or convulsions dominate (due to the vitamin B₁ destroying thiaminase), in cattle (which are capable of synthesizing vitamin B₁) clinical signs are chronic haematuria or 'bloody sweat'. In the case presented, the reduced microbiological status of hay has to be considered too, and pathogenic symptoms were most prominent as convulsions and colic.

Conclusion: This case report shows that roughage, harvested from extensive areas or fallow, should be proofed critically with regard to both microbiological status and poisonous plants, or plants with toxic constituents (secondary ingredients), before it is provided to horses and cattle.

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The influence of the ratio of structured/unstructured feed on oral disturbances in captive giraffids

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Oral disturbances such as tongue playing, are a common problem in zoo okapis and giraffes. In domestic animals these behaviours often have been associated with feeding practices: diets low in structured feeds (roughage like hay, browse, silage, grass) have been shown to make cattle more prone to behaviours like tongue rolling or licking on substrates.

In our studies, the behaviour and food consumption of three individual okapis and six giraffes (of which three were kept as a group) were recorded for 10 days each. The diets consisted mainly of lucerne hay *ad libitum*, smaller amounts of browse and grain based concentrate.

For the three okapis, concentrate/roughage intake ratios of 0.9, 1.1, and 2.3 (on DM-basis) were measured. Only the animal with the highest proportion of concentrate performed oral disturbances. The giraffes showed behaviours like tongue playing or wall licking to varying degrees (5-29% of observation time), but the behaviour was documented for all animals. The concentrate/roughage ratio varied between 2.3 and 3.7. A correlation was found between the proportion of intake of unstructured food and the amount of oral disturbances displayed. After dietary manipulation (addition of tannin-containing pellets), the consumption of more unstructured food led to the occurrence of more oral disturbances in the according animals.

The results indicate that the ratio of unstructured/structured food is a very important factor in the development of oral disturbances in giraffids. It has to be stressed that all animals had permanent access to good quality lucerne hay. Our data raise the question of the acceptance of roughage and requirements for structured foods of wild ruminants, especially that of browsers.

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Digestive tolerance of sorbitol in cats

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Sorbitol is a natural polyol also produced industrially by catalytic hydrogenation of glucose. Although sorbitol is commonly used in semi-humid petfood industry, especially as a humectant, very little information is available concerning its digestive tolerance in cats. This preliminary study was designed to observe the consequences on faeces quality, of different doses of sorbitol (Table 1) in a dry commercial food for cats in order to find a safe dose.

Table 1 - Sorbitol dose added to the different diets used in the study

Diet	S0	S1	S2	S3	S4
Sorbitol (%)	0	0.25	0.5	1	2
dose (mg/kgBW/d)	0	35	70	140	280

Ten queens, all neutered and receiving the same food (Hill's Feline Maintenance Adult chicken, Hill's Pet Nutrition, Topeka, KS) for several months, were randomly assigned to group A or B, with 5 animals in each group. The experimental design is described in Table 2:

Table 2 - Design of the study (each cell represent a week ; the grey bar between 2 diets, represents a 2 days dietary transition).

Group A	S0	S1	S1	S0	S3	S3	S0
Group B	S0	S2	S2	S0	S4	S4	S0
Feces Scoring	x		x			x	

The queens were housed in individual cages, with paper in the litter box in order to facilitate the collecting of the faeces. The queens had free access to water and received one meal per day at a maintenance amount. The palatability of the food was scored (from 1: very palatable eaten quickly, to 5: very unpalatable). Faeces were collected every morning before the meal, scored for color, texture and odor by the same manipulator, weighed and stored at -20°C until analysis for dry matter, nitrogen content and pH. Statistical analysis consisted of a one way analysis of variance (GLM procedure, SAS system).

Palatability of the food was not modified by sorbitol incorporation. No diarrhea was observed during the entire study. Instead, a slight green coloration of the faeces for doses S2, S3 and S4, and a slight softening of faeces in some queens on doses S2 and S4 was noted. However no significant change in the faeces color, odor and texture could be reported, due at least in part to the high individual variation of the results and the small number of animals in each group. No modification of faeces pH, dry matter and nitrogen content and faeces weight could be shown from the doses of sorbitol used in this study. Sorbitol appears to be tolerated well by the cats at the doses used in this study.

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Cats are more sensitive to the toxic effects of oral lipoic acid than humans, dogs, or rats

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Introduction: Antioxidant supplementation of pet foods necessitates investigation into safety in target species. Although cats may benefit clinically from antioxidant supplementation to reduce oxidative damage secondary to chronic diseases (e.g. diabetes mellitus or FIV), cats develop clinical toxicity to the antioxidant lipoic acid. This study describes the determination of the maximum tolerated dose (MTD) and toxic effects of a single oral dose of lipoic acid in cats.

Methods: Nine healthy adult male cats were housed in metabolism cages in a controlled environment. Three cats received 60 mg/kg lipoic acid (T), three 30 mg/kg (M), and three, no dose (C). Food intake was measured and urine collected. Prior to, 2 hours and 24 hours after dosing, concentrations of serum enzymes, bile acids, ammonia, plasma free amino acids, lipoic (LA) and dihydrolipoic acid (DHLA) were measured and CBCs performed. All cats were euthanized 24 hours after dosing and necropsied. Tissues were assayed for free amino acid, lipoic acid (LA) and dihydrolipoic acid (DHLA) concentrations, and examined histologically using an electron microscope (EM).

Results: By 24 hour after dosing, all T cats showed hypersalivation and ataxia, while T and M cats showed histological and EM hepatocellular changes and significantly increased concentrations of LA and DHLA in plasma, urine, and bile. Gut, liver, pancreas, and kidney LA and DHLA concentrations exceeded those in plasma in T and M cats. Within 2 hours of dosing, serum ALT and ammonia concentrations were significantly elevated while branched chain:aromatic amino acid ratios decreased significantly in the T cats within 2 hours of dosing.

Discussion: In cats, oral LA produces clinical and hepatocellular toxicity at lower doses than in dogs (126 mg/kg), rats (600 mg/kg), or humans (>2 g/adult). MTD for a single oral dose of LA in cats is < 30 mg/kg. Protein-bound LA and DHLA are primarily excreted in bile, but enterohepatic circulation returns the compounds to the liver. This recirculation could allow accumulation and further metabolism to toxic by-products that interfere with normal cellular metabolism and may trigger apoptosis of hepatocytes.

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Effects of dietary clenbuterol and cimaterol on performances, muscle composition and endocrine response in broiler chickens

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Dietary administration of beta-adrenergic agonists (e.g. clenbuterol and cimaterol) as energy repartitioning agents in animal nutrition is forbidden in European Countries. In domestic growing animals these molecules bring about an increase in protein deposition and/or a decrease in fat deposition. The effects in birds are less pronounced than in mammals and seem to be more evident in animals either very old or very young and/or to be related to dietary concentration of protein.

As illegal administration of beta-adrenergic agonists is assumed to occur in practice, we planned a trial to define the influence of these molecules on male broiler chicken. Fifty-four Ross 508 broiler chicken, 36 days old and of same initial mean live weight, were randomly divided into three experimental groups: control group, clenbuterol group (1 ppm) and cimaterol group (1 ppm). Diets were isonitrogenous and isoenergetic (E.M.: 12.85 MJ/kg; C.P.: 19.20%); animals were fed *ad libitum*. Body weight and feed consumption were weekly recorded to calculate feed conversion ratio (FCR). At the age of 57 days animals were slaughtered and carcass yields measured. Breast samples were analysed for chemical and fatty acid composition. Testicular androgen receptor (An-R) concentration were measured on cytosol fractions whereas cellular membranes of heart, lung and brain were used to evaluate β -adrenergic receptors (β -AR) levels.

Table 1: chemical composition of breast muscle

	control	clenbuterol	cimaterol	Growth performances (weekly weight gain, feed intake, FCR) and carcass yields were not influenced by dietary treatment even if in clenbuterol group leg
Crude protein (%)	22.93±0.659	23.66±0.535	23.60±0.847	
Ether extract (%)	1.38±0.274	1.19±0.120	1.39±0.211	
Ash (%)	1.18±0.10	1.20±0.05	1.22±0.11	
Dry matter (%)	28.29±1.91	26.86±0.87	26.96±0.89	

weigh was higher ($p < 0.05$). Chemical and fatty acid composition of breast muscle was similar among the groups showing a negligible influence of beta-adrenergic agonists on lipid and protein metabolism in chicken.

Table 2: β -adrenergic receptors concentration (fmol/mg) (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

	β -AR control	β -AR clenbuterol	β -AR cimaterol	Both dietary clenbuterol and cimaterol induced An-R down-regulation in testicular tissue: control
Heart	244±10	137±11***	177±18**	
Lung	906±16	728±28*	721±75*	
Brain	393±28	253±21***	259±23**	

33±2 fmol/mg of protein; clenbuterol 8±0.4 fmol/mg ; cimaterol 8±0.3 fmol/mg. β -adrenergic receptors concentration in heart, lung and brain are reported in table 2.

Data suggest that performances and muscle composition are poorly influenced by dietary administration of beta-adrenergic agonists. It would be useful to analyse the aspects related to endocrine response, as quantification of An-R and β -AR receptors, to identify illegal administration of these molecules.

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lipid metabolism

Chairperson : André Chwalibog (Denmark)

Interaction of dietary unsaturation level with linolenic acid and α -tocopherol deposition in poultry meat

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Over recent years consumers are more aware of the direct repercussion of food on their health. Some works have been studying the meat ω 3 polyunsaturated fatty acids enrichment, like linolenic acid (C18:3 ω 3), associated with prevention of cardiovascular diseases. However, the higher unsaturation level in meat leads to an increase in the susceptibility to lipid oxidation. In order to improve oxidative stability of such products, inclusion of tocopherol in animal feeds has been successfully used. Moreover, this supplementation increases the meat vitamin E content. Nevertheless, there are few data regarding the optimum dietary polyunsaturated fatty acids and vitamin E to achieve nutritional benefits in meat.

The present study was carried out to evaluate the effect of dietary unsaturation level and supplementation with α -tocopheryl acetate on linolenic acid and α -tocopherol content in raw poultry breast.

One hundred and ninety-six female broiler chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary polyunsaturated fatty acids (15, 34, 45, 61 g/kg) and 4 levels of supplementation with α -tocopheryl acetate (0, 100, 200 and 400 mg/kg). The unsaturation degree was achieved by replacing linseed and fish oil to a basal diet enriched with 9% tallow. Linolenic acid content for feed and breast was determined as previously described by Sukhija and Palmquist (1988) and Carrapiso et al. (2000), respectively, using C19:0 as internal standard for the quantification. α -Tocopherol from feed and breast meat was analysed using the method described by Jensen et al. (1999).

The dietary polyunsaturated fatty acids clearly affected the linolenic acid content of raw breast meat. Linolenic acid content in breast increased linearly with dietary polyunsaturated fatty acid inclusion of poultry feed ($y = 0.1106x - 0.0021$, $R^2 = 0.73$). However, the supplementation with α -tocopheryl acetate did not modify the linolenic acid content in raw breast.

α -Tocopherol content of breast meat increased linearly with dietary α -tocopherol supplementation ($y = 0.0412x + 1.1498$, $R^2 = 0.70$). Moreover, α -tocopherol content of breast meat was reduced as the inclusion of dietary unsaturation increased. Thus, increasing of 46 g dietary polyunsaturated fatty acid, significantly, decreased 1.86 fold α -tocopherol content in breast of poultry feed with diets supplemented with α -tocopheryl acetate.

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Relationship between dietary PUFA level and apparent absorption of vitamin E in poultry

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Vitamin E is the major lipid-soluble antioxidant present in nature. Its main function is to protect polyunsaturated fatty acids (PUFA) from peroxidation caused by free radicals. Some old works with rats (Weber et al., 1966, Gallo-Torres et al., 1971) have suggested that dietary PUFA interfere with the intestinal absorption of vitamin E so they increase vitamin E requirements not only because they are easily oxidized and need more protection but also because lower vitamin E intestinal absorption. More recent papers question this fact (Tijburg et al. 1997). The objective of our work was to evaluate to what extent dietary PUFA have an effect in the apparent absorption of α -tocopherol in broiler chickens.

One hundred and ninety-six female broiler chickens (8 d) were randomly distributed into 16 experimental groups resulting from the combination of 4 levels of dietary PUFA (15, 34, 45 and 61 g/kg) and 4 levels of supplementation of Vitamin E as α -tocopheryl acetate (0, 100, 200 and 400 mg/kg). The unsaturation gradient was achieved by replacing linseed and fish oil to a basal diet enriched with 9% tallow. A digestibility balance was carried out between days 18 and 22 of age. Feed consumption and total faecal excretion were measured. Faecal samples were collected, lyophilised, milled and stored at -80°C for subsequent analysis. Feed samples were also milled and stored at -80°C .

α -tocopherol and α -tocopheryl acetate content in feed and faeces were determined by HPLC using a direct solvent extraction method described by Lee et al. (1999). 1-Phenyldodecane was used as an internal standard for quantification. Total fatty acid content in feed and faeces was determined by GC following transmethylation in methanol-HCl, as described by Sukhija and Palmquist (1988). C_{19} was used as the internal standard for quantification. The apparent absorption of α -tocopherol was calculated as intake minus excretion of α -tocopherol equivalents (1 mg of *all rac* α -Tocopheryl acetate = 0.67 mg of d- α -tocopherol) expressed as a percentage of the intake. The apparent absorption of total fatty acids was calculated in the same way.

Apparent absorption of α -tocopherol decreased as the level of inclusion of α -tocopheryl acetate in the diet increased (41.7%, 37.4% and 30.8 % for 100, 200 and 400 mg/kg respectively, $p \leq 0.05$). Dietary PUFA affected apparent absorption of both total fatty acids (47.4% for 15 g/kg vs. 65.9% for 34 g/kg and 74.4% and 78.5% for 45 and 61 g/kg, $p \leq 0.001$) and α -tocopherol (21.4% for 15 g/kg vs. 40.7%, 42.1% and 42.3% for 34, 45 and 61 g/kg, $p \leq 0.001$). The low absorption of saturated fat impairs the absorption of other lipidic substances, as it has happened with α -tocopherol in the tallow-rich diet. Besides this, we couldn't find any significant difference in α -tocopherol apparent absorption among the rest of unsaturated treatments, suggesting there is no interference of the dietary PUFA level in α -tocopherol uptake in our levels of inclusion. Nevertheless, a possible degradation of α -tocopherol while protecting PUFA in the gastrointestinal tract should be considered.

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Influence of dietary lipid source and genotype on fatty acid composition of Muscovy duck meat

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Reported benefits of long-chain polyunsaturated fatty acid (LC-PUFAs), mainly EPA and DHA, on human health have increased the interest in animal products with high level of these molecules. Several studies showed the possibility to modify PUFAs composition of poultry meat by changes in the lipid composition of diet. The aim of this trial was to investigate the possibility to modify meat lipid composition of two different muscovy duck (*Cairina moschata domestica* L.) strains by dietary strategies. 42 female French broiler strain muscovy ducklings, 35 days old, and 42 female Italian rural strain muscovy ducklings, 42 days old, both of same initial mean live weight were randomly assigned each to two experimental groups (three replications/dietary treatment). The two isoenergetic and isonitrogenous diets (E.M.: 12.5 MJ/kg; C.P.: 16%) differed for lipid source (2% fish oil vs. 2% soybean oil). Breast samples were analysed for fatty acid composition. Multifactorial ANOVA was used to process data; the factors were dietary treatment and strain.

Fatty acid composition of breast samples (% of methyl esters; mean values \pm d.s.)

	French broiler strain		Italian rural strain		P level	
	soybean oil	fish oil	soybean oil	fish oil	diet	strain
C14:0	0.36 \pm 0.11	0.42 \pm 0.08	0.27 \pm 0.01	0.44 \pm 0.01	0.05	n.s.
C16:0	23.23 \pm 2.16	23.09 \pm 0.28	21.92 \pm 0.64	22.73 \pm 0.52	n.s.	n.s.
C16:1n7	1.02 \pm 0.35	0.97 \pm 0.18	0.68 \pm 0.09	0.96 \pm 0.18	0.05	n.s.
C18:0	14.76 \pm 2.09	15.21 \pm 0.78	16.23 \pm 0.46	15.94 \pm 0.02	n.s.	n.s.
C18:1n9	21.63 \pm 6.48	18.62 \pm 3.10	17.50 \pm 1.73	16.95 \pm 0.96	n.s.	n.s.
C18:1n7	2.40 \pm 0.14	2.77 \pm 0.15	2.69 \pm 0.23	2.81 \pm 0.04	n.s.	n.s.
C18:2n6 (LA)	17.96 \pm 3.12	15.85 \pm 2.44	18.29 \pm 1.77	15.03 \pm 2.22	0.09	n.s.
C18:3n3 (ALA)	0.37 \pm 0.07	0.27 \pm 0.04	0.35 \pm 0.03	0.24 \pm 0.09	0.05	n.s.
C20:0	0.12 \pm 0.03	0.14 \pm 0.02	0.15 \pm 0.01	0.14 \pm 0.01	n.s.	n.s.
C20:1n9	0.15 \pm 0.01	0.25 \pm 0.04	0.09 \pm 0.07	0.29 \pm 0.08	0.05	n.s.
C20:2n6	0.33 \pm 0.08	0.25 \pm 0.08	0.30 \pm 0.04	0.25 \pm 0.01	n.s.	n.s.
C20:3n6	0.75 \pm 0.19	0.74 \pm 0.15	0.78 \pm 0.03	0.71 \pm 0.16	n.s.	n.s.
C20:4n6 (AA)	9.97 \pm 2.40	7.97 \pm 0.72	12.88 \pm 1.44	9.57 \pm 0.71	0.05	0.05
C20:5n3 (EPA)	-	1.76 \pm 0.20	-	1.67 \pm 0.18	-	-
C22:5n3 (DPA)	0.57 \pm 0.21	1.42 \pm 0.24	0.62 \pm 0.27	1.46 \pm 0.43	0.01	n.s.
C22:6n3 (DHA)	0.70 \pm 0.13	6.56 \pm 1.39	1.21 \pm 0.10	7.36 \pm 1.31	0.01	n.s.
SFA	38.76 \pm 1.87	39.23 \pm 0.90	38.93 \pm 0.45	39.72 \pm 0.61	n.s.	n.s.
MUFA	25.70 \pm 6.52	23.27 \pm 3.23	21.37 \pm 1.91	21.68 \pm 1.38	n.s.	n.s.
PUFA	34.61 \pm 5.38	36.44 \pm 2.81	38.98 \pm 1.43	37.84 \pm 0.81	n.s.	n.s.
n6	23.00 \pm 3.23	18.46 \pm 2.55	23.92 \pm 0.63	17.53 \pm 1.76	0.05	n.s.
n3	11.61 \pm 2.65	17.98 \pm 1.86	15.06 \pm 1.75	20.31 \pm 2.31	0.05	n.s.
n3/n6	0.50 \pm 0.09	0.99 \pm 0.17	0.63 \pm 0.09	1.18 \pm 0.26	0.01	n.s.

Fatty acid composition of meat was influenced by dietary oil supplementation: fish oil supplementation reduced AA and ALA amount, increased n-3 LC-PUFAs content and n6/n3 ratio was deeply reduced. AA rate was influenced either by dietary treatment or genotype. In conclusion fatty acid composition of duck meat could be easily modified by dietary manipulation. Receptiveness to lipid modification appears very similar between the two examined strains, except for AA which synthesis appeared highest for Italian rural strain.

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Dietary supplementation of polyunsaturated fatty acids in cats

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All vertebrate species so far studied have been shown to have a dietary requirement for polyunsaturated fatty acids (PUFA) which are essential for growth, development and health. In vivo and in vitro experiments suggest that dietary intake of PUFA can affect bone remodeling directly or indirectly.

Like other mammals cats are not able to synthesize linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3). These fatty acids are further converted into their respective essential long-chain metabolites of the n-6- and the n-3-families by several reactions of desaturation and elongation. Unlike other mammals cats lack the enzyme Δ -6-desaturase (Rivers et al.) and thus, the capacity to convert LA into arachidonic acid (AA, 20:4n-6) and ALA into eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). As a consequence the adequate dietary provision of the above mentioned fatty acids presents a major point of interest in the nutrition of the cat.

The aim of this study was to investigate the influence of omega-3 fatty acids on the serum fatty acid pattern and the effect on bone metabolism in cats.

For this purpose a feeding study was performed with 16 European short-hair cats and a duration of 20 weeks (including a preperiod, test period 1, wash out period, test period 2). Two diets produced for this purpose and differing only in their source of fat were fed. Beef tallow was used in the control diet and fish oil in the test diet. Both diets contained nearly equal amounts of LA, ALA and AA whereas EPA and DHA were 0,095 % and 0,058 % in the test diet compared to 0,006 % in the control diet. Both diets were considered balanced in their mineral, vitamin and amino acid content. The animals were separated into two groups with regard to their age, sex and weight. After a preperiod (4 weeks) with the control diet group 1 was fed for 4 weeks the test diet and group 2 the control diet, followed by a 6 week wash-out period with the control diet. In test period 2 (4 weeks) group 1 was fed the control diet and group 2 the test diet. All animals were regularly clinically examined and found in a good condition.

Blood and urine samples were taken at the end of every period. Alkaline Phosphatase (serum) as a marker of bone formation and desoxyypyridinoline (urine) as a marker of bone resorption were assessed. Serum fatty acids from blood samples, taken at the beginning and at the end of the test period 1, were analysed by gas chromatography.

Under our experimental conditions, in contrast to what has been reported in humans and in rats, no influence of omega-3 fatty acids on biochemical markers of bone turnover was observed.

In both groups at least a 13-fold increase in EPA and a 4-fold increase in DHA was found. The results indicate that the serum level of n-3 polyunsaturated fatty acids may be increased by feeding a diet rich in n-3 fatty acids in healthy cats.

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Reverse cholesterol transport in dogs

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Objective: In humans, the transport of cholesteryl ester (CE) from plasma to the liver (reverse cholesterol transport) involves 3 pathways : two direct ways mediated either by apoA-I or by hepatic scavenger receptor B-I responsible of selective HDL-CE uptake and an other indirect way where HDL CE is exchanged for VLDL and LDL triglycerides by cholesteryl ester transfer protein (CETP). The aim of our study was first to determine CETP activity and then to evaluate the importance of these 3 pathways in dogs, a species where HDL are the main carrier of cholesterol.

Materials and methods: CETP mass was determined by isotopic CE transfer assay in lipoprotein-deficient plasma and its activity was measured in plasma by fluorimetry in eight healthy dog and in three humans as reference. ¹³C-acetate and D₃-leucine as labeled precursors of cholesterol and apoA-I, were infused over 8 h intravenously to dogs. Blood samples were obtained during the perfusion and during the 16h after ending the perfusion. VLDL, LDL and HDL were isolated by ultracentrifugation. After isolation of CE in all lipoprotein fractions and isolation of unesterified cholesterol and apoA-I in HDL, isotopic enrichment was measured by GC-C-IRMS for cholesterol and GC-MS for apoA-I. Compartmental modeling (SAAM II) was used for the analysis of tracer data.

Results: Dogs had approximately the same CETP mass as human (8.0 and 10.6 % of lipoprotein-independent CE transfer rate in dogs and humans respectively) but its activity was 3-fold lower (0.018 %·h⁻¹ versus 0.048 %·h⁻¹ of CE transferred by CETP in dogs and humans respectively). These results suggested that dog was a species poor in CETP activity leading to negligible CE transfer among HDL, VLDL and LDL. Indeed, VLDL and LDL CE concentrations were very low with a high rate of CE catabolism of $6.37 \pm 1.76 \text{ h}^{-1}$ and $0.038 \pm 0.002 \text{ h}^{-1}$ for VLDL and LDL respectively. HDL kinetic analysis allowed to measure the activity of cholesterol esterification ($0.18 \pm 0.03 \text{ h}^{-1}$), rate of apoA-I catabolism ($0.008 \pm 0.003 \text{ h}^{-1}$), HDL CE turnover ($0.078 \pm 0.013 \text{ h}^{-1}$) and CE selective uptake ($0.070 \pm 0.013 \text{ h}^{-1}$).

Conclusion: Our results suggested that, due to the low CETP activity, reverse cholesterol transport in dogs, which concerns essentially HDL, is characterized by a high rate of esterification and selective HDL CE uptake (89 % of HDL CE turnover). Therefore, the dog can be considered a useful model to study the nutritional modulation of this process.

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amino acids & protein

Chairperson : Quinton Rogers (USA)

Six months taurine or methionine supplementation in Newfoundland Dogs suffering from low whole blood taurine

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Low blood taurine has recently been documented in a substantial proportion of Newfoundland dogs.¹ In that population, blood taurine levels were found to be significantly lower in dogs with dilated cardiomyopathy (DCM) compared to normal dogs. The rationale for this study was therefore to investigate whether taurine deficiency in Newfoundlands could be corrected by taurine and/or methionine supplementation.

Material and methods: Fifty three dogs with whole blood taurine (<200nmol/ml) were identified and echocardiography was used to classify dogs as either normal, having echocardiographic abnormalities that may precede dilated cardiomyopathy (such as decreased contractility or left ventricular enlargement) or having dilated cardiomyopathy. The dogs with DCM received 1000mg crystalline taurine PO q12h and the remaining dogs were age and sex matched before being randomly assigned to receive 250mg taurine PO q12h or methionine 750mg PO q12h and 4 dogs were assigned to receive a proprietary giant breed diet. Heperanized whole blood and urine were collected initially (T₀) and after 3 (T_{3m}) and 6 (T_{6m}) months of supplementation. Samples were frozen at -20°C pending analysis. Taurine was measured in plasma with an amino acid analyser following protein precipitation and filtration. Urine creatinine was assayed with a commercially available enzymatic kit. Data were analyzed by repeated measure ANOVA. Data are expressed as a mean ± SEM.

Results: 48 dogs completed the study. Three dogs were excluded because of other pathologic conditions and 2 dogs were lost to follow-up. Whole blood taurine increased in all dogs from 144±8 nmole/ml at T₀ to 324±14 nmole/ml at T_{3m} and 275±10 nmole/ml at T_{6m}. No difference was observed based on source and amounts of supplementation. Urine Taurine/creatinine ratio was minimal at baseline and increased significantly following methionine or taurine supplementation. More taurine was excreted in urine of dogs given the highest level of taurine.

Discussion: These results suggest that 250 mg of taurine or 750 mg of methionine PO q12h as well as a dry expanded diet containing 1000 mg taurine/kg, as fed, will normalize taurine status in taurine deficient Newfoundland dogs. In the dogs of this study, low taurine status could not be explained by higher taurine losses nor their inability to use methionine as a precursor.

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A model for the development of taurine deficiency in dogs by the use of cholestyramine

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Cholestyramine is a resin used in human medicine to lower plasma cholesterol by enhancing bile salts excretion. Since taurocholic acid is the main bile salt secreted by the dog, we hypothesized that by feeding cholestyramine to dogs, taurocholic acid excretion would be enhanced and negative taurine balance would occur. The purpose of this study was to determine whether the use of cholestyramine depletes body taurine pools in dogs. Twelve adult dogs (29 - 43 Kg BW) were fed an experimental lamb-meal and rice-bran diet (18% Crude Protein, 14% Crude Fat, DM) to maintain body weight constant. At week 12, the experimental group (n=6) was given 4 grams of cholestyramine/day, added to their diet. Twelve weeks later the dose was increased to 8 grams of cholestyramine/day. The control group (n=6) received no cholestyramine throughout the study. At week 28, both groups were switched to a second batch of food (16% Crude Protein, ~ 21% Crude fat, DM) to enhance the rate of taurine depletion. Blood was collected every 4 weeks and muscle biopsies were taken at weeks 0, 4, 12, 20, and 36. Free catch urine was collected at weeks 0, 12, 28, and 36. Taurine concentrations were determined in plasma, whole blood, skeletal muscle and urine. Plasma taurine concentrations in the control group did not change over 40 weeks. In contrast, plasma taurine concentrations from the experimental group dropped to 21 nmol/mL by week 40. At this point, five of six dogs were below the critical range for taurine deficiency in plasma (< 40 nmol/mL). Whole blood taurine concentrations from the control group initially increased, then decreased to the initial values by week 20, and remained constant thereafter. By week 40, three of six dogs in the experimental group had whole blood taurine concentrations below 150 nmol/mL (range: 32 – 184 nmol/mL), whereas all dogs in the control group were above 210 nmol/mL (range: 216 – 270 nmol/mL). Muscle taurine concentrations and urinary excretion of taurine were also significantly lower in the experimental group compared to the control. These results indicate that it is possible to induce taurine deficiency in healthy adult dogs. This model for taurine deficiency promises to be an useful aid in studying the metabolic and pathological effects of taurine deficiency in dogs.

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The minimal dietary lysine requirement of kittens for maximal growth

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Three experiments using purified diets were conducted to determine the minimal lysine requirements of kittens for maximal growth. The diets used in all experiments were of the same basic composition (g/kg diet): isolated amino acids 240, chicken or turkey fat 250, starch 290.6, glucose 150, mineral mixture 40, sodium acetate 16.4 (to balance the hydrochlorides in arginine, histidine and lysine), vitamin mixture 10 and choline chloride 3. Diets were maintained isonitrogenous by substituting alanine for lysine. Kittens had free access to the diet at all times. Before entering the experiments weaned specific-pathogen-free kittens were accustomed to a complete purified diet.

In the first experiment, duplicate 5 x 5 Latin squares (one square for male and the other for female kittens) with 10 day periods were used. The five concentrations of lysine (free base) used were 5.5, 6.0, 6.5, 7.0, and 11 g/kg diet. Daily body weight gain was linearly related to lysine concentration in the diet from 5.5 to 7.0 g /kg diet ($r^2 = 0.980$), but kittens given the diet containing 11g lysine/kg had higher weight gains (mean \pm SEM) of 15.2 ± 1.7 versus 13.3 ± 3.3 g/day respectively. These results indicate that the lysine requirement is greater than 7.0 g/kg diet.

In experiment 2, duplicate 6 x 6 Latin squares were used, one square allocated to six male kittens and another to six female kittens with 10 day periods. The six concentrations of lysine (free base) in the diets used were 4.0, 5.0, 6.0, 7.0, 8.0 and 11 g/kg. Body weight gain (g/day) was linearly related ($r^2 = 0.985$) to lysine concentration in the diet for the treatments 4 to 8.0 g lysine/kg diet. Rate of gain was not significantly different between kitten given 8.0 and 11.0 g lysine/kg diet. These results indicated that the requirement for lysine is not greater than 8 g/kg diet.

In the third experiment a duplicate 4 x 4 Latin square design was used with dietary concentrations of 6.0, 7.0, 8.0 and 9.0 g lysine (free base)/kg diet. When kittens were given the diet that contained 8.0 g lysine/kg diet they had significantly higher rates of daily gain than when given diets containing 7.0 g lysine/kg, but not significantly different than kittens given the diet containing 9.0 g lysine/kg.

These three experiments indicate that the lysine requirement of kittens for maximal growth is 8.0 g (free base) lysine/kg diet in the free form. Higher dietary concentrations of lysine would be required in practical diets that have a lower lysine bioavailability than free lysine.

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Hair pigmentation can be affected by diet in dogs

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There are reports from breeders that coat color of dogs changed from black to reddish brown when fed certain commercial petfoods. Until recently, this « Red Hair Syndrome » has been considered as anecdotal and unfounded. Lately, it has been reported that levels of tyrosine (Tyr) and phenylalanine (Phe) required to support maximal melanin synthesis in cats were above current recommendations for growth. (Yu et al. 2001, Morris et al. 2001) The purpose of this study was to evaluate if a similar observation could be made in dogs.

Materials and methods : Twelve black puppies (6 Newfoundlands (6 sisters), 6 Labradors (3 brothers and 3 sisters from 2 different litters) were weaned on a large breed commercial puppy diet. They were then divided in 3 groups matched for breed and sex, fed 3 diets with similar guarantee analysis (A,B,C) but with levels of Phe+Tyr in diet A = 1.9 and diet B = 2.6 times the requirements recommended for growth by the American Association of Feed Control Official (AAFCO). Diet C was Diet B coated with free Tyr so to reach 3.2 times AAFCO requirements. Food intake, bodyweight and coat color were monitored for a year. Hair samples were collected monthly, pictures taken bimonthly and plasma samples for amino acid profiles every 3 months.

Results : Dogs remained healthy and showed similar growth patterns on the 3 diets. After 2 months, hair was red in animals fed diet A, and was darker in animals fed diet C than in those on diet B. After 5 months of feeding, the coat of dogs fed diet A appeared reddish brown, and it was possible for a blinded researcher to distinguish between dogs fed diet B or C based on the intensity of the black color. Over the growth period, plasma tyrosine dropped in dogs fed diet A and B whereas it raised in dogs fed diet C supplemented with free Tyr.

Discussion : In dogs, diet can affect hair pigmentation and could explain the « red hair syndrome » reported by breeders. Addition of free available Tyr to the food can prevent the « Red hair syndrome » and optimize hair pigmentation, even for diets that have a level of Phe+Tyr above current recommendations.

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Feeding type (conventional versus organic) and lysine/energy ratio influence meat and carcass characteristics of organic fattening pigs

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Introduction

As organic pigs have more space allowance and an outdoor area, and as the stable is not climatized, a higher basal energy requirement can be expected for organic pig fattening through increased energy needs for activity and thermoregulation. Because of specific feed formulation and the production of the ingredients according to organic guidelines, this can also affect meat and carcass characteristics. The results of two experiments on this topic will be presented.

Material and methods

In the first experiment, 2 groups of 32 pigs, the one grown and housed in a conventional way, the other in an organic way, were divided in 8 groups of 4 animals. In each stable, 4 groups received a conventional feed, the others an organic one, implying four replicates per diet and housing combination. The organic feed was formulated following the European regulations on organic farming, and the digestible lysine content was formulated to a 15% lower lysine content.

In the second experiment, a group of 36 pigs was divided over 9 groups of 2 barrows and 2 gilts and randomly divided over three diets with either a high a medium or a low lysine content, implying three replicates per diet. The lysine content of the low lysine feed was 20% lower than the high lysine feed. In both experiments there was measured a set of carcass and meat characteristics.

Results

In the first experiment, the organic diet led to a higher ultimate pH in loin and ham, a higher CIELAB a* score (redness) and a lower CIELAB L* score (lightness). The drip losses tended to be lower on the organic feed. Intramuscular fat content was higher on organic than on conventional feed. Meat percentage did not differ between the groups.

In the second experiment, dietary lysine content influenced meat percentage and fat thickness. Differences in meat quality parameters could not be demonstrated.

Discussion

These data allow to conclude that dietary lysine/energy ratio does not have a major influence on meat quality within the tested range, whereas it can influence meat percentage by changing fat thickness. Organic feeding can influence meat colour and ultimate pH as well as intramuscular fat content. This might be due to the ban of synthetic amino acids in the organic feed and to the lower lysine content.

This study was supported by the Belgian Ministry of Small Enterprises and Agriculture.

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BioProtein as protein source to growing mink kits and slaughter chickens -energy expenditure and nitrogen metabolism

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BioProtein is a bacterial protein grown with natural gas as carbon and energy source and ammonia as nitrogen (N) source. The crude protein content is ca 70% and the amino acid composition is resembling that of fish meal although the lysine content is somewhat lower. The tryptophan content is high. BioProtein contains ca 7% RNA and 2% DNA. Its apparent faecal N-digestibility is 79% (mink), and 81% (chicken) (Skrede et al., 1998). The objective of this study was to evaluate the effect of dietary supply of BioProtein on N-balance and energy expenditure (EE) in mink and chickens as representatives of rapidly growing animals.

Materials and methods

Both studies were carried out in 5 balance periods each with a 4 days total excreta collection period and comprising a 22 h respiration experiment by means of indirect calorimetry in an open-air circulation system. The mink study was carried out with 16 male mink kits of the standard brown colour type measured from the age of ca 2 months until they were 6 months old. Four dietary treatments were used and BioProtein replaced fish meal (LT quality) on an iso-N and iso-energetic basis. BioProtein supplied 0, 20, 40 and 60% of the digestible protein of the respective diets. The chicken study comprised 4 diets, each in 3 replicates. BioProtein replaced fish meal (LT), and made up respectively 0, 2, 4 and 6% of the diets. The initial 72 animals in week 1 were reduced to 24 in week 5. During weeks 1 and 2 groups of animals were measured in the respiration chambers (6 and 5, respectively), but from week 3 single animals were measured. SAS procedure MIXED was used for repeated measures analyses.

Results and conclusion

Food intake and live weight of the mink kits were not significantly affected by dietary level of BioProtein, but there was a tendency for decreasing values with increasing BioProtein supply. The apparent N digestibility (ADN) of the diets decreased with increasing BioProtein content whereas the amount of retained N (RN) was similar for all diets. The utilization of the digested N (DN) expressed as RN/DN was lowest for the BioProtein free diet while diets providing 20 and 40% of DN from BioProtein showed significantly higher RN/DN values. The age of the animals had a strong influence on ADN, which increased as the kits grew older, RN which declined and RN/DN which first declined and then levelled out. Energy expenditure was almost identical for the 4 diets and ranging from 647 to 660 kJ/kg^{0.75}, whereas period effects were strongly significant and values decreasing from 828 (period 1) to 476 (period 5) kJ/kg^{0.75}. In the chicken experiments animal food intake and gain were independent on diet, but strongly affected by period. Similar to in the mink experiment neither N metabolism nor EE were affected by diet, but strongly affected by period. Again, EE was almost identical between diets (range 849 to 869 kJ/kg^{0.75}) but declining significantly from period 2 onwards with progressing age of the chickens (847, 975, 944, 824 and 688 kJ/kg^{0.75} in periods 1 through 5). In conclusion, BioProtein at the dietary levels used here did not affect N metabolism and energy utilization of growing mink kits and chickens.

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Rumen bypass of free methionine and lysine for double-muscle Belgian Blue bull

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Double-muscle Belgian blue (dmBB) bulls have higher amino acid (AA) requirements than other beef cattle (Froidmont et al., 2000, 2001). Methionine (Met) and lysine (Lys) are the most limiting AA in several dietary conditions. Because rumen protected AA are not economical for such animals, our study investigates the proportion of these AA, supplied on a free form, susceptible to escape from rumen fermentations and be digested in the small intestine.

Six growing dmBB bulls (257 ± 9 kg), fitted with fistulas in rumen, duodenum and ileum, received a diet containing 13% straw and 87% concentrate, at an intake level of $85 \text{ g/kg}^{0.75}$. Animals were submitted to three 1-d treatments consisting to supply in the rumen, during the morning meal, 40 g Met, 60 g Lys or 40 g Met + 60 g Lys in a cross-over design. Two days separated each treatment. At the beginning and at the end of the cross-over periods, two control periods were realized during which animals did not receive any AA supplement. Concentrations of Met and Lys in the rumen fluid were measured 1, 2, 4, 6, 8, 10 and 12 hours after AA administration. Plasma Met and Lys contents were also determined 3 h after treatments. CoEDTA was used as liquid phase marker in order to determine the volume and the outflow rate of rumen fluid as well as the outflow kinetics of AA according to equations proposed by Volden et al. (1998).

During control periods, free Met and Lys contents in the rumen fluid were negligible. Volume and outflow rate of rumen fluid did not vary between treatments and reached respectively 28.9 L and 9.5 %/h.

Total amounts of free Met escaping from rumen fermentations reached 15.7 ± 2.5 g/d and 13.9 ± 2.6 g/d when this AA was supplied alone or simultaneously with Lys and did not differ between these treatments ($P > 0.05$). Amounts of Lys leaving the rumen intact reached respectively 27.5 ± 5.5 g/d and 26.6 ± 5.5 g/d for individual or simultaneous supply and did not differ with the mode of administration ($P > 0.05$). Comparatively to control periods, a supplement of Met and Lys increased their plasma concentrations by 513 and 133%, respectively. This confirms the high availability of free AA bypassing the rumen.

In this study, 37% of Met and 45% of Lys escaped from rumen fermentation and arrived into the duodenum. Bypass rates of AA were higher than those mentioned previously for dairy cows (Volden et al., 1998). The reason could come from the smaller rumen volume in growing dmBB bulls (29 vs 70 L), causing higher AA concentrations in the rumen fluid for a given AA supply. High AA concentrations decrease desaminase activity in rumen fluid and could increase, by this way, the proportion of free AA bypassing the rumen. Since the price of free AA are until five times lower than protected ones, their utilization in dmBB bull feeding appeared to be interesting to correct the AA pattern of metabolizable protein.

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Nitrogen requirements for maintenance of captive *Aceros*- and *Buceros* Hornbills

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In recent years hornbills have been identified as a group in need of proper husbandry guidelines and a priority for captive breeding, because of their threats in the wild and low reproductive successes (successfully raised chicks) in captivity. Diet could be a key factor in developing successful programs¹ However, actual nutrient requirements for hornbills have not been determined; evaluations of diets therefore can only be extrapolated from other model species. Dietary protein is metabolically important because it can supply essential amino acids and through intermediary metabolism, it can give rise to energy. Excess protein in captive diets may result in nutritional imbalances, which may lead to stress in kidney function, reduced health and breeding condition. High levels of dietary protein cause acidosis, and may contribute to a variety of conditions in birds, including poor bone mineralization, thinning of eggshells, and poor growth³. We quantified protein intake and utilization (following methods of⁴ and⁵) in captive *Aceros* and *Buceros* hornbills. The goal of the study² was to determine protein requirement (nitrogen requirement x 6.25) at maintenance level and to test differences in this requirement within *Aceros* (frugivorous) - and *Buceros* (moderately omnivorous) hornbills. The requirements were obtained from non-invasive feeding experiments. Over two months, seven feeding-trials were conducted at St Catherine's Island, GA, USA. Three *Aceros* and two *Buceros* species were fed homogenous isocaloric diets. A mixture of bird of paradise pellet (Zeigler Bros., Gardners, PA, USA), grapes and raisins was offered to the birds as grape-sized balls, supplemented with diced cantaloupe to maintain hydration. To vary protein level (range 10,8 – 22,6 %) within the diets, different amounts of a powdered soy protein supplement (Protein booster, Naturade, Inc., Irvine, CA, USA) were added to the mixture. Test diets were fed for three consecutive days. Birds were weighed at the beginning and the end of each trial by training onto a perch-scale. A loss of > 5% of the bodymass would indicate insufficient dietary protein (tissue catabolism) and would halt the feeding trial. All defections and diet samples were collected for nitrogen (Kjeldahl) analysis. A four-day period, in which the bird's regular diet was fed, separated the feeding trials. Data were analysed for nitrogen balance, nitrogen equilibrium (regression of nitrogen intake vs. nitrogen excretion), body mass and endogenous nitrogen losses. Nitrogen equilibrium occurred at 0,284 g N/BW^{0,75}/d. Endogenous loss was 0,139 g N/BW^{0,75}/d. No differences in N balances were found between *Aceros* and *Buceros* hornbills, indicating a similar requirement for nitrogen. The methodology of this study suggested a dietary crude protein requirement of 5,2% DM in a diet containing 16,8 kJ or 13 mg protein/ Kcal (GE) on a dry matter basis. However, this value was determined by extrapolation and not has not been experimentally determined to be adequate. In this study, the hornbills maintained body mass on a diet containing 10,8% CP (4 Kcal/g DM).

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Blood serum branched chain amino acids and tryptophan modifications in horses competing in long distance rides of different length.

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The role of protein catabolism, and the contribution of different amino acids (in particular branched chain amino acids, BCAA) to the energy supply during long distance exercise is now well documented for human athletes. The above mentioned catabolism could lead to an increase in the Tryptophan/BCAA ratio. This could subsequently induce a higher serotonin production in the brain and an early onset of the “central fatigue” during exercise. Based on these considerations, we studied the modifications of blood serum BCAA and tryptophan (Try) levels in endurance horses competing in long distance rides of different length, during exercise. We considered 4 groups of horses (GA, GB, GC and GD); GA and GB were composed of 6 horses each, aging from 6 to 12 years, competing respectively in 20 and 50 km rides. GC was composed of 10 horses aging from 7 to 10 years, competing in a 32 km lasting ride; GD was composed of 8 horses aging from 6 to 10 years, competing in a 72 km lasting ride. All horses were Arabian or Anglo Arabian; blood samples were drawn from jugular vein both before and just after the end of the ride for all the horses.

	GA (20 km)		GB (50 km)	
mmol/l	At rest	End race	At rest	End race
Leu	98.8± 8.0	152.8±10.9*	129.6±34.8	278.8±69.5°
Val	142.5±27.5	240.6±65.3°	171.4±37.8	254.8±47.5°
Iso	70± 33.9	92.3±52.6	112.8±30.4	115.8±28.3
Try	19.7±3.6	23.3±4.9^	15.1±1.4	12.9±3.0
Try/BCAA	0.06±0.008	0.04±0.01	0.035±0.004	0.022±0.005
	GC (32 km)		GD (72 km)	
mmol/l	At rest	End race	At rest	End race
Leu	139.1±23.3	179.3±31.6°	134.4±15.0	69.8±5.7*
Val	148.5±19.4	186.3±36.8*	175.6±17.4	123.6±17.7°
Iso	87.3±11.4	94.3±15.1°	132.4±14.5	102.4±32.2°
Try	21.3±1.8	39.6±3.9*	15.4±2.7	30.8±8.4°
Try/BCAA	0.06±0.004	0.08±0.009	0.038±0.002	0.10±0.003°

Legend: * p<0.001; ° p<0.01; ^ p<0.05

All blood serum samples were analysed for their leucine (Leu), Valine (Val), Isoleucine (Iso) and Try levels, by amino acid derivation and HPLC detection. Mean values and their standard deviations are shown in the table. Student's T test for paired data was used to study the differences between the different sampling moments for each group (at rest vs. after race). The horses in the 72 km race had an increase in serum try and a decrease in BCAA such that the Try/BCAA ratio increased about 2 fold, perhaps indicator of fatigue in long distance rides and long lasting exercise in general.

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digestive kinetics

Chairperson : NancyLou Conklin-Brittain (USA)

Influences of rabbit breed on relative size of the intestinal tract and the composition of its content

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While in laboratories and in meat production primarily large rabbit breeds are used, smaller breeds (e.g. dwarf rabbits) are predominantly kept as pets. At the same time, data on nutrition physiology and digestive capacity generally gathered for larger rabbit breeds are sometimes indiscriminately applied to smaller ones. But in feeding studies on digestibilities in different rabbits breeds (smaller and greater ones) dwarf rabbits showed a higher digestion capacity than New Zealand White or Belgian Giants (1). Aim of this study, therefore, was to illustrate physiological parameters in the intestinal tract (IT) and possible breed-related peculiarities to explain such differences and to take these into account in optimizing the nutrition of dwarfs.

Material/Methods: 5 adult female rabbits each of the breeds Belgian Giant (BG; \bar{X} 7.27 \pm 0.18 kg BW), New Zealand White (NZ; \bar{X} 4.36 \pm 0.60 kg BW) and Dwarf rabbit (DR; \bar{X} 1.82 \pm 0.30 kg BW) were available. The rabbits were fed a pelleted complete diet on the basis of green meal (offered restrictively: 3 g DM/100 g BW). After an adaptation of 10 days the rabbits fasted about 24 hours. On the next day they got 1 g diet per 100 g BW and were sacrificed 6 h ppr. to obtain the chyme. The individual compartments (stomach, duodenum, caecum, proximal/distal colon, rectum) were ligated, weighted and the content extracted. The chyme was analyzed using standard methods of analysis (nutrients, pH, NH₃, FFA, lipopolysaccharides). Particle sizes in chyme and feces were determined via wet sieve analysis. The following table shows some of the **results**:

	BG		NZ		DR	
• weight of IT (excl. chyme)						
- total (% of BW)	5.70 \pm 0.76		5.93 \pm 0.50		5.55 \pm 0.58	
- stomach (% of BW)	1.29 \pm 0.39		1.80 \pm 0.34		1.34 \pm 0.34	
- caecum (% of BW)	1.34 \pm 0.15		1.34 \pm 0.09		1.47 \pm 0.18	
• amounts of chyme						
- total (g DM/100 g BW)	1.61 \pm 0.21		1.52 \pm 0.24		1.41 \pm 0.24	
- proportion in stomach (%)	40.4 \pm 10.9		47.0 \pm 6.70		34.0 \pm 6.12	
- proportion in caecum (%)	40.3 \pm 11.0		37.5 \pm 4.06		46.6 \pm 4.25	
• particle size (%)						
- stomach ($\geq 1.0 / \leq 0.25$)	61.1 \pm 9.92	27.7 \pm 8.36	51.0 \pm 2.55	35.2 \pm 5.23	50.1 \pm 3.39	30.4 \pm 6.4
- feces ($\geq 1.0 / \leq 0.20$)	69.7 \pm 15.8	10.1 \pm 6.01	60.4 \pm 2.11	22.7 \pm 2.84	55.7 \pm 5.14	24.0 \pm 6.9

Microbiological investigation of caecum chyme showed – without breed differences - following results (data in Cfu/g): Cl. perfringens: 10^2 – 10^7 ; E. coli < 10^2 ; Lactobacillae < 10^2 ; Enterococcus: < 10^3 and gramneg. Bacteria 2.6 – 7.1×10^8 . Data on pH, ammonia (slightly higher levels in dwarf rabbits) or FFA in the chyme did not indicate breed influences.

Summary: Dwarf rabbits showed the lowest weight of ITT. The stomach was slightly lower and the chyme left them earlier than in other ones. Most of total chyme is located in the large ITT (especially caecum). Furthermore the chyme (stomach) of DR is characterized by a high level of well grounded particles (chewing intensity[↑]?). It seems, that dwarfs follow up the strategy to channel quickly the chyme from stomach to caecum. The apparently higher transit time leads to a higher intensity of caecotrophy and therefore to a higher digestion capacity.

(1) B. ZUMBROCK, P. WOLF and J. KAMPHUES (2001): Investigations on ingestion behaviour and digestibility of organic matter in rabbits of different breeds. Proc. 5th ESVCN-Conference, 13th–14th September 2001, Sursee, Switzerland

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Relationship of genotype, body composition and sexual maturity in females of different rabbit breeds

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Trial using young female twenty-six, 6-week-old New Zealand White and a twenty-two 7-week-old Hungarian Giant rabbit was carried out to establish the total body composition and sexual maturity in function of feeding intensity. Animals in each breed were divided into two groups: the control ($n_{\text{NZW}}=13$; $n_{\text{HG}}=11$), which was fed *ad libitum* (AL) while their sisters' ($n_{\text{NZW}}=13$; $n_{\text{HG}}=11$) feeding was restricted exactly the 70% of *ad libitum* intake (RS). The energy concentration of pelleted feed mixture was 11.5 MJ/kg DE and that of crude protein 15.9%. The trial lasted between the age of 6 to 18 weeks of the NZW and 7-24 ones of HG animals. To establish the basic values at the beginning of experimental feeding, another analogue 5-5 rabbits were analysed for major chemical components in both breeds. At the end of the trial the total body's major chemical composition has been measured in all rabbits. In order to follow ovarian and pituitary actives, hCG and GnRH hormonal treatments were used for four times in each animal during the trial. Clinical observation (colour and swelling of the external genitalia) has been made on days 2 and 5 following the hormone treatments. Blood plasma progesterone levels (P4) were determined from samples, collected 8 days after hormone treatment. At the end of the experiment, the average body weight was significantly lower in RS-Group (NZW: 84.4%; HG: 89.7%) when compared to AL-Group. During the trial the average body weight gain were in the AL-Groups 80.9% in NZW and 87.5% in HG of RS-Groups. As a result of restricted feeding in both breed groups the ash and protein content expressed both in total body and dry matter were increased, while the fat concentration decreased. In Hungarian Giant Females till 11 weeks, and in New Zealand Whites till 13 weeks of age the pituitary and ovarian response to exogenous HCG and GnRH administration failed. In the late maturing Hungarian Giant the intensity of feeding exercised effect from 10-11 weeks of age during the growing period, while in the early maturing New Zealand breed this response could be observed only from 12-14 weeks of age on. Amongst the individuals of both breeds, those animals became pregnant the earliest, which had the highest body fat content. HCG promoted sexual maturity in both breeds in case of *ad libitum* feeding, while in case of restricted feeding no such effect was observed. In case of GnRH administration there was no difference between breeds and feeding levels regarding sexual maturity. It was concluded that restriction of feeding intensity of growing breeding rabbits is a delicate procedure and a 30% rationing exerted unfavourable effect on the body composition, and consequently, slowed down the reproductive maturation of future does.

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Influence of body size on fermentative activity and faecal consistency in dogs

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Objective: Observations on food tolerance in dogs have shown that large breeds have a higher faecal water content and a greater frequency of soft stools. This could be explained by significant differences in colonic functions i.e. a limited water absorption and/or an important fermentative activity. The aim of this study was to investigate the fermentative aspect of this theory by comparing gut microflora activity in dogs differing in body size.

Materials and methods: Adult dogs of four breeds, with body weights varying from 4 to 55 kg, were included in the study: six miniature poodles (MP), six medium size schnauzers (MS), six giant size schnauzers (GS) and six great Danes (GD). The faecal moisture content and faecal consistency were scored daily for two weeks. Fermentative activity was evaluated through the intensity and appearance kinetics of the sulfapyridine (SP) in blood during 30 h, after oral administration of sulfasalazine (SLZ). The maximum concentration of SP and the area under the curve were considered representative for the fermentative intensity in each animal. Lactic acid and volatile fatty acid (VFA) faecal concentrations were also evaluated as well as the total fibre (TDF) digestibility.

Results: A poorer faecal quality (higher moisture and looser consistency) was recorded in large breed of dogs. Larger SP concentration and area under the curve were shown in GS and GD than in MS and MP. The measurement of fermentation products in stools revealed significant higher lactic acid and VFA concentrations in GD (23 and 299 mmol/kg faeces as is, respectively) than in MP (7 and 159 mmol/kg faeces as is, respectively) ($p < 0.0001$). In the same way, TDF digestibility was dramatically higher in large dogs (52.5% vs 38.7% in GD and MP respectively) ($p < 0.0001$).

Conclusion: This study confirms the predisposition of large breed dogs to have soft stools. Greater concentrations of SP and faecal fermentation products in GS and GD suggest a higher fermentative activity in large and giant dogs. This inference is confirmed by a higher TDF digestibility in GS and GD than in MS and MP. These results suggest that a high fermentative activity is a possible cause of poor faecal quality in large breed of dogs.

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Age-related changes in digestibility and gastric emptying in growing dogs

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Objective: Many changes occur in morphology as well as in function of the gastrointestinal tract during growth. It could be thus hypothesized that age could affect the transit time (overall or sequential, gastric, intestinal or colonic). These changes could also modify the digestive process and efficiency and lead to significant nutritional consequences. The aim of this study was therefore to assess eventual changes in both the digestibility of a dry diet and its gastric emptying time using radiopaque markers in growing dogs.

Materials and methods: Twenty four dogs from very small to giant breeds, 6 miniature poodles (MP), 6 medium schnauzers (MS), 6 giant schnauzers (GS) and 6 great Danes (GD) were studied at 12, 22, 36 and 60 weeks of age. The same diet was used for the overall period. Digestibility coefficients were determined over a 7-day period in each dog at each age. One week after each digestibility trial, dogs received 30 radiopaque markers (BIPS[®], 1.5 mm diameter, Chemstock Animal Health Ltd, New Zealand) mixed with the diet. Abdominal radiographs were made hourly for 12 h and the time (T50) at which 50% of the markers had left the stomach was then determined for each dog.

Results: The mean apparent digestibility coefficient of organic matter, crude protein, fat and gross energy significantly increased between 12 and 60 weeks of age in the four breeds. The gastric emptying time significantly increased with age in all dogs so that T50 was significantly longer in adult dogs (3.4 ± 0.4 vs 6.4 ± 0.5 h in MP, 4.4 ± 0.3 vs 6.5 ± 1.2 h in MS, 5.2 ± 0.9 vs 7.8 ± 0.7 h in GS and 4.3 ± 0.6 vs 6.4 ± 1.1 h in GD at 12 and 60 weeks of age respectively).

These results show (1) a higher nutrient utilization in adult dogs and (2) a slowing down of gastric emptying time during growth. That suggests that the faster gastric emptying of puppies could take part in their lower digestive efficiency.

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Seasonal changes in digestive tract function in marmots

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Marmots are the largest members of the rodent family Sciuridae (squirrels). They are primarily herbivorous, while nearly all other sciurids are granivores. The dietary strategy of herbivory is made possible for marmots by their large body size and therefore large capacity of the digestive tract. The digestive tract of all sciurids is relatively simple, so that at the small body sizes typical of chipmunks (*Eutamias* spp.), digestive efficiency is limited by short mean retention times of food in the gut (Hume, Morgan and Kenagy 1993). This restricts them to easily digested foods such as seeds. The larger digestive tract of marmots overcomes some of the limitations of a simple tract morphology by increasing digesta mean retention times, enabling them to expand their dietary niche to include the structural parts of grasses and forbs.

However, the dietary strategy of herbivory precludes the caching of food such as seeds and nuts to sustain the animal during the annual winter hibernation as do most sciurids, and instead marmots store energy as body fat. Entry into hibernation appears to be triggered by the attainment of a certain level of body fat, in addition to an optimal level of polyunsaturated fatty acids in the stored fat. Marmots appear not to feed during their periodic arousal periods throughout the winter hibernation which, for alpine marmots (*Marmota marmota*), lasts for 6 months or more.

The digestive tract is expensive to maintain, so if it is not being used during this long period it can be hypothesised that it should be reduced in size and/or activity. This hypothesis was tested during a marmot control program in Switzerland. The gastrointestinal tracts of 76 alpine marmots shot during this program were analysed for patterns of change in morphology and function over the active season (April to September) of 1999 and 2000 (Hume *et al.* 2002). Dramatic increases in fresh tissue mass (by 105% in the stomach to 259% in the small intestine) and in the pool size of short-chain fatty acids in the caecum (12-fold) between emergence from hibernation in April to mid-summer (July) were followed by decreases between July and September in anticipation of re-entry into hibernation.

It appears that a low level of activity of the marmot gastrointestinal tract is maintained during winter, the main substrates in both the small and large intestine being of endogenous origin. The main signal to increased levels of activity in the spring seems to be ingested food rather than some endogenous signal. The earlier increase in tissue mass in the hindgut than the small intestine reflects the importance of microbial fermentation in the marmot digestive strategy. The delayed up-regulation of the small intestine in spring, together with its earlier down-regulation in autumn before re-entry into hibernation, are consistent with the high costs of maintaining this section of the gastrointestinal tract.

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Effect of different fibre types on the digestibility of nutrients in cat

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Objectives. In the last few years research about the role of dietary fibre in cats' diet have been actively pursued. Until now, fibre was primarily added to commercial cat diets for two reasons: to alter stool consistency and to provide indigestible components helping slimming diets. In the present study the effect of different fibre types was investigated on the digestibility of nutrients in cat.

Methods. Dried sugar beet pulp (source of hemicellulose and pectin), alfalfa meal (source of cellulose) and peanut hull (source of lignin) were mixed to a poultry meat based control diet at a level of 10% on dry matter (DM) basis. The digestibility of the major nutrients of the following four feed mixtures were determined. Diet (control): poultry meat based diet; Diet 2: Diet 1 (control) supplemented by 10% of dried sugar beet pulp; Diet 3: Diet 1 supplemented by 10% of alfalfa meal and Diet 4: Diet 1 supplemented by 10% of peanut hull. Digestibility trials were conducted by the same 10 castrated, adult cats (self-control study). Cats were housed individually, cages allowed the exact registration of the feed intake, and the quantitative collection of the faeces. Cats had free access to tap water. Animals were fed individually by providing 200 g original matter, resulting 80 g DM intake per day. The length of the test (collection) period lasted 6 days per treatments.

Results. Fibre supplementation did not influence the daily dry matter intake of cats. Perhaps it is due to the high palatability of the diets. Related to the Diet 1 (control) the digestibility of the dry matter content was lower in the Diet 2 and in the Diet 4 (76.74; 73.82, and 74.04, respectively). Surprisingly, by feeding Diet 3, the digestion coefficient (76.36%) practically did not differ ($p < 0.05$) from the control. According to this results, Diet 3 containing cellulose, known to be poorly fermentable fibre, proved not to be of decreasing the dry matter digestibility of the feed mixture.

Conclusions: According to this trial not only the absolute fibre content of the diet but also the type of the fibre, has to be taken into consideration, when evaluating the possible role of fibre fractions, as components of weight loss products.

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Digesta kinetics in feral pigeons (*Columba livia*)

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The experiment was conducted to determine the digesta kinetics of liquid phase and particles in feral pigeons (*Columba livia*). All animals were individually caged. Mean retention times (MRT) were estimated in seven adult pigeons (Bodyweight 275 – 320 g) fed a pulse dose of Co-EDTA (Co 8 mg/animal), Cr-mordanted fibre (Cr 1 mg/animal, particle size <2 mm) and the n-alkane hexatriacontane (C₃₆ 3 mg/animal, as labelled food pellets). For details concerning the marker analysis and the calculation refer to Hatt et al. (2001) and Hatt et al. (in press). Individual samples were collected every 1 ½ h for 12 hours and one more sample was collected 24 h after marker application. Average MRT for the liquid phase marker Co was 5.3 h (± 3.18). For the particle phase markers Cr and C₃₆, MRT was 6.8 h (± 2.87) and 8.4 h (± 3.25), respectively. Transit-time (TT) was in all birds < 1 h. This was shorter than the 2 ½ h published by Vogel (1980)

The retention times of all three markers differed significantly. This is surprising since it as expected that the particle markers would not differ in their kinetic behaviour. As a possible explanation may be selective retention. The pellets which contained the n-alkane C₃₆ could have dissolved into particles that were much smaller and easier to digest than the Cr-mordanted fibre. For a bird such as the pigeon, which flies long distances it may be of importance to eliminate less digestible food faster, to reduce weight. Further studies will have to test this assumption using particles of different size and digestibility.

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Dietary management of dental disease in the dog and cat

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Periodontal disease is one of the most common problems affecting companion animals. It is the most common oral disease and a major reason for them to be presented at clinics. Periodontal disease includes both gingivitis (plaque induced inflammation) and periodontitis. Tooth brushing is effective in reducing plaque build-up but owner compliance towards this is very low. Plaque that is not removed can eventually be converted to dental calculus. Calculus can only be adequately removed by professional dental prophylaxis but its build-up can be reduced by specific dental diets.

Dietary formulations that maximize oral-health care through reducing plaque and calculus build-up, increased owner compliance, and addressing the masticatory habits of companion animals, are available. One strategy involves using a mechanical scraping action while the pet chews the food. Some studies have shown a 19% reduction in plaque, and 32% reduction in calculus using such a diet in dogs. Mechanical abrasion from these specialized diets occurs where the food actually contacts the tooth surface.

Relatively new technology to companion animal foods allows for mineral sources of phosphorus, in the form of polyphosphates, to enhance the properties of the dry kibble (pellet). The polyphosphates coat the outer surface of the dry food. Their benefit in dogs in reducing calculus build-up has already been demonstrated using both dry food and mouth-rinses. More recent studies have shown an average of 55% reduction in calculus build-up in dogs and an average of 45% reduction of calculus build-up in cats. These enamel-safe minerals are used successfully in human dentifrices in helping reduce calculus build up.

There are two phases to the effect of food coated with polyphosphate crystals:- (1) during chewing the hard food can help scrub away plaque (2) after chewing the polyphosphates embed into the plaque and help prevent calculus build-up through sequestering or complexing calcium within plaque forming soluble calcium complexes that diffuse into saliva. The benefit of the barrier approach is that polyphosphates can provide whole mouth benefits as they are released from the diet during mastication and are then carried throughout the oral cavity. The polyphosphates also provide benefits to non-chewing surfaces. The nutritional safety of polyphosphates is due to their conversion into orthophosphates which are utilized by the host. There is no detrimental effect on the nutritional value of the food as they make up part of the normal phosphorus dietary content.

Polyphosphates offer an alternative strategy in helping reduce calculus build-up in companion animals when applied to the surface of complete and balanced diets.

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Fluid and particle passage rate in captive black rhinoceros (*Diceros bicornis*)

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In contrast to ruminants, hindgut fermenters are considered more flexible in their digestive response to different amounts of available forage. As they are not intake-limited due to the selective particle retention of a forestomach system, they can adapt their passage to the available food for intake to a distinctively higher degree. Recently, the feeding of captive rhinoceros has received considerable attention. As part of several different feeding trials, therefore, we measured the passage rate in captive black rhinoceros.

Seven animals from different zoological institutions were given a pulse-dose of a combination of cobalt-EDTA as a fluid and chromium-mordanted fibre (particle size ≤ 2 mm) as a particle marker. Faeces were collected continuously for 15 hours before to 80 hours after marker application. Food intake was measured simultaneously. Marker concentration of faecal subsamples was determined by atomic absorption spectrometry. Mean retention times (MRT) were calculated according to the method of Thielemans et al. (1).

The average fluid and particle MRT were 35.0 (± 8.1 , range 27.4-48.0) and 42.5 (± 13.8 , range 31.0-67.1) hours, respectively. The particle marker was retained 1.1-1.4 times longer than the fluid marker. Animals that had a higher dry matter intake had faster passage rates.

These results were considerably faster than the values obtained by Foose (2) of 51-60 hours. The difference is most probably due to the increased sampling interval of our investigation and indicates that black rhino MRT are closer to the time of 35 hours Demment and Van Soest (3) calculate for a reasonable exploitation of fast-fermenting plant material.

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Morphological correlates of accelerated passage rates in very large herbivores

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The largest extant mammalian herbivore, the elephant, is characterised by comparatively short ingesta passage rates and low digestibility coefficients. We intended to search for morphological correlates of this obvious deviation from the general concept that an increase in body mass should enhance longer passage rates and hence more thorough digestion.

There is a surprising paucity of anatomical data on the gastrointestinal tract (GIT) of large mammalian hindgut fermenters. We collated data on tapirs, equids, rhinoceroses and elephants. The few available data suggest the following trends: larger hindgut fermenters do not show an according increase in GIT length; the diameters of GIT sections tend to increase in larger species; the relative capacity of the caecum tends to be reduced in larger species.

Although evidently more data is needed to substantiate these preliminary observations, they are in accord with the comparatively fast passage rates of the largest extant herbivores, and could be indicative of the trend the even larger extinct mammalian herbivores – all of which are thought to have been hindgut fermenters – would have had to follow.

The reason for this adaptation probably lies in the fact that the fermentation of plant material cannot be optimized endlessly; there is a time when plant fibre is totally fermented, and another when energy losses due to methanogenic bacteria become punitive. Therefore, very large herbivores would need to evolve adaptations for a comparative acceleration of ingesta passage. To our knowledge, this phenomenon has not been emphasized in the literature to date.

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Nutrition, physiological adaptation and re-introduction

A case study of the knot (*Calidris canutus*)

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The ability to adapt to changes in the environment plays an important role in the survival of species. These adaptations can take place within short time lapses, resulting in repeated physiological changes of the organism within one lifespan. The adaptation of migratory bird digestive tracks and flying muscles is an example. The ability to show these adequate changes in physiology may determine the success of individuals in a competing environment, which will be most important in re-introduction programs.

Size reduction of the knot's (*Calidris canutus*) digestive track takes place before the start of the migration period. At the same time the flying muscles as well as the heart muscles are increased in size. This adaptation is directed toward the effective use of energy. The trade-off between physiological capacity and environmental requirements is essential for individual as well as long term species survival.

The natural diet of the Knot consists out of small shells and other organisms. The shells are found in the top sediment layer of the wetlands where they forage. This prey is swallowed as a whole. The shells are crushed using stomach muscles. This requires strong stomach muscles. Training induces the increase of the stomach musculature. Taking in larger prey incites these muscles to increase. Reduced food intake during the migration period results in reduced stomach size. During this period the Knot relies on smaller, softer shelled prey.

Research showed that the food intake rate in the Knot depends on prey size and shell mass. Stomach size did not influence food intake rate. To crack larger prey specimen stronger and thus larger stomachs are required.

The foraging behaviour of the Knot in-situ is adapted to this physiology.

Translating these results to the management of the Knot ex-situ, and to comparable bird species there are two major lessons to be learned. Especially so where re-introduction is a long-term option.

First the willingness of the birds in captivity to accept pelleted food must not automatically lead to the use of such food. The advantage of controlled diet composition, reduced risks of diseases and such must be balanced against the possible long-term consequences of such easily digested pellets. The lack of stimulation of the stomach muscles when feeding pellets can prove to have long term lasting effects on the survival rate of the re-introduced animals.

Second the increased handling time of natural food is a distinct advantage in the prevention of stereotypic behavior. Diets complemented with natural food must be considered. Providing shells hidden in a sandy substrate in a well-controlled manner is not only environmentally enriching, but also assures natural behaviour and physiological development.

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The influence of L-carnitine on nutrient retention in pigeons (*Columba livia domestica*) fed corn or peas

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The vitamin-like substance L-carnitine is an intermediary factor in fatty acid metabolism, which can be useful in racing pigeons. On the other hand, little is known about the utilisation of nutrients from different feedstuffs by these animals.

This study wanted to clarify whether L-carnitine supplementation could affect the nutrient retention of corn and peas in non-active, adult racing pigeons.

Two groups of eight female adult pigeons (*Columba livia domestica* L.) were fed restrictedly (25 g/d) with 100% corn or 100% peas respectively. Within each group, four pigeons received drinking water that was supplemented with L-carnitine (Lonzagroup., Basel, CH) at 2.5 g/L. After a 7d adaptation period, total collection of the excreta for each individual bird was performed daily during a 7d collection period.

Retention coefficients of corn and pea with and without L-carnitine supplementation.

Retention %:	Pea		Corn		P-value
	L-Carnitine dose		L-Carnitine dose		
	0 g/L	2.5 g/L	0 g/L	2.5 g/L	
Dry matter	63.3±2.9 ^a	65.9±2.3 ^a	84.4±0.9 ^b	83.1±1.1 ^b	Feed : > 0.001
Crude protein	37.2±1.7 ^a	38.1±6.7 ^a	61.1±2.2 ^b	47.6±4.8 ^c	Feed*Dose : 0.006
Ether extract	76.8±1.7 ^a	76.9±3.4 ^a	84.5±3.9 ^b	84.1±2.5 ^b	Feed : < 0.001
Crude fibre	14.2±6.9 ^a	22.0±3.1 ^a	17.9±4.5 ^a	12.9±6.5 ^a	Feed*Dose : 0.038
N-free extract	85.4±1.0 ^a	85.1±0.9 ^a	92.2±0.1 ^b	92.4±1.0 ^b	Feed : < 0.001
Organic matter	67.9±1.3 ^a	68.4±2.3 ^a	86.3±0.6 ^b	84.8±0.9 ^b	Feed : < 0.001

^{a,b,c} different indices in a row indicate a significant difference at p<0.05 (Scheffé-test).

Both on dry matter basis and on organic matter basis, corn was 30 % more digestible in pigeons than peas, which was consistent in the retentions of every analysed organic component except for crude fibre. For the latter an interaction between L-carnitine supplementation and the feedstuff used was significant, although no significant differences were found between the four groups. L-Carnitine supplementation had no effect on the crude protein retention in peas, but caused a significant decrease of crude protein retention in corn. It can be hypothesised that L-carnitine improved the efficacy of energy utilisation from fat and carbohydrates in corn, thus limiting the uptake of digestible amino acids for energy production. As peas are far higher in protein (and lower in fat), this effect could have been absent due to a higher need to use protein as an energy source. Nevertheless, this hypothesis should be checked in further research.

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Changes in gastrointestinal transit time and pH in piglets around weaning.

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Management of the newly weaned piglet presents one of the most significant challenges to swine producers. The stress of weaning and movement to another environment increases the risk for disease, especially diarrhea, decreases the food intake and can lead to nutritional disorders (Pulske et al., 1997; Lecce et al., 1979). To develop oral medications to treat piglets, a good knowledge of gastrointestinal (GI) parameters is needed. The objective of this study was to determine the GI transit time of pellets and the local pH in piglets at different moments around weaning.

The transit time of non-disintegrating radio-opaque pellets was measured by radiography. The radiographs were analysed with a software programme to calculate the number of pellets present in the different parts of the GI tract. The pH was measured after slaughter at different sites along the GI tract with a pH probe. In suckling piglets, the gastric emptying was faster and the colonic retention was greater than in weaned piglets: 75% gastric emptying was obtained during the first 1.5 to 3.5 h in suckling piglets, whereas 60% in 18 h, 58% in 15 h, and 73% in 7 h, were obtained 3 days, 1 week and 2 weeks postweaning, respectively. In suckling piglets, colonic accumulations to 73% were found, whereas in the weaned piglets the pellets maximally accumulated to 48%. Immediately after weaning, the transit times were markedly prolonged and subsequently shortened with the time postweaning: the 85 % excretion times were 175.5, 72.5 and 50.5 h, at 3 days, 1 week and 2 weeks postweaning, respectively. Three weeks postweaning, the transit seemed no longer affected by weaning, as the transit times were similar to values previously reported in growing and adult pigs (Gregory et al., 1990; Potkins et al., 1991; Davis et al., 2001; Clemens et al., 1975) and retention appeared to be restricted to the stomach and the colon. In the stomach compartments of distinct pH were discerned. Along the first half of the small intestine and in the caecum, a negative correlation was found between the pH and the age of the piglet in contrast to the colon and rectum.

It can be concluded that weaning prolongs the transit times whereas the pH is not noticeably affected. These data are of crucial importance in the design and formulation of coating materials for orally administered vaccines and therapeutics.

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Seasonal variation of grazing available forage for domestics and wild ungulates in different alpine areas

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Introduction

In the last years Alpine pastures have been progressively abandoned, because of the decreasing number of herds led to mountain summer pasture.

The pastures in the mountains have both a productive and ecological role: grazing, when rational and controlled, may be a “natural” way for the conservation of floristic diversity and grassland management, especially in the protected areas.

Objectives

The aims of this work were:

- to determine qualitative and quantitative characteristics of the grassland in the studied areas;
- to verify how grazing management affects the quality and the quantity of herbage mass.

Materials and methods

Three Alpine areas were chosen for this study, all in Piemonte region: two inside the Gran Paradiso National Park (Valli Orco and Soana) and one adjacent to Orsiera Rocciavre Natural Park (Val Chisone). The following surveys were performed:

- to determine the herbage mass available for grazing, and cattle and sheep grazing efficiency, 120 samples were collected from sample areas inside and outside enclosure cages by cutting with a portable lawnmower;
- organic matter digestibility (OMD; pepsine-cellulase method) and crude protein (CP, Kjeldhal method) were determined on fortnight-collected samples, and the variation of the grassland quality during summer was assessed;
- fodder values (Pasture Value method, Daget & Poissonet, 1972) for each vegetation type (*ecofacies*) were calculated (Cavallero *et al.*, 2002);

Results

The quality of the grassland was variable in the different areas and during time: OMD ranged from 41% to 77%, and CP ranged from 8% to 27%. The highest nutritive values were found during May and June, regardless the vegetation type, where cattle exploited the grassland twice during the grazing season. The lowest values were found in ungrazed areas.

The herbage mass available for grazing was very variable too, ranging from 1,7 t ha⁻¹ to 4,5 t ha⁻¹. Its highest values were found in the areas grazed by cattle.

Discussion

A great influence was found on the vegetation composition due to grazing management.

In most areas, summer grazing by cattle seemed to improve fodder quality, for their ability to completely exploit the grassland, resulting in good quality regrowth (high leaf/stem ratio). On the contrary sheep, which are very selective, might worsen botanical composition on the long term and induce a decrease of fodder quality during summer.

Domestic animals exploit the grassland when the nutritive value has already declined. This means that wild ungulates are offered the best periods of the season for grazing.

Finally, the rational grazing, in the present conditions, might have positive effects on wild ungulates feeding management and on environmental conservation.

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Starvation of rumen bacteria and their shift-up growth under different condition

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The rumen liquor has been taken from a mature dairy cow with permanent rumen cannula, fed 50:50 mixture of grass and legume hays. The bacteria cells were separated from the rumen liquor by use of a centrifuge and kept on a phosphate buffer for thirty days.

Two concentrations of glucose and two concentrations of isoacid (valeric and isovaleric) along with glucose were used for shifting-up growth after starvation. A roll tube method was used for maintenance and cultivation of bacteria and changes in turbidity were used as an indicator for reduction or increase of bacterial mass at various times. Results showed that bacteria cells could survive in buffer medium without any nutrient for 30 days, but after this period they needed a considerably long time for shift-up growth compared to normal conditions. The nutrient type influenced the time needed for shift-up growth. In low glucose concentration (3.3 mmol/ml of media) shift-up growth started after 12 hours of solution injection to the tubes and logarithmic growth kept on for the next 12 hours. In a high glucose concentration (6.6 mmol/ml of media) logarithmic growth started after 18 hours of solution injection and continued for the next 29 hours along with a higher bacteria concentration. Isovaleric acid (25.5 μmol + 23 μmol glucose per ml of media) and valeric acid (29 μmol + 23 μmol glucose per ml of media) initially showed the same effect on shift-up growth, but bacteria growth in valeric acid continued for 14 hours more than isovaleric acid along with a higher bacteria concentration.

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feed intake

Chairperson : Andrea Fidgett (UK)

Diet selection by the White-naped Pheasant Pigeon *Otidiphaps nobilis aruensis* at the Barcelona Zoo

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In 1999 the Barcelona Zoo held 53% of the total European captive population of white-naped pheasant pigeons *Otidiphaps nobilis aruensis* (a columbiforme endemic from Aru Isle - South-West of New Guinea). There is very little information about the biology and wild status of this subspecies, and it is poorly represented in captivity. This study was performed with 11 pheasant pigeons born at the Barcelona Zoo. A 14-days intake study was carried out and samples of the diet offered and refused by the birds were collected for proximate and crude energy analyses. Based on the generic food preferences of columbiformes, the pheasant pigeons of the study received a wide range of food types (10), allowing them to feed close to *ad libitum*. The aims of this study were to determine (1) the nutrient profile of the diet consumed by the captive population of the white-naped pheasant pigeons at the Barcelona Zoo, and (2) some of the factors that could have an influence on their diet selection.

Due to the small sample size, there was a great variability among individuals. However, only the age and the origin of the birds had significant effects on some of the dependent variables (diet items, nutrient and water intake). Younger animals consumed significantly less grains than older animals. However, when looking at the whole group behavior, it was found that white-naped pheasant pigeons selected a diet with a great percentage of grains (over 35%). The average nutrient composition of the diet consumed was 18.9% crude protein, 8% fat, 4.6% crude fiber and 5.4% ash (on a dry matter basis). Comparing the results to the nutrient requirements published in the literature, the birds of the study consumed a diet that covered their energy, protein and fat requirements.

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Energy and nutrient intake, feeding behaviour and activity budget of Douc langurs (*Pygathrix nemaeus nemaeus*) in captivity

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Douc langurs are very difficult to keep in captivity (Ruempler, 1998), which is mainly due to nutrition-related problems. Our study aims at providing first quantitative data on the nutrition and feeding behaviour of captive douc langurs, thus supporting the development of suitable diets for colobines in captivity.

Individual food intake was recorded quantitatively over a period of three months in nine captive douc langurs. Nutrient and energy concentrations of the consumed diets were calculated and compared to results from relevant studies on other colobine species. Determinants which might influence food intake in the study group were assessed, an activity budget was drawn up, and dominance hierarchies were described.

The diet consumed by the langurs consisted of 33% vegetables, 21% leaves, 21% fruits, 12% salad and 14% other food items. The results showed that the different foodstuffs differed in their acceptance by the animals. Leaves always had the highest amounts of leftovers. A greater diversity of leaves offered, however, correlated with a higher leaf intake. Regarding the nutrient composition of the consumed diet, a high content of water-soluble carbohydrates and a low crude fibre content were obvious when compared to the diets of wild colobines. The amount of energy consumed by any of the studied douc langurs was lower than the calculated energy requirement.

During the daily observation time, the animals spent 56% with resting and sleeping, 22% with eating, 6% with locomotion, and 3% with grooming.

The results are mainly discussed with regard to the presumably sub-optimal energy intake of the studied douc langurs. The fact that a considerable proportion of the offered leaves was not consumed by the animals might be indicative of the leaves' quality (palatability, taste etc.) not matching with the requirements of the langurs.

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Epidemiologic study of hospitalized dogs voluntary food intake

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Unusual environment as veterinary hospital can generate different stresses in dogs. Often considered as less relevant clinically in dogs than in cats, stress induced by hospitalization conditions could reduce voluntary food intake in hospitalized dogs.

The aim of the present study was to investigate the daily spontaneous food intake in hospitalized dogs and to define the external factors that could be responsible for a lesser intake. This experiment has been performed as an open trial, including all dogs admitted in the National Veterinary School of Toulouse that required a more than 48 hours hospitalization. All dogs requiring enteral tube support or parenteral nutrition were excluded. Forty-three dogs were included during a period of 16 days. Notification of the nature and quantity of daily food intake in standard hospitalization conditions was used to evaluate spontaneous food intake. From a clinical point of view, body weight, pain score and clinical parameters were recorded every day.

Included dogs were 6.0 ± 4.4 years old [0.3-14 years] and were classified in large breed dogs (19/43), medium sized dogs (4/43) and small breed dogs (20/43) including poodle dogs (5/43). Considering their initial body condition, dogs were thin (35%), normal (51%), overweight (7%) and obese (7%). Twenty three per cent of them had clinical muscular atrophy. Hospitalization causes were gastrointestinal disorders (10/43), general surgery (10/43) and ophthalmology troubles (8/43). Other dogs were hospitalized for neurological or orthopedic disorders (6/43), or for infectious (3/43), urologic (2/43), cardiologic (2/43), endocrine (1/43) and skin (1/43) diseases.

In these conditions, mean spontaneous food intake was 7.61 ± 0.69 g/kg/d (dry matter basis) during hospitalization period. During the four initial days of hospitalization, voluntary intake covered 40.3, 68.5, 62.7 and 73.9% of the dog's resting energy requirement (RER = $70 \cdot BW^{0.75}$). After the fourth day, voluntary intake appeared above the RER, but remained under the daily energy requirement¹ for ill dogs, except of the 6th day of hospitalization (DER : as described¹). The body weight was slightly decreased during hospitalization (maximal decrease -4.4% at the tenth day). In all evaluated contributing factors, pain score or Elizabethan collar presence failed to influence spontaneous food intake. Casuals of hospitalization analysis revealed that dogs with gastrointestinal and ophthalmology disorders as well as dogs submitted to general anesthesia had a lower energy intake, than dogs suffering other diseases or not submitted to anesthesia. We observed that small (< 10 kg) and great (> 30 kg) breeds ate less than medium breeds. Physical aspect of meal significantly influenced voluntary food intake, since spontaneous intake was 8.42 ± 1.07 g/kg/d and 12.60 ± 1.41 g/kg/d (dry matter basis) with canine dry food and canned food, respectively.

Thus, our study shows that canine feeding behavior can be significantly influenced by hospitalization conditions. Different risk factors such as breed size, anesthesia procedure and nature of disorders appear to be clinically relevant in their influences on spontaneous food intake. Those factors must incite veterinarian to monitor food in hospitalized dogs.

1 Crowe: Vet Med Small Anim Clin 1988; 83:1224-1249.

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Some Preliminary Observations on Herbivorous Insect Composition: Nutrient Advantages from a Green Leaf Diet?

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Most insectivores maintained in captivity are fed a limited variety of species that have been raised on grain-based diets, comprising primarily the larvae of meal beetles (*Tenebrio molitor* or *Zophobas morio*), crickets (*Gryllus* sp.) of various instars, and the larvae of the wax moth (*Galleria mellonella*). Almost all published reports of invertebrate composition demonstrate them to be a poor source of calcium (Anderson, 2000; Klasing et al., 2000), with an imbalanced Ca:P ratio. Chemical analysis of silk moth larvae (*Bombyx mori*) and stick insects (*Baculum extradentatum* and *Eurycantha calcarata*), raised on a diet of white mulberry (*Morus alba*) leaves, revealed high Ca concentration (silk moth larvae 0.9%, gravid female stick insects >2% Ca, dry matter (DM) basis) as well as Ca:P ratios >1:1. Similar to other insects analyzed (Dierenfeld et al., 1995; Barker et al., 1998), carotenoids were present but minimal or no vitamin A activity was detected in extracts from these samples. In contrast, vitamin E concentration was high (>450 IU/kg DM) in both silk moth larvae and stick insects. Mulberry contains high levels of Ca and vitamin E (Dierenfeld et al., 1990; Zootrition, 2001), and may be a good source of these nutrients for herbivorous invertebrates. Dietary fibre (measured as neutral detergent fibre) ranged from moderate to high (12 to 50% of DM in silk moth larvae and stick insects, respectively), and encompassed ranges reported for different life stages of invertebrates consumed by anteaters and other insectivores in nature (Redford & Dorea, 1984; Oyarzun et al., 1996). Fibre has been shown to provide energy to insectivores, and promotes improved fecal quality and gastrointestinal health (Graffam et al., 1998). Additionally, the dietary bulk of chitinous exoskeletons and/or even the plant cell walls found in gut contents of herbivorous insects may provide a nutrient dilution effect to alter otherwise excessively energy-dense diets. The larvae of the silk moth and adult stick insects, both feeding on mulberry, may be useful insects for feeding captive insectivores. Proximate constituents are comparable with those of other invertebrates commonly used in zoo feeding programs; a diet of mulberry leaves, however, may enhance dietary fibre and particularly vitamin E and Ca concentrations in these alternative feeder insects.

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The role of insects in primate nutrition: how is chitin utilized?

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The study of mammalian insectivory has been neglected in contrast to folivory, frugivory and grazing mammals. A major component of insects is their chitinous exoskeleton, but there is little evidence of how mammals may break down large structural carbohydrates, such as those found in the chitin. Insects form a large part of the diet of callitrichids, small, New World primates. Data on the nutrient composition of insects consumed by callitrichids is scarce and the proportion of insects digested is not known. This information is important for the conservation of callitrichids both in the wild and in captivity.

The study focused on members of the callitrichid genus, the range providing an overview of their insectivory. Work on the anatomy, mechanical, biochemical and microbial processes was carried out on individuals culled for purposes other than this study. The chitin content of a variety of insects were analysed and a chitin budget determined for the callitrichid species. The results were assessed in relation to captive and wild diets.

The proportion of chitin in insect body parts was determined and related to preference of their ingestion. Methods were established to examine chitin digestion by microbial, biochemical and mechanical means. Ten samples of four insect species were given to each primate species. The course of chitin digestion through the gastro-intestinal tract of callitrichids was mapped. Results produced an exact baseline chitin budget from 200 samples from 10 common marmoset, *Callithrix jacchus*, and an estimated chitin budget for other callitrichid species, including Geoffroy's marmoset, *C. geoffroyi*, pygmy marmoset, *Cebuella pygmaea*, cotton top tamarin, *Saguinus oedipus*, golden headed lion tamarin, *Leontopithecus chrysomelas*, Goeldi's monkey, *Callimico goeldii*. Analyses of gut content revealed how callitrichids digest insects and the importance of insects in their diet was determined.

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Open trial to determine voluntary food intake in hospitalized cats

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Known to induce clinical complication, anorexia appears as a frequent sign in ill cats. The feeding behavior of cats would be deeply modified in a stressful environment, however the incidence of caloric deficiency remains unclear in cats hospitalized in standard conditions.

The aim of the present study was to investigate the daily food intake in hospitalized cats.

This pilot experiment has been carried out as an open trial, including all the cats admitted in the National Veterinary School of Toulouse which required a more than 48 hours hospitalization. Cats receiving enteral nutritional support (or tube feeding) or parenteral nutrition were excluded. Twelve cats were included on a period of 16 days. Spontaneous food intake was evaluated through the notification of nature and quantity of daily-ingested food in standard hospitalization conditions (i.e. T° around 20°C, cat in specific individual cage without contacts with dogs, frequently proposed meals, specific feline food changed every 2 to 3 hours, medical examination twice a day). In a clinical point of view, body weight, pain score and clinical parameters were recorded every day.

The included cats were 6.1 ± 4.9 years old [0.7-15.5 years]. They were mainly European shorthair (8/12). Their initial body weight was 4.4 ± 1.3 kg [2.6-7.2 kg] and 9/12 cats presented a normal body score, one being thin with a muscular amyotrophy, one overweight and one obese, respectively. They were hospitalized mainly for major surgery (7/12) and uro-nephrologic troubles (4/12).

In these conditions, the spontaneous food intake was 4.01 ± 0.74 g/kg/d (dry matter basis) corresponding to 17.8 ± 3.2 kcal/kg/d. Concurrently, we observed a decrease of body weight during hospitalization (-11.7% at the sixth day). Regarding spontaneous intake, we noticed that 100% of hospitalized cats were unable to cover their own resting energy requirement (RER = $70 \text{ kcal} \cdot \text{BW}^{0.75}$) or their daily energy requirement (DER = k.RER). The pain score appeared without direct influence on feeding behavior (pain score 4.2 ± 0.4 vs 4.9 ± 0.6 respectively in cats covering less or more than 50% of their RER). Presence of intravenous fluid therapy failed to modify percentage of daily caloric cover (34.5 ± 7.4 vs $34.9 \pm 10.6\%$ with or without, respectively).

This preliminary study shows that standard hospitalization conditions can dramatically inhibit feeding behavior of cats. Even though this approach should be carried out on a larger scale, it appears that clinicians must keep a permanent watchful eye on the daily caloric intake in hospitalized cats.

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Feed choice in pigeons with or without L-carnitine supplementation

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Former studies (Janssens et al., 1998) have demonstrated that L-carnitine supplementation enhances energy utilisation efficiency in pigeons. This study wanted to investigate whether the L-carnitine driven change in energy utilisation would be able to induce differences in feed intake or feedstuff selection by means of a cafeteria experiment in pigeons.

Six groups of adult pigeons (*Columba livia domestica* L.) - three female and three male groups - were housed per group and used in a double latin square design : per sex three doses of L-carnitine (0, 40 and 80 mg/day/pigeon) (Lonzagroup, Basel, Switzerland) were combined with three groups of four randomly allotted pigeons over three 1 week periods. The periods were buffered with one week for adaptation to the new L-carnitine dose.

The daily supplementation was accomplished by intubation of L-carnitine in 0.5 mL distilled water. Fresh water was ad libitum available. The choice feeders consisted of four similar metal troughs that were put in line. Each of them was filled with either corn, wheat, peas or sunflower seeds. These feedstuffs were supplied ad libitum. Each day the feedstuffs were randomly allotted to the four troughs in order to exclude the effect of the trough ranking. In the beginning and at the end of each period, the individual body weights and the intake of the water and the different feedstuffs were measured weekly by weighing.

The trial gives data on the food preferences of non-active racing pigeons. However, no significant differences were seen in the selection of feedstuffs or total feed intake between the L-carnitine supplemented and the control group. It is suggested that L-carnitine does not play a significant role in feed intake or feedstuff selection in pigeons.

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The use of n-alkanes to estimate diet composition, intake and digestibility in sheep fed mixed diets

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The nutritive value of a forage will depend on its intake (understood as both daily amount and composition of the diet, in terms of proportions of the different botanical fractions actually consumed by the animals) and digestibility, the estimation of these parameters becoming difficult in grazing conditions. The use of the n-alkanes as internal markers for this purpose has given good results with mixtures of two temperate forages (Dove and Mayes, 1996), but has not yet been tested with other species or mixed diets. The aim of the present experiment was to compare observed values of intake, digestibility and diet composition obtained in sheep fed different proportions of barley grain and straw to their n-alkane estimates.

Sixteen adult sheep were randomly assigned to four diets with different proportions of barley grain and straw (60/40, 45/55, 30/70 y 15/85) and fed at a level which avoided refusals. Intake, digestibility and diet composition (proportion of grains, leaves and stems consumed) were recorded for seven days. The contribution of leaves and stems to total straw intake was estimated from the comparison of their respective n-alkane profiles. From 14 days before until the end of the balance period the animals were dosed once a day (9 h) with 1.5 g of paper pellet containing equal proportions of tetracosane (C₂₄) and dotriacontane (C₃₂), used as external markers to provide for faecal recovery, and hexatriacontane (C₃₆) used for faecal production calculations. N-alkanes were extracted from samples of grains, leaves, stems and faeces, and their concentrations analysed by means of gas chromatography. Diet composition was estimated according to the procedures proposed by Mayes et al. (1994), using C₂₅ (pentacosane), C₂₈ (octacosane) and C₃₀ (triacontane) as internal markers, and dry matter intake following Mayes et al. (1986). Digestibility was calculated from estimated intake and faecal production. Observed and estimated proportions of grain in the diet were compared by regression, and differences within each treatment assessed by the paired t-test procedure. This last method was also used to compare observed and estimated intake and digestibility values.

Estimates of proportions of grain in the diet were very accurate (estimated = 1.0063 observed - 2.4720; $r^2 = 0.99$; $P=0.0001$), and mean differences between measured and estimated parameters ranged from -6.9 to 2.7% for dry matter intake, and from -4.6 to 2.1% for dry matter digestibility, depending on treatment ($P<0.05$ for diets 60/40 and 30/70). There was not a consistent relationship between the magnitude of the deviations and the proportion of grain in the diet.

Due to the scarce differences between observed and estimated values, it is concluded that the n-alkane technique is a powerful tool to estimate intake, digestibility and diet composition of mixed diets and hence may be used in sheep grazing mature whole cereal crops.

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Food intake and body weight development of captive roe deer (*Capreolus capreolus*) fawns fed diets of different tannin content

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It has been shown that roe deer do not totally reject artificially designed, tannin-containing food, even if the same food without the tannins is offered. Potentially positive effects of tannins that could explain this behaviour comprise antioxidant effects, action against gastrointestinal helminths and pathogenic bacteria, or an increased escape of valueable dietary protein from ruminal degradation. In order to better understand the food choices of roe deer, we wanted to test whether any beneficial effects of tannin feeding could be demonstrated.

Two groups of four hand-raised roe deer fawns were fed a pelleted feed only. One group served as control; the other received the same feed mixture with the addition of 3 % (original weight) of commercial tannic acid or quebracho tannin. Animals were weighed regularly, and the food consumption of the groups was monitored. Animals were dewormed on a regular basis.

The tannin group showed a significantly greater increase of body weight, both in absolute values and in relative weight gain. After eight months of tannic acid feeding, the tannin group had achieved a mean relative weight gain of nearly 25 %, in contrast to the control group with a mean of about 14.5 %. The food consumption of the two groups did not differ significantly. There was no difference in relative weight gain between the groups after two months of quebracho feeding.

The findings suggest that tannic acid increased the food conversion efficiency. This is most likely due to a protein-protecting mechanism by the tannins, which has repeatedly been reported for domestic ruminants on a low-dose tannin regime. The question remains whether such an increased food efficiency could be a reason for the animals to actively select a low dose of dietary tannins in preference trials.

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Investigations on ingestion, amounts and composition of casting and digestibility of organic matter in different birds of prey (kestrel falcon, common buzzard, and eagle owl)

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Birds of prey have been kept in human custody for centuries (hawking/zoos). Also, more recently so-called "hospices" have been established whose purpose is to temporarily care for injured animals until health is restored and subsequently return them to their natural habitat. The birds are usually fed day-old chicks and mice (sometimes enriched with vitamin-supplemented minerals).

Aim of this study was to gain qualitative and quantitative insights regarding feed consumption, nutrient supply, size and composition of castings (pellets), and feed digestibility.

Material/Methods: In this study 3 adults each of the species kestrel falcon (Ø 140- 220 g BW) and common buzzard (Ø 750 - 1,200 g) as well as 4 adult eagle owls (Ø 1,600 - 1,800 g) were observed. The birds received day-old chicks (Ch: 251±2.30 g dry matter/kg FM; in dm (g/kg): crude ash: 75.1±1.80; crude protein: 673±24.8; crude fat: 167±50.8) or mice (M: 340±38.4 g dm/kg FM; in dm (g/kg): crude ash: 105±6.00; crude protein: 536±67.9; crude fat: 273±103) in toto and ad libitum. Body weight was recorded at the beginning and end of every trial (7 days of adaptation, 5 days of collection). In calculating digestibility of organic matter (om, %), feed consumption was defined as the consumed quantity of feed animal (calculated by offered and refused amounts) minus castings (pellets). Corrections were made for uric acid in feces (mutes).

Results:

<i>feed animals:</i>	<i>kestrel falcon</i>		<i>common buzzard</i>		<i>eagle owl</i>	
	<i>Ch</i>	<i>M</i>	<i>Ch</i>	<i>M</i>	<i>Ch</i>	<i>M</i>
feed consumption (g DM/100 g BW/d)	5.03 ± 0.87	7.69 ± 1.35	2.20 ± 0.56	2.43 ± 0.52	2.01 ± 0.16	2.26 ± 0.09
Casting						
- quantity (% of DM-intake)	5.97 ± 0.28	6.09 ± 0.75	4.03 ± 0.43	6.09 ± 0.75	3.45 ± 2.88	10.9 ± 0.32
- composition						
crude ash (% DM)*	5.90	15.2	2.00	15.7	6.96	38.9
crude protein (% DM)	68.0 ± 8.98	63.3 ± 2.22	78.2 ± 3.25	68.5 ± 1.10	81.1 ± 2.81	52.3 ± 2.15
crude fat (% DM)	1.77 ± 0.23	2.63 ± 0.28	6.37 ± 4.03	2.64 ± 0.15	3.18 ± 0.50	1.75 ± 0.18
digestibility (om, %)	75.1 ± 0.30	82.5 ± 1.83	76.9 ± 0.97	81.5 ± 0.98	72.9 ± 4.75	84.0 ± 2.15
development BW (%)	- 2.10 ± 0.51	3.07 ± 1.15	-0.12 ± 0.34	1.17 ± 0.65	0.11 ± 0.06	0.77 ± 0.02

Ch = day-old chicks M = mice * average

Summary: As to be expected, relative consumption declined with rising body weight. Real feed consumption (at steady body weight) was comparable to values observed in pet birds or poultry. With regard to digestibility, effects were not so much species- as feed-related, i.e. mice were generally better digested than day-old chicks. The data will also allow quantitative ideas on energy and nutrient supply, that is needed to optimize nutrition in birds of prey.

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session contributed to the
EAZA Atlantic Rainforest campaign

Chairperson : Kristin Leus (B)

EAZA Rainforest Campaign

European Zoos' Commitment to Conservation of the Atlantic Rainforest

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It may appear odd to dedicate a session of a nutrition conference to the EAZA Atlantic Rainforest Campaign. Perhaps a few words of explanation are in order.

Until the end of September 2002, the European Association of Zoos and Aquaria is running an awareness and fundraising campaign for the South American Atlantic Rainforest. Together with Copenhagen Zoo, Dublin Zoo and the Durrell Wildlife Conservation Trust (Jersey), the RZSA is one of the driving forces behind the EAZA campaign. As such, we are trying to take every opportunity to draw attention to the campaign.

*The Royal Zoological Society of Antwerp (RZSA) has a special bond with the Atlantic Rainforest, mainly through our activities with the golden-headed lion tamarins (GHLTs) (*Leontopithecus chrysomelas*). Since 1993, the RZSA has co-ordinated the International Studbook and European breeding programme (EEP) for this species. As the current studbook keeper I am a member of International Committee for the Conservation and Management of the Lion Tamarins, an official advisory committee to the Brazilian federal environmental agency. Intensive *ex situ* research has been conducted on the GHLTs housed at Antwerp Zoo since 1987, under the co-ordination of Dr. Linda Van Elsacker. In 2002, as part of the expanding activities of our Centre for Research and Conservation, Dr. Kristel De Vleeschouwer will start an *in situ* project in the Una Biological Reserve, in close collaboration with the University of Maryland (Dr. J.M. Dietz and B. Raboy). Her research will focus on feeding ecology and the use of different habitats by GHLTs, and the effects of forest fragmentation.*

For all of the above reasons, we felt it appropriate to dedicate a session of this joint nutrition conference to the campaign. In this way we hope to raise awareness about the plight of the Atlantic Rainforest, as well as to stimulate work on the nutrition, nutritional ecology and physiology of (South American) rainforest animals. This in turn will contribute to the conservation of this habitat.

The Atlantic Rainforest is commonly divided into two main regions: the coastal Atlantic forest (a 50-100 km wide strip along the Northeast coast of Brazil) and the interior Atlantic forest (stretching 500-600 km inland into south-eastern Brazil and extending into eastern Paraguay and the extreme north of Argentina (Misiones)). The Atlantic Rainforest or "Mata Atlântica" as it is locally called, has very high levels of biodiversity and endemism and is the fourth 'hottest' of the world's 25 threatened biodiversity hotspots^{1,2}. It has a higher level of diversity and endemism per unit area than the Amazon. 160 out of 261 mammal species (61%) are endemic to the Atlantic Rainforest, 73 out of 620 birds (12%), 60 out of 200 reptiles (30%), 253 out of 280 amphibians (90%) and 6,000 out of 20,000 terrestrial plants (30%)^{1,2}. Currently only 7.5% of the former range of the Atlantic forest remains. The main threats to the region include: habitat destruction and fragmentation (due to logging, agricultural expansion, urbanisation, industrialisation and associated road building); hunting, and wildlife trade; and the collection of flora from the forest such as heart of palm ("palmito"), bromeliads, orchids and tree ferns. More than 70 species of animals living in the Atlantic Rainforest are kept in EAZA zoos and a number of them are managed in breeding programmes and/or studbooks.

Through the campaign, EAZA zoos hope to raise awareness of the plight of this unique forest and to raise funds for the Lion Tamarins of Brazil Fund (LTBF). By doing so the Atlantic Forest as a whole will benefit through the synergistic effect of support to high-profile conservation projects in the area. The Lion Tamarin programmes are typical examples of how originally species-based activities have developed into ecosystem programmes with strong socio-economic components. The Atlantic Rainforest is under a high degree of threat. However, by making a relatively small contribution to well functioning local conservation activities in which many zoos are already involved, we can make a real difference. The 'world' of nutrition research can make its own contribution to this effort. After all, a better knowledge of the nutritional ecology and nutritional needs of key species is often essential for the planning of short and long-term conservation measures for the ecosystem in which they live.

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KEY-NOTE LECTURE

Nutrition of marsupials in captivity

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Successful captive management of marsupials, as with any animal, depends on sound knowledge of the animal's natural habits, including diet. The natural diets of marsupials span almost the same spectrum as those of eutherian mammals. Thus there are insectivorous marsupial moles, carnivorous marsupial tiger cats (quolls), nectarivorous marsupial gliders, omnivorous bandicoots, and herbivorous kangaroos, wallabies, wombats and arboreal possums. Captive diets for many species are based on artificial ingredients designed to satisfy all known (or estimated) nutrient requirements, but in some cases successful captive maintenance is still dependent on some or all natural food ingredients.

This paper surveys the known natural diets of marsupials and highlights cases in which specific nutrients need attention in the captive situation. A common problem is obesity caused by overfeeding with diets much richer in digestible energy than natural diets likely to be encountered in the wild. On average, the metabolic rates of marsupials are below those of equivalent eutherians and thus their energy and protein requirements for maintenance are also relatively low. On highly digestible diets the stomach empties faster, with virtually no indigestible residues to maintain stretch of the gastric wall. Consequently the amount of energy ingested before the stomach fills is likely to greatly exceed that ingested on a natural wild diet, leading to excessive energy intakes and obesity when opportunities for exercise are limited by captivity. The consequences of obesity include musculo-skeletal problems and impaired reproductive success.

Deficiencies or excesses of specific nutrients are likely to be manifested in more specific ways. Of great importance to captive marsupials is the role of vitamin E in the prevention of nutritional muscular dystrophy when requirements for the vitamin are increased by confinement stress. Simple remedies for this and for other nutritional problems in captive marsupials are discussed.

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HACCP based bacteriological study of the feed of fruit-eating birds in the Rotterdam Zoo

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A HACCP (Hazard Analysis and Critical Control Point) based inventory study of the presence of bacteria in the feed of tropical fruit-eating birds during processing and feeding, demonstrated that several critical control points could be identified. Feed samples were taken out of two selected cages present in the Victoria voliere, a large tropical greenhouse of the Rotterdam Zoo. The birds in the selected cages were all fruit-eating birds. The diluted samples were plated on different media e.g. Plate Count agar and Violet Red Blue agar to investigate for *Total Aerobic Count*, *Enterobacteriaceae*, *Staphylococcus Aureus*, *Salmonella*, *Faecal Enterococcus sp.*, *Lactobacillus*, *Yersinia*, *Pseudomonas*, moulds and yeast. High bacterial numbers were present in the feed of the tropical birds. A trend of increasing bacterial numbers was found during the day. The number of bacterial species also increased over time. The total aerobic count developed during the day from log 5.3 at the start to log 7.8 at the end of the day. Enterobacteriaceae numbers rose from log 3.6 to log 6.4. The mixing moments and the bacterial growth during the presentation of the feed to the birds were critical control points.

To limit the bacterial growth, an experiment was done reducing the time the feed was presented to the birds. Clean feed was served in clean trays instead. The experiment demonstrated a visible drop in bacterial numbers.

The results of this experimental study showed that a HACCP based bacteriological study was able to identify critical control points in the processing and feeding strategy of tropical birds in the Royal Rotterdam Zoo. Changing processing methods at these critical control points resulted in a decrease in bacterial numbers.

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Evaluation of the use of organic formulated bird foods for large psittacines.

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The use of formulated diets for birds has been associated with high rates of infertility, poor hatching, weak, non-thriving chicks (with a high percentage of gram negative bacterial problems), yeast excesses, bent legs, crop emptying problems and high chick mortality. In addition, there have been problems associated with parents failing to incubate eggs, resulting in decreased hatchability, decreased parent-raising if eggs hatch, and an increase in the time spent hand-rearing and hand-feeding chicks. Reports of breeder mortality have also been recorded. This study evaluates the efficacy of formulated bird foods composed of organic ingredients for health and productivity of large psittacines maintained at two different commercial aviaries.

At aviary 1, eight psittacine species including: African grey parrots (*Psittacus erithacus*), Amazons (*Amazona* spp), cockatoos (*Cacatua galerita*, *C. moluccensis*, *C. galerita* triton), eclectus (*Eclectus roratus*) and macaws (*Ara* spp and *Anodorhynchus* spp), were maintained on a variety of commercial bird pellets composed of nonorganic ingredients for over 15 years, before being transferred to commercial products composed of organic ingredients (HBD Adult Lifetime Coarse for maintenance and HBD High Potency Coarse for breeding) in 1994. This resulted in an increase in breeding success from 0.87 chicks per pair (n = 150 pairs) to in excess of three chicks per pair, with a reduction in embryonic death, poor chick development and adult aggression, and an improvement in parental care eliminating the need to hand-rear chicks, which is not only costly but can also result in compromised immune systems. The nutrient compositions of the two organic products were compared to four nonorganic products (Kaytee Rainbow Exact, Mazuri Parrot Maintenance, Pretty Bird African Special). Variations in nutrient composition include: higher levels of preformed vitamin A (5-15 IU/kg vs. 0.4-5.7 IU/kg), copper (15-17 mg/kg vs. 7-13 mg/kg), iron (110-214 vs. 103-124 mg/kg) and zinc (95-118 mg/kg nonorganic, 70-81 organic breeding, 43-45 organic maintenance) in the nonorganic products with higher levels of fat detected in the organic diet for breeders (20-29% vs. 11-21% nonorganic). Vitamin E values were comparable for two of the nonorganic and organic maintenance diets (153-253 mg/kg), with higher values in one of the nonorganic diets and the organic breeding diets (223-305 mg/kg).

At aviary 2, 30 pairs of Amazons were maintained on nonorganic diets. Only one chick was produced and diagnosed to be of poor health. Productivity increased to 120 chicks when transferred to the organic products, all in good health. Birds were transferred to nonorganic diets during the nonbreeding season and only returned to the organic diets just prior to the breeding season, resulting in only nine chicks, indicating a requirement to maintain birds on diets of superior quality all year round. It is unclear whether the organic nature of the products in these two studies, or variations in nutrient composition are implicated but nutritionally balanced diets composed of organic ingredients are certainly indicated. These studies highlight the need to better understand the nutritional requirements of psittacines and consider the implications for the effects of pesticide contamination on the health of birds maintained in captivity on formulated diets composed of nonorganic ingredients.

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Global wildlife nutrition database: Howler monkey (*Alouatta pigra*) prototype

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The development of a global wildlife nutrition database has been initiated in response to the absence of information easily and quickly available on the Internet, which allows solutions to the resolution of questions and problems of a nutritional nature relative to populations of wild animals either in captivity or in nature. This model represents a first step for the fulfilment of the proposal contained in the CBSG Wildlife Nutrition Database Report (Boardman and Dierenfeld, 2001) where the concept was defined: "...to develop the infrastructure of an Internet-accessible database of nutritional information to fill some of these current data gaps, providing a global network for contributors as well as end-users, administered through the Conservation Breeding Specialist Group of the IUCN." This specific project was launched as a prototype through a grant awarded from Columbia University's Centre for Environmental Research and Conservation to the Wildlife Conservation Society. A planning meeting involving representative possible users of this envisioned system was hosted at the Wildlife Conservation Society February 2001, with feedback from staff of Zoo Conservation Outreach Group, Wildlife Information Network, Wildlife Trust, the New York Botanical Garden, and the host institution. Howler monkeys (*Alouatta pigra*) in Belize were targeted as a likely prototype species for development of a database module for a number of reasons: overlap with human uses of plant resources exists, long and short-term field data on movement patterns, habitat use, and nutrient composition of food plants have been collated. While the conservation issues of howlers may not be as pressing as those of other primates, there is, nonetheless much interest in nutritional management of howler monkeys both in US and Latin American zoological facilities, and the biodiversity index of plant use within Belize itself may have implications for non-primates. Such a prototype, therefore, could logically and realistically be developed based on datasets that are already published. Phase 1 (Prototype) model development incorporated the field data of Silver (Silver, 1997; Silver et al., 2000) and Ostro (Ostro, 1998; Ostro et al., 1999). Bibliographic information on howler monkeys in general and specific characteristics of *A. pigra*, along with these field data, defined the primary keys of the model structure. Hence animal species, plant species, or bibliographic data can initiate a search to retrieve more detailed information on any of the other primary keys. Under this integrated model, mechanisms will be investigated to effectively link a) detailed information on composition of plants consumed by howlers to the database structure of Zootrition™ software (Zootrition, 2001) b) botanical uses and toxicological data to extant plant databases, and c) published natural history and health information within the WildPro Multimedia electronic encyclopaedia and library of the Wildlife Information Network. Such linkages would magnify the functionality and capacity of a Global Nutrition Database, and accessibility world-wide, while at the same time making most effective use of collaborating partners.

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A glutenfree diet for callitrichids and marmosets in Emmen-zoo, the Netherlands

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Callitrichids living in zoos are prone to developing several symptoms that belong to the “Wasting Marmoset Syndrome”. Research has been done, but the cause of the Syndrome is not completely known and opinions about this differ. Possible causes that are mentioned are a lack of energy, incorrect nutrition, infections and stress. In the Noorder Dierenpark in Emmen, Holland, it is believed that an intolerance for gluten might be one of the causes. The symptoms of the “Wasting Marmoset Syndrome” are similar to the symptoms of gluten intolerance (coeliac disease) in people. These symptoms are: intestinal problems, a swollen abdomen, chronic stinking diarrhoea, stomach-ache, anaemia, fatigue, listlessness, weight loss, osteoporosis, enamel deviation, and in children, growth retardation.

Coeliac disease is a condition in which the lining of the small intestine is damaged by gluten. Gluten is a protein found in wheat, rye, oats and barley. In people who are sensitive to gluten, gluten damages the mucous membrane of the small intestine. The damage which occurs considerably impairs the absorption of nutrients from the small intestine, causing several problems. When a patient follows a strictly gluten-free diet the intestinal villi can restore and after a few weeks the symptoms will decrease. However, it takes 1 to 2 years for the mucous membrane to fully recover. The disease cannot be cured, which is why the patient has to follow the diet strictly during his whole life. Considering the possibility that some of the Callitrichids might suffer from coeliac disease, they probably cannot be cured either. The only possibility is that the mucous membrane of the small intestine of the Callitrichids fully recovers, providing that the damage of the intestinal villi was not too progressive. A monkey who is thought to have “Wasting Marmoset Syndrome” probably cannot be saved, (whether or not it is coeliac disease), because the damage of the intestinal villi already is too far ahead. It may be advisable to give the remaining Callitrichids of a group from which one monkey died from “W.M.S.” a gluten-free diet, even though they do not have any symptoms of the disease yet, since coeliac disease is thought to be hereditary. By providing the rest of the group with a gluten-free diet these animals have a chance to recover from damage in the lining of the small intestine; it is just preventive and a gluten-free diet is not harmful anyway.

A six-month pilot project was conducted at Edinburgh Zoo in 1998, regarding the influence of wheat on the health of Callitrichids. The research found that a gluten-free diet had a positive influence on the health. This was one reason for the Noorder Dierenpark to introduce a gluten-free diet for the Callitrichids in January 1999. We also observed positive effects: the incidence of diarrhoea, hunger, wasting, and other symptoms described earlier are greatly reduced. This is not an illogical result when you keep in mind that cereals don't grow in the natural habitat of these animals.

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Handfeeding of young parrots – techniques, diets and recommendations

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In recent years handrearing of pet birds has become a standard procedure for breeding valuable pet birds (e.g. African greys). In this case nestlings are fed with a special powdered complete diet mixed with water (i.e. 1st to 4th day of life: 1 part diet diluted with 6 parts water; 5th to 8th day of life: 1 part diet diluted with 4 parts of water; from the 9th day of life: 1 part of water : 2 parts of diet). This prepared solution is administered directly by a syringe into the crop during the first few days of life or afterwards into the beak by a teaspoon. The labeled ingredients of commercial handrearing diets comprise only a few nutrient contents (e.g. crude protein or crude fat), whereas data on amino acids or minerals are frequently absent.

Therefore this investigation will evaluate the energy and nutrient contents in commercial handrearing diets (n=11) in order to assess the suitability for rearing young pet birds.

Nutrients	nutrient contents ¹⁾ of handrearing diets ²⁾			necessary nutrient contents ³⁾ for	
	$x \pm s$	min.	max.	budgerigars	lovebirds
crude protein	14.4 ± 1.82	12.4	17.8	9.54	8.90
Lysine	0.735 ± 0.110	0.491	0.880	0.329	0.316
met + cys ⁴⁾	0.462 ± 0.184	0.234	0.695	0.469	0.368
Arginine	0.729 ± 0.143	0.591	1.140	0.484	0.430
Calcium	0.616 ± 0.243	0.349	1.145	0.276	0.271
phosphorus	0.312 ± 0.121	0.175	0.518	0.156	0.160
magnesium	0.086 ± 0.024	0.053	0.128	0.011	0.012
Sodium	0.133 ± 0.095	0.049	0.356	0.028	0.036
Potassium	0.346 ± 0.119	0.236	0.552	0.069	0.072

¹⁾ g/MJ ME ²⁾ MJ ME/kg dry matter: \bar{x} 15.2 ± 1.16 (min.: 13.0; max. 16.8); crude fat: 7.38 ± 1.10 g/MJ ME; starch: 29.5 ± 3.16 g/MJ ME; sugar: 2.48 ± 2.47 g/MJ ME; ³⁾ derived from growth rates of young as well as the body composition (KAMPHUES et al. 1996); ⁴⁾ methionine + cystine

A comparison of the analysed nutrient contents and the factorially derived necessary nutrient contents in handrearing diets for budgerigars and lovebirds shows sufficient crude protein, lysine and arginine contents. On the other hand some of the products showed a lack of the sulphureous containing amino acids methionine and cystine (feather growth ↓).

All minerals met the requirements of the young, but some mineral concentrations were higher than needed (i.g. high calcium contents → possible interactions with copper or zinc; high sodium contents among a limited water intake). Furthermore some of the handrearing diets contain an excessively high content of vitamin A (up to 47,000 IE/kg diet).

In general the results verify, that on the one hand the requirements of sulphureous amino acids for feather growth in nestlings are frequently underestimated and that on the other hand the calcium requirements (mineralisation of skeleton) is often overestimated. However, the primarily problem of handrearing in pet birds is most likely not caused by insufficient energy and nutrient contents of the diets, but to the consistency of the suspended diets within the gastrointestinal tract (e.g. stasis of the crop content).

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Experimental data on feeding extruded diets in parrots

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The feeding of pelleted/extruded diets to parrots has been a controversial subject. Some bird fanciers dislike the use of these diets because of body weight losses of their parrots during conversion from existing seed mixtures to formulated diets. Also, a reduced time for feed intake is linked with behaviour disorders (feather biting/picking) due to boredom, and a reduced attrition of the beak. In spite of those reservations pelleted/extruded diets allow the composition of a well-balanced diet that meets the nutritional requirement of the parrot at each stage of life. A pelleted or extruded diet also prevents the selection of individual ingredients within the offered feed (i.e. the choice of seeds like sunflower seeds, that are characterized by a high fat and energy content increasing the risk of obesity). Another positive aspect of feeding a pelleted diet is the improved hygienic quality due to the commonly used ingredients (mostly based on cereals). In feeding trials with amazons, grey parrots and cockatoos the parameters mentioned above were tested. The comparison of the chemical composition shows great differences between formulated diets and commercial seed mixtures based on fatty seeds like sunflower seeds, safflower, hemp, pumpkins, peanuts etc. (see Table 1). Compared to seed mixtures the pelleted/extruded diets are characterized by lower crude protein, fat, fiber and energy contents, but higher amounts of carbohydrates (diets are based on maize, wheat and oat especially). In particular, the calcium content of 10.9 g/kg dry matter (on average) indicates a high calcium supply. The calcium:phosphorus ratio was well-balanced. Sodium contents point to a supply, even if amounts of 1-2 g sodium/kg dry matter are not recommended.

Table 1: Chemical composition of pelleted/extruded diets compared to seed mixtures

<i>data per kg dry matter</i>	<i>pelleted/extruded diets (n=16)</i>	<i>seed mixtures (n=10)*</i>
crude protein (g)	196 ± 38.7	243 ± 63.6
crude fat (g)	81.6 ± 24.9	383 ± 125
crude fiber (g)	32.0 ± 14.0	38.2 ± 25.1
carbohydrates (g)	566 ± 56.4	284 ± 30.9
energy (MJ ME)	15.8 ± 0.60	21.4 ± 3.51
calcium (g)	10.9 ± 4.10	1.79 ± 1.24
phosphorus (g)	5.68 ± 1.26	9.08 ± 3.59
sodium (g)	3.21 ± 1.90	0.54 ± 0.24

*related to the 'kernels', that means the real intake after dehusking/shelling of the seeds

Conversion of parrots from usual seed mixtures to unknown mixed diets was done within a short time and without any problems (i.e. refusal of the diet combined with body weight losses). Offering seed mixtures ad libitum a typical rhythm of feed intake could be observed (higher ingestion activities in the early morning and in the afternoon), whereas formulated diets were ingested continuously during the whole day. The time spent for feed intake (measured in minutes/g feed) did not differ significantly between pelleted/extruded diets and seed mixtures. When mixed diets were fed, digestibility of organic matter varied between 76 and 84% (in comparison: 78% ingesting fatty seeds and 87% fed seeds rich in carbohydrates). In general the results do not support the reservations held against formulated diets, but for a final determination long term studies over several year are required.

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Feed composition and digestive capacity in parrots

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Nutrition in many species of parrots is traditionally based on seeds, feeds offered as mixtures (the composition depends on species) and supplemented by fruits, vegetables and plants. Byproducts of animal origin (food like cheese etc.) are also used. The aim of several investigations on parrot nutrition performed during the last 10 years, was the characterization of different ingredients by commonly used parameters of feed science. Of special interest is the proportion of husk and kernel in seeds, and the content of crude nutrients (including starch and sugar) in the part of feed that is actually ingested, i.e. the kernel. The protein quality (content of amino acids), the mineral and vitamin contents as well as the palatability of various ingredients (for example in choice trials) were tested. Historical data published on the composition of seeds in toto (including their husks or shells) can often result in an incorrect determination of the nutritive value of the part of the seed really ingested. Of importance in formulation complete diets for different species of parrots is the observation that some of preferred seeds and ingredients fed traditionally to parrots have characteristic patterns of amino acids (for example: peanuts and hemp contain high levels of arginine). The mineral content of seeds used in parrot feeding are characterized generally by low calcium concentrations (in kernels of starchy seeds: < 0.7 g Ca/kg dry matter [DM]; of fatty seeds: 0.9-3.6 g Ca/kg DM) and high phosphorus levels (in starchy seeds: 1.5-6.4 g P/kg DM, in fatty ones: 7-16 g P/kg DM), whereas the sodium content is low in general (0.1–0.6 g/kg DM).

Further studies were done to characterize the supply of granivorous pet birds with β -carotene, tocopherols, thiamine, riboflavin and vitamin B-6 in more detail. Evidently, β -carotene and riboflavin requirements of pet birds cannot be met by exclusive feeding seeds. Under certain feeding conditions (e.g. when only few species of seeds are offered or birds develop a selective feeding behaviour) vitamin E and B-6 may be in short supply (without supplementation of the seed mixture). Only the relative high activity of analyzed seeds for thiamine is expected to meet the animals' demand.

In recent studies the effect of crude fibre in the diet on digestibility of organic matter was tested in different species of pet birds (in comparison to hens). Digestibility of organic matter of identical diets clearly revealed species-specific effects (digestibility for love birds > cockatiels > budgerigars > canaries > hens > amazons). There may be differences in the enzyme concentration within the intestinal contents or the species differences could be based on anatomical conditions (size and proportion of the hindgut) and potential differences in intestinal microflora. Of special interest is the adaptation in enzyme secretion when the dietary fat or carbohydrate intake is changed, as was demonstrated in canaries and budgerigars.

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An overview of captive *Aceros* and *Buceros* hornbill diets in some Dutch- and US facilities

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Hornbills are very popular and spectacular exhibit animals in collections of zoos and aviculturists. In recent years the birds have been identified as a group in need of good husbandry guidelines and a priority for captive breeding, because of their threats in the wild. Unfortunately, captive reproductive success is not very high. Diet could be a key factor in developing successful programs (Dierenfeld *et al.*, 1991) and it would be useful to determine nutrient requirements in order to compose or evaluate (current) hornbill diets. However, as with most zoo species, actual requirements have not been previously determined.

To obtain more insight in the current status of captive *Aceros* and *Buceros* hornbills nutrition, a survey was conducted among several Dutch and US facilities which keep these species. Ten facilities were visited and in each facility an interview concerning the hornbill diets took place. The nutritional composition of 12 diets was calculated using Zootrition software (Wildlife Conservation Society, 2001), and compared with available literature concerning nutrition of frugivores as well as results from the study on nitrogen requirements by Foeken and de Vries (2001).

Preliminary results of the analysis show a considerable spread in ingredients used and nutritional composition (i.e. different types of offered domesticated fruits and vegetables, meat and commercial diets, protein levels, Ca, Ca/P ratio and iron levels). All data obtained will be presented as an overview of captive *Aceros* and *Buceros* hornbill diets in some Dutch- and US facilities.

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ruminants

Chairperson : Joeke Nijboer (NL)

KEY-NOTE LECTURE

Allometry and ecology of feeding behavior and digestive capacity of herbivores: a review revisited

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Herbivores have evolved in response to plant composition and morphology. Plants in turn have evolved defenses as well as exploitation of the animal presence. Plants offer food to herbivores and in return have obtained seed dispersal and altered chemical composition to favour animals. However, plants have also evolved protection through resistant structures fiber, lignin and secondary compounds that limit digestion. These resistant substances are at the expense of photosynthesis. Herbivores in return have developed means of coping with less digestible food. One of these is increased body size which promotes greater digestive capacity. Animal gut capacity forms a linear function (power one) relationship with body weight, while energy requirements are related to metabolic size (power three-quarter of body weight). The interaction of these two power relationships favour digestive capacity to increase at the one-quarter power of body weight. Thus small animals are less efficient utilisers of slower digesting fibre. The indigestible fibre must pass through the digestive tract or else be avoided by selective feeding. Monocots (grasses) are more difficult to selectively feed upon than dicotyledonous species because of morphology. Selective passage occurs in rabbits and lemmings. Intraspecific comparative digestive trials are often confused by failure to measure the fibre and lignin content of uneaten food. Avoidance of the more lignified structures by small herbivores allows higher digestibility and longer retention times. Ruminating animals with foregut fermentation are more efficient in extracting energy from fibre relative to non ruminants of the same size. Rumination allows a smaller animal to achieve the digestive capacity of larger non ruminant herbivores.

Plant fibre varies widely in nutritive quality, decreasing with plant maturity and warm climates. Geographic variation creates a difficulty for zoo nutritionists because local sources of feed may not match the adaptations of specialised animals from foreign habitats. Pelleting fibrous feeds forces the animal to consume the lignified matter and effectively limits selective feeding. This can be a problem for small selectors. Grinding also eliminates the “scratch factor” needed by larger bulk and roughage eaters, and compromises the rumination process in ruminants.

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Body size limitations in ruminants, or why buffalo defecate pies not pellets

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While the lower body size limit of ruminants has been explained convincingly, the upper body size range attainable for ruminants still poses a theoretical problem. In this contribution, we focus attention on the morphophysiological consequences of ruminant foregut fermentation. Ruminant forestomachs were designed to delay ingesta passage; as a side effect they limit food intake. Therefore, with increasing body size their relative capacity has to increase in order to compensate for this intake limitation. It seems that the foregut fermenting ungulates did not evolve species in which the intake-limiting effect of the foregut could be reduced, e.g. by special bypass structures, and hence this digestive model imposed an intrinsic body size limit. This limit will be lower the more the natural diet enhances the ingesta retention and hence the intake-limiting effect. Therefore, due to the mechanical characteristics of grass, grazing ruminants cannot become as big as the largest browsing ruminant, and our model precisely predicts the observed difference in maximum body weight attained by grazing and browsing ruminants.

Ruminants are not absent from the very large body size classes because their digestive physiology offers no particular advantage, but because their digestive physiology itself intrinsically imposes a body size limit. We suggest that the decreasing ability for colonic water absorption in large grazing ruminants and the largest extant foregut fermenter, the hippopotamus, are an indication of this limit, and are the outcome of the competition of organs for the available space within the abdominal cavity. Our hypotheses are supported by the fossil record on extinct ruminant/tylopod species that did not surpass extant species in maximum body size.

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Browse silage: the solution for browsers in wintertime?

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According to Hofmann (2000), ruminants can be divided into concentrate selectors/browsers, intermediate types and grass/roughages eaters. The group of browsers consists of fruit eaters, animals with a high amount of herbaceous dicots in their diet, and tree and shrub eating animals. Several diseases in browsing ruminants are linked to nutrition like gastrointestinal disorders, rumen acidosis and phytobezoars. There is also a difference in the physiology between grazers and browsers (Hummel and Clauss, 2001).

Giraffes, kudus, bongos, dikdik's and duikers belong to the group of concentrate selectors or browsers which are often found in zoos. They are mostly fed a diet which consists of 'browser pellets' as well as alfalfa and some greens and fruits. However, browse should be a major part of their diet in order to prevent acidosis (Hummel and Clauss, 2001).

If available in summertime, browse often consists of willow or poplar. Especially in temperate climates, it is difficult to feed fresh browse during wintertime to browsers. If a zoo intends to feed browse in wintertime, it should be harvested in autumn and properly stored until winter. This presentation will summarize the ways browse can be stored – fresh, frozen and dried.

Another option is to produce silage. Silaging is an anaerobic process. Anaerobic bacteria ferment the browse, and lactic acid is set free. At a pH of 4.2, the silaging process stops and the browse is ready to be fed. In Zurich (Hatt and Clauss, 2002), willow, hazel and maple are processed in a chafcutter and stored under anaerobic conditions in plastic containers. The product is fed in wintertime to rhinoceros. Analyses showed no significant alteration in composition before and after silaging.

At Rotterdam Zoo, in autumn 2001, willow branches and leaves were bailed by a bailing machine under high pressure and automatically double wrapped in plastic. The 70 bails of willow and 15 of poplar weighed between 45 and 50 kg. The dimensions of the bails were 100 x 50 x 35 cm. The silage was fed to the browsers in the wintertime. All the browsers (okapi, giraffe, kudu, tuffed deer and bongo) preferred the silage browse rather browse which had been frozen. In the future, it is planned that browse silage will be an important part of the diet in both winter- and summertime.

The process and conditioning of the silaging as well as the palatability of willow browse and other browse species will be discussed in detail. More research concerning the digestion of browse silage is in preparation.

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Irregular patterns for low amounts of dietary tannins in captive roe deer (*Capreolus capreolus*): Implications for the validity of preference trials

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Recently, it was demonstrated that captive roe deer actively select for a low amount of dietary tannins in preference trials (1). However, the periods of these trials comprised mostly 5-8 days only. Other ruminants, e.g. goats, have been shown to develop a food aversion to dietary tannins after only a few days (2). We intended to replicate the results with roe deer in longer trials, and with different types of tannins.

Three groups of adult and two groups of growing roe deer were submitted to series of long-term preference trials in which different combinations of a regular pelleted feed and the same feed with the addition of 3 or 6 % of tannic acid or quebracho were tested. Food consumption of each group was determined by weighing offered components and leftovers the next day.

We could not elucidate a consistent preference pattern for the roe deer groups used in this study. One group consistently preferred the highest available tannin concentration, whereas the other groups mostly preferred the tannin-free feed, but included tannins in differing amounts. There seemed to be a general pattern that tannin consumption peaked after 5-10 days but declined afterwards again.

The most important implication of these findings is that - at least where tannins are concerned - results of short-term preference trials might be misleading. If the trials would have been terminated after 5-10 days, most groups would have shown a continuous increase in tannin consumption. Similarly, one group showed a distinct tannin rejection during the first 5 days and increased their consumption afterwards. Obviously, long-term diet choice needs to be considered when designing preference trials. If results from preference trials are to be used in a conclusive way, the scope, and possibly the reasons, for immense individual differences need to be further elucidated.

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Grazing, ruminating and resting in *Bos taurus*, when herbage availability is limiting.

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In intensively managed, temperate pastures herbage availability may become limiting when sward height, sward bulk, or a combination of sward height and bulk, drop below certain critical values. For a grazing ruminant there are three major time-consuming, daily maintenance activities: grazing to apprehend and ingest herbage, ruminating to facilitate herbage digestion, and rest/sleep. Other important maintenance activities, drinking, urination, and defaecation, represent a very small daily time investment.

When herbage availability becomes limiting, and there is insufficient time in the day (24 hours) to ingest adequate food, to ruminate the ingested food and to sleep, the time spent on one or more of these activities must be reduced. Time spent sleeping by cattle at pasture is rarely observed to drop below four hours a day, with approximately 0.5 hours of rapid eye movement (REM) or paradoxical sleep, and 3.5 hours of non-REM or slow wave sleep (Ruckebusch et al., 1975). Time spent ruminating is affected both by the quality, defined as neutral detergent fibre (NDF %DM), and quantity of the herbage consumed (Bae et al., 1983). So when the grazing animal cannot eat enough in the time available, the time spent grazing and consequently the time spent ruminating are reduced. It is also suggested by certain recently published data (Gibb et al., 1999) that the time spent ruminating each kg NDF consumed may also be reduced, thus leaving more time for grazing.

Using a basic model which describes daily maintenance activities in grazing beef cattle (Finegan et al., 2001), two scenarios of response to time constraint (where daily time spent grazing, ruminating and sleeping would need to exceed 24 hours to achieve adequate feed intake) have been investigated. For scenario 1, predicted grazing and ruminating time in excess of 24 hours was deleted in proportion to predicted total grazing and ruminating times. For scenario 2, the time spent ruminating each kg NDF was reduced by 15% (based on an evaluation of data from Gibb et al., 1999), prior to proportional deletion of any grazing and ruminating time still in excess of 24 hours. The predicted total daily dry matter intake (DMI), recalculated after reducing daily maintenance activities to 24 hours, was 3.1, 4.2, and 5.3 % higher (for cattle of 200, 400 and 800 kg live weight) when ruminating time /kg NDF was reduced (scenario 2).

Little is known about the circumstances under which grazing cattle may reduce ruminating time /kg NDF. Such a reduction in ruminating time might be expected to be associated with some reduction in feed digestibility, if the increased quantity of feed ingested moves more quickly through the digestive tract (Van Soest, 1994). It is also not known whether the increased DMI would more than compensate for any decrease in digestibility. It is possible that a strategy of reduced rumination to allow time for increased DMI would be advantageous to the grazing animal if the quality of herbage on offer was good, but not if it was poor and difficult to digest.

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Considerations on feeds used for zoo ruminants

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According to several authors, the nutrition of wild ruminants in captivity still poses some challenges. Factors like concentrate/roughage ratio and the distribution of concentrate consumption over the day have to be considered important when planning a suitable feeding schedule for these animals. Another obviously very important factor for the planning of a diet is the chemical composition and the fermentative pattern of the feeds.

To be able to quantify their fermentative pattern, the gas production of several feeds fed to captive okapi in different facilities was measured via the HFT (Hohenheimer Futterwerttest), a common in vitro technique for evaluating feedstuffs for ruminants. The HFT relates the gas produced by a feed/rumen inoculum mixture to the energy yield of a feedstuff. Gas production is highly correlated to the production of fatty acids. It was recorded after 2h, 4h, 6h, 8h, 10h, 12h and 24h to get information about the rate of fermentation during the first 12 h of fermentation and of the energy yield after 24h.

As expected, feeds like banana or apple resulted in a rather high fermentation rate; during the first 4 hours, these feeds showed higher fermentation rates than all other feeds due to their high sugar content. Fermentation rate of unmolassed beet pulp (6-7 % sugar) was comparable to that of oats and wheat. In this study, dried forage meal (e.g. on a lucerne/clover basis) had a rather low energetic value, even when compared to the lucerne hay used in the facilities.

When feeding ruminants reported to show selective foraging behaviour in the wild, there are often speculations about significantly higher demands of these animals for easy digestible feeds. Anyway, they seem to be prone for the known problems occurring in ruminants when fed diets high in sugar and starch. Results will be discussed in respect of their significance for dietary planning and suggestions for favourable concentrates will be made. It is concluded that in vitro techniques like the HFT can be an important tool in evaluating zoo feeds.

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The effect of dietary sugar content on glucosuria in a female okapi (*Okapia johnstoni*)

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As early as 1980, Glatston & Smith reported that urine of many healthy adult okapis can contain high amounts of glucose. In Antwerp Zoo 5 adults older than 8 years old and one young animal of 3 years old showed glucosuria, whereas 5 other young animals (between 1.5 and 4 years of age) remained negative. The reasons for chronic glucosuria in healthy ruminants remain unexplained yet. Theoretically, a continuous high dietary intake of sugars might be the cause, provided that a ruminal bypass and / or escape exists in this concentrate selector (as suggested for the roe deer, Rowell-Schäfer 1999). In the wild, okapis browse more than 100 species of plants and prefer fast growing heliophilics, but they do not eat mushrooms (Hart & Hart, 1988). In captivity okapis are fed several fruits and vegetables containing high amounts of sugars (mainly bananas, apples and carrots). In order to examine the role of the diet on the glucosuria, a diet with a reduced sugar content was administered during 170 days to an adult female okapi. Over the first 90 days the initial amount of 2.4 kg of bananas, apples and carrots (800 g each) was gradually changed to 0.4 kg carrots and maximum 0.5 kg other vegetables (endives, celery, chicory, tomato, courgette, cucumber and aubergine). Afterwards, on average 0.150 kg more luzern hay per day was consumed. The decrease in dietary sugars amounted to approximately 0.2 kg. This was mainly due to a lower (i.e. 3.76 %) dry matter intake in the second diet, although the sugar content on dry matter base of the two diets was almost the same. Once a month at normal micturation, midstream urine samples were taken and stored at -20°C. The five samples were analysed simultaneously. Urinary glucose (mg/dl) and creatinine (mg/dl) were quantitatively determined using dry biochemistry (Kodak - Johnson & Johnson). To compensate for the variations in volume of the excreted urine, the ratios of glucose and creatinine in each sample were calculated (Finco, 1989; Spieker, 1989). These ratios were 13.56, 9.95, 7.21 and 6.32 after 1, 2, 3 and 5.5 months, respectively. Just before the end of the observation period, the animal had to be anaesthetised for hoof trimming and urine was obtained afterwards. The ratio was then 8.88, but a likely explanation of this is the administration of medetomidine, since hyperglycemia is one of the known side-effects of an α_2 -agonist (Lees, 1991). These preliminary results seem to indicate an impact of decreasing dietary sugars on the glucosuria. However, more data of captive okapis are needed. Obviously, it would be very interesting to compare data of urine samples from captive okapis with those from okapis in Epulu (Central Africa) living in more natural conditions.

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Blood parameters of captive roe deer (*Capreolus capreolus*) fawns on diets of different tannin content

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Potentially beneficial effects of dietary tannins have received increasing attention in human health sciences during recent years. Due to reports on a deliberate ingestion of dietary tannins in roe deer, we wanted to determine potentially advantageous factors of long-term, low-dose tannin feeding in this species by evaluating blood parameters.

Two groups of four hand-raised roe deer fawns were fed on a pelleted diet only. For eight months, the tannin group was supplemented with tannic acid as 3 % of the original feed mixture; for another two months, quebracho was added accordingly. Blood was collected during immobilisation after both periods, and analysed according to standard laboratory procedures.

After tannic acid feeding, the roe deer had significantly lower hemoglobin concentrations, packed cell volumes (PCV), lower glucose and higher total protein values. The general trend for tannin animals to have lower mineral concentrations was only significant for zinc after the tannic acid period. After tannic acid feeding, tannin animals had higher glutathione peroxidase (GSH) ($p=0.051$) and S GSH ($p=0.050$) concentrations; after quebracho feeding, control animals had higher malondialdehyd values. After tannic acid feeding, tannin animals had lower thyroxin values.

The small sample size precludes most generalizations. The reduction in mineral levels and parameters of iron metabolism (hemoglobin, PCV) could be expected from similar reports in other species. The lower thyroxine and glucose values could be indicative of a generally lower metabolic turnover in tannin-fed animals, which could have contributed to the higher feed conversion efficiency observed in these animals. For several parameters, the trend observed among our animals was in accord with reported values for free-ranging roe deer (that have a hypothetically consistent tannin intake). The influence of tannins on antioxidant status and its relevance for captive animals should be further investigated.

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Ruminants: why browsers are non-grazers

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The validity of Hofmann's classification of ruminants into different feeding types (BR = browsers, IM = intermediate feeders, GR = grazers) has repeatedly been challenged and recently refuted on the base of Hofmann's own morphological data. Due to experiences with captive wild ruminants, we intended to test whether a grass-avoidance, similar to the observed hay-avoidance in captivity, could be found in data on free-ranging BR as well, and whether an anatomical feature could be isolated that accounts for such a forage discrimination.

The analysis of two available data sets on the foraging patterns of free-ranging ruminants showed that, while GR species ingest also browse in differing proportions, BR species have a significantly narrower range of foraging diversity, i.e. they include grass in their diets to much lesser proportions. A comparison of data on the thickness of the rumen pillars in different ruminant species showed that BR have significantly weaker rumen pillars than GR.

These results can be interpreted in terms of the mechanical characteristics of the different forages in the rumen: grass tends to induce a rumen contents stratification, and therefore GR had to evolve a strong rumen musculature. The natural forage of BR, however, does not induce a rumen contents stratification, these animals therefore never needed to evolve a strong rumen musculature, and consecutively will avoid stratifying forages, i.e. grass. While it is no mechanical problem to ingest browse when adapted to the characteristics of grass, vice, versa, there is.

We propose that the mechanical properties of the different forages, in particular the tendency of grasses to stratify, were the main driving forces of ruminant feeding type diversification, and are responsible for the increased particle retention, increased particle comminution and fibre digestibility observed in GR.

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The attribution of a feeding type to a ruminant species based on morphological parameters: the example of the okapi (*Okapia johnstoni*)

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While Hofmann's work provides a plethora of morphological parameters by which he attributed the different ruminant species to one of the three feeding types (BR = browsers, IM = intermediate feeders, GR = grazers), he did not set up a scheme by which potential followers could investigate further species, or rank his measurements according to importance. We intended to replicate the classification of a species of known free-ranging foraging behaviour but which had not yet been classified according to Hofmann, by the use of different morphological measurements.

An adult, captive, female okapi had to be euthanized due to chronic illness and old age. Several measurements of the feeding apparatus and the digestive tract were performed according to standard anatomical practices.

According to the size and structure of its forestomach – a relatively small rumen capacity, low reticular crests, a small omasum and weak rumen pillars, the okapi would be classified as a typical BR. Measurements of the tongue and the incisor arcade supported this classification. In contrast, the relative weights of both parotid salivary glands and liver did not fit the pattern claimed for BR. deviations from Hofmann's original scheme have been reported previously especially for the salivary glands.

The question of whether Hofmann's classification can be replicated in anatomical terms is an academic one. For the management of a species, all available information – anatomical, physiological, and from observations in the wild – should be collated. In this respect, the classification of a species as a BR, with a relatively smaller and weaker rumen, should be regarded as an indication of particular proneness to digestive problems.

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Faecal dry matter content in captive wild ruminants: implications for the browser/grazer-dichotomy

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Among the feeding types introduced by Hofmann (BR = browsers, IM = intermediate feeders, GR = grazers) the IM/GR have been able to conquer a greater variety of habitat niches than the evolutionary older BR. One would expect such a difference in habitat variation to be reflected by a variety in morphophysiological adaptations.

We therefore investigated the faecal dry matter (DM) content in the faeces of 245 individuals of 81 captive wild ruminant species. Animals sampled were adult, did not have diarrhoea, and had *ad libitum* access to drinking water. DM content was determined by drying cleaned faeces to constant weight.

While there was no difference in average faecal DM content between the feeding types, frugivores and BR had a much narrower range of faecal DM contents than IM, which in turn, although they comprised the majority of the species sampled, had a narrower range of faecal DM content than GR.

As it has been shown that faecal DM content correlates to the length of the *colon descendens*, and the animals investigated in our study were not subjected to varying heat stress, the faecal DM content could be interpreted as a surrogate measure for the colon descendens length. The data are in accord with the observation that among the GR, the *hippotraginae* have very long, and the *bovinae* very short hindguts. Thus, the greater variation in faecal DM content can be interpreted as indicative of a greater variation in anatomical hindgut design in GR. Integrating the knowledge of forestomach differentiation, GR are therefore considered 'morphophysiological progressive ruminants', while BR are regarded as 'morphophysiological conservative ruminants'.

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Reaction of a group of captive giraffe (*giraffa camelopardalis*) to the introduction of a tannin-containing pelleted diet

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As it had recently been reported from preference trials that captive roe deer (*Capreolus capreolus*), a small ruminant browser, deliberately included tannins in its diet we intended to test the effect of offering a tannin-containing diet to captive giraffe. Three individual adult giraffe and a group of three growing animals were used. Food consumption was determined for a week before and two weeks after the addition of a tannic acid-containing pelleted feed to the regular diet. Food and faecal samples were taken throughout the trial period to determine digestibility coefficients by the lignin method.

The giraffe differed enormously in their response to the additional tannin feed. One adult animal refused the tannin feed completely. The growing animals and another adult animal ingested differing proportions of the tannin feed, and the last adult animal preferred the tannin feed over the regular pelleted diet in constantly increasing amounts. However, even this animal reduced its tannin intake after 16 days. The total dry matter intake of the two adult animals that ingested the tannin feeds increased significantly after the introduction of the new diet item. Digestibility coefficients for the different periods did not differ consistently.

The results indicate that individual feeding preferences can make it difficult to determine species-specific ones. Although the results of two adults and the subadult group seemed to suggest a preference for a certain amount of tannins at first, this trend was reversed after 10-15 days. With respect to the increased feed consumption, one should not exclusively consider effects of the tannins, but of the general feed variety. As an increase of food ingestion could be a desired effect in captive giraffe, further studies on the effect of dietary variation in this species should be investigated.

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Tannin-binding salivary proteins in three captive rhinoceros species

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It has been demonstrated that the presence of salivary tannin-binding proteins (TBP) in mammalian herbivores is related to their dietary habits, with browsers having higher concentrations. As the three main representatives of the rhinoceroses differ in their natural diet – the white rhino (*Ceratotherium simum*) a strict grazer, the black rhino (*Diceros bicornis*) a strict browser, and the Indian rhino (*Rhinoceros unicornis*) a mixed feeder with grass as the major portion of its natural diet, we intended to demonstrate an according pattern in salivary TBP distribution.

Saliva was collected from 9 white, 10 black and 8 Indian rhinos from different zoological institutions and analysed for TBP by tannin binding assay, using a hydrolyzable (tannic acid) and a condensed (quebracho sol) tannin as standards.

Black rhino saliva bound significantly higher concentrations of both hydrolyzable and condensed tannins than white rhino saliva. Indian rhino saliva had a capacity to bind hydrolyzable tannins similar to that of the black rhino, but a significantly higher capacity to bind condensed tannin than black rhino saliva.

Whereas results for white and black rhinos are as expected, the high condensed tannin-binding capacity of Indian rhino saliva is surprising. In evolutionary terms, the Indian rhino possibly adapted to a grass diet later than the white rhino, and the high salivary TBP might be an evolutionary leftover from relatively recent times when browse was a larger constituent of the Indian rhino's diet.

This study was partly supported by a grant of the International Rhino Foundation and SOS Rhino to MC.

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Induction of salivary tannin-binding proteins in captive black rhinoceros (*Diceros bicornis*) by dietary tannins

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In some rodents and in goats, it has been demonstrated that salivary tannin-binding proteins (TBP) can be increased by dietary tannin levels, whereas dietary levels in browsing ruminants cannot influence their concentration. Although the black rhinoceros is a strict browser and tannins have been found in its natural diet, potential seasonal shifts in dietary tannin concentrations have not been investigated to date. If such shifts are hypothesized, these animals would benefit from a tunable mechanism to produce TBP.

Six black rhinos from three different zoological institutions were sampled for this study. Each feeding period lasted for three months. Animals received their regular zoo diet, or the same diet with an addition of 5 % tannic acid (hydrolysable tannin) or quebracho (condensed tannin) to the pelleted ingredient of their diet. Saliva samples were analysed by tannin binding assay, using tannic acid and quebracho as standards.

There was a significant increase in tannic acid-binding capacity both after tannic acid and after quebracho feeding. Tannic acid feeding did not increase quebracho-binding capacity. After quebracho feeding, there was a trend of increased quebracho-binding capacity, however this was not significant.

The results indicate that the black rhinos investigated increased their production of salivary TBP in response to increased levels of dietary tannins. The fact that TBP capable to bind hydrolyzable tannin were more responsive to dietary stimulation could indicate that hydrolyzable tannins play a greater role in the black rhino's natural environment than condensed tannins.

This study was partly supported by a grant of the International Rhino Foundation and SOS Rhino to MC.

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Salivary tannin-binding proteins are not affected by mid-term feeding history in captive roe deer (*Capreolus capreolus*)

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Whereas in some monogastric rodents, salivary tannin-binding proteins (TBP) have been shown to vary according to dietary induction, there has been contradictory evidence among ruminant species: deer showed no increase after tannin feeding, but goats did. In order to test whether the constant exposure to a tannin source could influence the development of TBP in growing roe deer, we performed an experiment with eight hand-raised roe deer fawns on two different dietary regimes.

Both groups received a pelleted diet only. For eight months, the tannin group was supplemented with tannic acid as 3 % of the original feed mixture; for another two months, quebracho was added accordingly. Saliva was collected during immobilisation after both periods, and analysed for TBP by tannin binding assay. There was no difference in the salivary TBP concentration between the two groups.

As roe deer are strict browsers, the presence of salivary TBP could be part of the overall genetic makeup of the species, and this could be the case in the other deer species previously investigated. In contrast, goats, as intermediate feeders, can include different forages in their diet, and therefore should profit from the potential to adapt their salivary proteins to the type of forage actually ingested. The presence of an induction mechanism for TBP could therefore be indicative of the evolutionary feeding history of an animal species.

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general nutrition

Chairperson : Jean-Michel Hatt (Switzerland)

Eggs, endothermy and milk: A novel scenario for the evolution of lactation

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Lactation is a primary mechanism for transfer of nutrients from mother to young in all mammals, but its evolutionary origin is shrouded in mystery. The synapsid branch that was to produce mammals separated from the sauropsid branch (ancestors of turtles, squamates, tuataras, crocodylians and birds) in the Carboniferous, more than 310 million years ago (mya). Mammary glands may be an ancient synapsid feature, but there are no preserved intermediate structures in living or fossil taxa that demonstrate how or when they came into being. Herein I report a new evolutionary scenario for their origin.

Early synapsids, like other early amniotes, produced flexible-shelled eggs that would require an environmental source of water, as do extant squamate eggs. The global increase in temperature and aridity in the Permian, coupled with the gradual development of endothermic metabolism among therapsids, would have exacerbated this need. Yet if incubated eggs had to be thermally isolated, access to environmental moisture would have been limited. I hypothesize that secretions of abdominal skin glands became an important moisture source for eggs, and were essential for endothermic incubation in advanced therapsids. [Birds produce eggs with highly calcified shells that are resistant to water vapor flux, and thus do not face the same constraints.] In monotremes and marsupials the mammary glands develop in intimate association with hair follicles, suggesting that the evolution of hair and mammary glands are linked. It is possible that hair evolved as a means of retaining and dispersing glandular secretions needed by eggs, and only subsequently proliferated over the body to provide insulation.

These secretions subsequently became important for hatchlings as an ingested fluid ("milk"). Evolutionary modification of lysozyme to alpha-lactalbumin made synthesis of lactose and its oligosaccharide derivatives possible, milk lipids came to be secreted as membrane-bounded globules, and novel casein proteins provided amino acids and calcium to the young. These supplemented and then supplanted yolk nutrients. Suckling per se may have required development of a secondary palate, as seen in therocephalian, dicyonodont and cyonodont therapsids. Lactation must have been well established in advanced cynodonts in the Late Triassic (ca. 200-225 mya), because early mammaliaforms appear to have produced altricial young. This conclusion is based on the presence of epipubic bones, the evolution of diphyodonty (reduction of tooth replacement to two sets), and the energetic consequences of small body size. Adult mammaliaforms in the Late Triassic and Early Jurassic were mostly very small; a recently discovered form was 2-3 g in mass.

Early mammaliaforms probably sucked from an areolar area that lacked nipples, as do extant egg-laying monotremes. Evolution of a nipple may only have become possible when it was no longer necessary to disperse secretions for egg uptake, i.e. with the development of live-bearing after therians (ancestral to marsupials and eutherians) split off from the monotremes more than 125 million years ago.

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The protein-to-fiber ratio does not predict feeding selectivity among primate frugivores

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The ability to dependably predict feeding selectivity would have many theoretical and practical applications. The protein-to-fiber ratio has been used, with varying degrees of success, to predict feeding selectivity in many species of animals. In primatology, this ratio has been used with a similarly sporadic success rate. In this report we will present new data from four species of frugivorous primates, and discuss why this ratio frequently fails, both here and in other literature reports.

The most common form of this ratio used in primatology is crude protein divided by acid-detergent fiber (CP/ADF) and compared to some measure of selectivity. Here we will use time spent feeding on the different food items. Our results, from Kibale Forest National Park, Uganda, show that CP/ADF did not predict the feeding choices made by chimpanzees (*Pan troglodytes*; $r = 0.031$, $p = 0.6542$, $n = 212$), Group 30 blue monkeys (*Cercopithecus mitis*; $r = 0.071$, $p = 0.3447$, $n = 178$), Group 14 mangabeys (*Cercocebus albigena*; $r = 0.023$, $p = 0.7333$, $n = 230$), Group 30 mangabeys ($r = 0.024$, $p = 0.7042$, $n = 260$), and Group 30 redbellied monkeys (*Cercopithecus ascanius*; $r = 0.095$, $p = 0.173$, $n = 206$). However, it did predict Group 14 blue monkeys ($r = 0.287$, $p < 0.0001$, $n = 211$), and Group 14 redbellied monkeys ($r = 0.195$, $p = 0.0054$, $n = 203$).

In neither of the cases where the ratio predicted feeding time was protein by itself predictive ($r = 0.075$, $p = 0.2873$ and $r = 0.038$, $p = 0.5864$ respectively). The predictive power was probably coming from the fiber fraction ($r = 0.304$, $p < 0.0001$ and $r = 0.245$, $p = 0.0004$ respectively). As an interesting twist, in three of the five cases where the ratio was not predictive, the fiber fraction did (negatively - as expected) significantly predict time feeding but the protein fraction also, negatively, significantly predicted time feeding.

Overall, inconsistent results like these and those from the literature indicate that the CP/ADF ratio is not a dependable predictor of frugivore diet selection.

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Australian marsupials eat *Acacia* !

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Evaluation of Australian marsupial diets always emphasizes *Eucalyptus*, however, there is another food source of major importance – *Acacia*. *Acacia* species play a major role not only as a food source, but also in determining the characteristics of Australian landscapes. *Acacia* are legumes, relying on symbiotic bacteria for nitrogen fixation. Because *Acacia*'s ability to fix nitrogen, its nutritive value might be expected to be higher than most browse species (Gutteridge and Shelton 1994). Due, however to the phyllode leaf structure and high tannin content; Australian *Acacia* tends to be lower in nutritive value - high fiber and low digestibility. *Acacia* rarely has toxic effects on animals, but some *Acacia* contain secondary metabolites that may be feeding deterrents. Secondary metabolites found within various *Acacia* include tannins, oxalates, cyanogens and fluoroacetate. Evaluation of Australian marsupials and how they utilize *Acacia* to compliment other dietary ingredients is a unique opportunity into animal nutrition, gastrointestinal physiology, behavior and environmental circumstances. We will briefly look at *Acacia* gum, foliage, arils, seeds, pollen or flowers and the animals that have adapted to an *Acacia*-based diet. In order to utilize *Acacia* gum as an energy source, it is essential that mammals have the necessary gut flora capable of fermenting complex polysaccharides found in gum (Lindenmayer, 1996). These microflora are retained in enlarged fermentation chambers in the gastro-intestinal tracts of these marsupials, specifically the caecum and forestomach (Hume 1999). There is a direct relationship between hindgut caecum size and proportion of *Acacia* gum consumed. The largest caecum sizes are found in animals consuming large amounts of gum. Conversely, those with smaller caecums consume little or no gum. *Acacia* foliage is consumed by few marsupial species except in times of drought or low food shortages. Dried *Acacia* leaves are more commonly consumed than fresh *Acacia*, probably due to a lower tannin concentration. The low intake may be due to high fiber content or secondary compounds that the animal would have to metabolize for detoxification. *Acacia* seeds are consumed only by a few species, and the lipid-rich arils consumed preferentially by lactating mahogany gliders. *Acacia* is critical to the survival of many native Australian marsupials as a food source - gum, foliage, arils and seeds, in addition to habitat. Many of these unique creatures have developed gastrointestinal compliments to enable them to ferment, digest and detoxify *Acacia*, having a seemingly innate ability to select those components needed. Often the use of *Acacia* species is opportunistic chosen, or is linked to environmental conditions, but there is no denying the role *Acacia* plays in the survival of these species and the Australian landscape.

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Brookfield Zoo Databank Overview

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Currently nutritional challenges remain for those commonly managing many captive species and usually no indications of nutritional status exist. Even when biochemical nutritional status is assessed, limited information is available with which to compare to determine if a particular animal or nutrient is within normal ranges. Determinations of circulating levels of lipids (total cholesterol, HDL-cholesterol, measured LDL-cholesterol, and triacylglycerides), vitamin D metabolites and vitamins A and E, carotenoids and minerals can provide a base for examining the nutritional status of animals. Additionally, as free-ranging populations become more fragmented and resources more scarce, assessment of nutritional status in healthy populations will serve as a baseline from which to compare questionable populations. The purpose of examining nutritional status in many animals representing numerous species was to establish normal values for captive exotic animals. The nutritional status data bank currently holds data for up to 8 canid species (n=45), 17 felid species (n=83), 25 primate species (n=235), 4 ursid species (n=22) and 22 miscellaneous grouped species (n=62) which were housed at four zoos (Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens, and North Carolina Zoological Park) for over 5 years. However, due to the quantity of blood needed, all analyses were not performed for all animals. These measurements are unique because they are derived from healthy animals with known diets and because they represent comparatively large groups of animals for most species measured. Several species-specific anomalies in the measured blood parameters became evident. It is important to examine these data to determine the potential impact on both zoo animal nutrition and ultimately animal conservation. Some of the irregularities include the following: 1) Spectacled bears had the greatest total cholesterol (369 mg/dl (n = 7)) and triacylglycerides (1079 mg/dl (n = 7) levels of all species measured, 2) Compared to other canids, the maned wolves and the African wild dogs had the greatest total cholesterol (268 mg/dl (n=9) and 261 (n = 5), respectively) while the maned wolves possessed the greatest HDL-cholesterol (209 mg/dl (n = 5)) levels, 3) Sand cats had substantially greater retinyl palmitate (3149 nmol/L) and retinyl stearate (4882 nmol/L) than all other feline species measured, 4) Cholesterol and LDL-cholesterol were the highest in gorillas (247 mg/dl n=33 & 144 mg/dl n=32, respectively) and second highest in spider monkeys (195 mg/dl n=13 & 137 mg/dl n=12, respectively), and 5) Vitamin D deficiencies in certain primate species despite adequate levels in diet. While examining the nutritional status of captive animals is important, often there are not data on free-ranging counterparts available for comparison. In addition, some research studies have shown that the nutritional status data obtained from free-ranging animals are somewhat different compared to those of their captive counterparts. Thus it is important to note that some inferences made on nutritional status of captive animals may not apply to free-ranging animals and vice versa. In summary, the nutritional status project provides a databank that gives a unique collection of information on normal circulating blood values in a large number of captive animals. These data provide a baseline to understanding the health and nutritional needs of captive animals. With the aid of future cooperative research studies in this area, the conservation of both free-ranging and captive species may come closer.

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Recent activities of the United States National Research Council Committee on Animal Nutrition

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The Committee on Animal Nutrition (CAN) oversees *The Nutrient Requirements of Domestic Animals* covering approximately 30 species of economically important farm animals, laboratory species, wildlife, and companion animals. Reports are used as a standard worldwide by government agencies for research and regulatory purposes, by universities for teaching and research, by extension personnel, by food and feed industries and by veterinarians, livestock producers, and pet owners. In addition to the consensus reports in this series, CAN also addresses prevailing and emerging issues through *Special Reports and Events*. The primary focus of CAN changes over time in response to emerging issues and changing national needs. Early on, CAN's focus was improving animal nutrition to ensure an adequate food supply for the population during times of war. Today, the major focus of its work is centered in environmental quality, animal production, food safety, and animal and human health. Advances in science and technology, such as genetically improved animals, require continual reassessment of nutrient requirements. Changes in management strategies to address environmental concerns necessitate on-going review of animal nutrition and feeding approaches.

Several reports that were published recently by CAN serve as examples of the continuing work of this committee and its response to emerging issues. *Nutrient Requirements of Dairy Cattle, Seventh Revised Edition* has expanded greatly in scope from earlier editions and is based on the wealth of information that has emerged in the past decade. The second edition of *Nutrient Requirements of Nonhuman Primates* is a much-needed comprehensive volume that addresses primate feeding, digestive physiology, and nutrient requirements of primates, many of which are endangered and threatened species. *Scientific Advances in Animal Nutrition* is a special report by CAN that is structured to highlight the critical role of animal nutrition in the future for a diverse readership. Responding to rapidly growing public concerns about air emissions from confined animal feeding operations in the United States, CAN's report, *The Scientific Basis for Estimating Air Emissions from Animal Feeding Operations*, identifies the scientific criteria needed to ensure that estimates of air emission rates are accurate, assesses emission-estimating mitigation techniques, and identifies best management practices.

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Nutritional aspects of the dry-season diet of mountain gorillas in Bwindi Impenetrable National Park, Uganda: preliminary results

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Plants (N=128) and fungi (N=2) eaten by highly endangered mountain gorillas (*Gorilla gorilla beringei*) in Bwindi Impenetrable National Park, Uganda were collected, processed, and analysed for their chemical composition. Plant parts and fungi were identified as food items when gorillas were observed feeding on them or by their trail remains. Foods were collected within the home range of the gorilla group observed and were dried, packaged, and transported to Cornell University's animal nutrition laboratories in New York, USA and the nutrition laboratory at the Wildlife Conservation Society, New York, USA. Moisture content was recorded and foods were analysed for acid and neutral detergent fiber, lignin, crude protein, soluble carbohydrates, condensed tannins, and total phenolics using standard methods. Preliminary results indicate that Bwindi mountain gorillas eat protein rich leaves (4% to 26% crude protein) compared with their overall diet (2% to 14% crude protein). Neutral detergent fiber ranged from 22% to 78% and acid detergent fiber ranged from 9% to 42% dry matter in food parts. Preliminary results reveal that lignin values in foods were 2% to 22% dry matter. Compared with Virunga gorillas, Bwindi gorillas eat more foods containing condensed tannins. The chemical analysis of common forest plants that are not consumed is in progress to assess the importance of chemistry in food selection. We plan to analyze foods collected in other seasons and conduct observations of food intake to quantify frequency of consumption of each food item. The combination of chemical analyses and observations of food intake will provide the data for calculation of nutrient intake of the Bwindi gorillas. Understanding the feeding ecology and nutritional needs of Bwindi gorillas is essential to their management and conservation *in situ*, and provides useful comparative data for optimizing *ex situ* feeding management of gorillas.

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vitamins

Chairperson : Eduardo Valdes (USA)

Vitamin A nutrition of cockatiels

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The vitamin A (VA) requirements for Psittacine birds have not been experimentally determined, and often are extrapolated from the VA requirements for growth and egg production of chickens, turkeys, domestic ducks and Japanese quail. However, due to substantial differences between digestive physiology, rates of embryonic and post-hatch development, and desired performance of psittacine species, it is difficult and potentially erroneous to infer psittacine VA requirements based upon those of commercial poultry. Therefore, the purpose of these experiments was characterize the responses of adult and growing Psittacines (Cockatiels, *Nymphicus hollandicus*) to various dietary vitamin A levels. Adult female cockatiels at maintenance were randomly assigned to 1 of 4 dietary treatments, containing 0, 2000, 10000, or 100000 IU VA/kg. Birds were monitored for ~ 700 days for signs of VA deficiency or toxicity by a variety of parameters. Additionally, newly hatched cockatiel chicks were randomly assigned to 1 of 3 dietary treatments, containing 0 IU VA/kg, 4000 IU VA/kg, or 2.4 mg β -carotene/kg diet. Birds were fed their assigned diets for approximately 5 weeks, and were monitored for a variety of parameters.

After 269 days of feeding, adult cockatiels fed 100,000 IU VA showed poor conditioning (reduced feather quality and increased prominence of keel bone), although no significant change in body weight was observed due to dietary treatment ($P=0.30$). In contrast, birds fed 0, 2,000 or 10,000 IU VA maintained body weight and conditioning for 700 days. VA level influenced vocalization patterns; in general, 0 or 100,000 IU VA altered the number of vocalizations, and reduced the average length and peak frequency of each vocalization ($P<0.05$). Plasma VA levels were not affected by 0 IU VA, but were increased by 10,000 or 100,000 IU VA ($P<0.05$).

After ~35 days post-hatch, cockatiel chicks fed 0 IU dietary vitamin A developed signs of vitamin A deficiency, including poor feathering on the head, neck and breast regions, as well as dermatitis on the face. However, body weights of chicks were not affected by dietary treatment through day 36 post-hatch.

These data demonstrate that adult cockatiels at maintenance are much more susceptible to vitamin A toxicity than to vitamin A deficiency. Considering their wild-type diet, which is seed-based and would likely contain low levels of pre-formed vitamin A, adaptation to low dietary VA levels, and efficient storage of VA is not surprising. Growth trial results demonstrate that 0 IU VA is insufficient for chick growth and development. 4,000 IU VA or 2.4 mg β -carotene was sufficient to prevent signs of vitamin A deficiency, providing valuable information regarding this psittacine birds' ability to utilize carotenoids as a vitamin A source. Based upon the apparent susceptibility to vitamin A toxicity, it may be prudent to include β -carotene as a vitamin A source in psittacine diets.

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Determination of 25-Hydroxy Vitamin D in seed fed grey parrots

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Vitamin D deficiencies are common in captive birds kept indoors in a UV deficient environment with insufficient dietary vitamin D. The domestic fowl does not have a dietary requirement for vitamin D if it receives adequate UV light in the 285-315nm spectrum. Psittacines are commonly fed a seed based diet deficient in vitamin D. In addition psittacines kept indoors receive inadequate UV for cutaneous synthesis. It is well established that grey parrots are particularly susceptible to hypocalcaemia. It is postulated that seed based diets deficient in vitamin D are a possible contributory factor to this condition leading to nutritional secondary hyperparathyroidism but no study has measured vitamin D in psittacines. The aim of this study was to assess the vitamin D status of a colony of grey parrots fed an unsupplemented seed diet.

Vitamin D status is best assessed by assay of 25-Hydroxy Vitamin D due to its long half life compared with other vitamin D metabolites. Traditionally radio immunoassays (RIA) have been used to assay 25-Hydroxy Vitamin D but more recently enzyme immunoassays (EIA) have become available with the advantages of both convenience and economy.

100 grey parrots were fed on a mixed seed diet with no additional supplementation. The group were kept indoors under artificial lighting. 40 birds were selected at random and blood samples taken from the brachial vein under sevoflurane anaesthesia. The blood samples were subjected to a standard haematological and biochemical profiles and the birds had a standardised clinical examination: any birds considered unhealthy were withdrawn from the study. This left a sample size of 34 healthy grey parrots.

The IDS OCTEIA 25-Hydroxy Vitamin D kit was used on the samples for the quantitation of 25-Hydroxy vitamin D. Each sample was assayed in duplicate. The results indicated a range of 25-Hydroxy Vitamin D between 5.1-380nmol/l with a mean of 119nmol/l.

The results show a wide variation in the level of 25-Hydroxy Vitamin D in seed fed grey parrots. In mammals vitamin D levels below 50nmol/l are considered a vitamin D deficiency. Chronic vitamin D deficiency would lead to hypocalcaemia due to nutritional secondary hyperparathyroidism.

Analysis of the diet using the Zootrition program indicated a vitamin D level of 0.1%. It is suggested that minimal levels for vitamin D in the domestic fowl is .4%.

This study suggests that the vitamin D levels were low in 18 of the 34 birds sampled. All the birds had normal ionised calcium levels at the time of the study. Further studies on the same group are being carried out to analyse the effect of increasing levels of UV light in the 285-315nm spectrum and dietary changes to pelleted diets on the vitamin D levels. Initial observations on 5 grey parrots kept on pelleted food (Harrisons High Potency Course) under the same husbandry conditions indicate higher levels of 25-hydroxy vitamin D compared with the seed fed birds.

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Differential absorption of natural versus synthetic alpha-tocopherol in Asian and African elephants

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alpha-Tocopherol (α -TOH) is the major antioxidant vitamin for mammalian species and the primary vitamer in the diet of free-ranging elephants. However, knowledge regarding the daily requirement, absorption and elimination of α -TOH in elephants is sparse. An investigation of the differences in absorption and elimination of α -TOH in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants was conducted using differentially deuterium-labeled natural and synthetic α -tocopheryl acetate and a single dose pharmacokinetic experimental design. An equimolar mixture of deuterated d_3 -RRR- α -tocopheryl acetate (natural vitamer) and d_6 -all rac- α -tocopheryl acetate (synthetic vitamer) for a total of 4 g tocopheryl acetate was administered to healthy non-lactating adult female elephants (n=5 per species). Blood samples were collected at baseline (pre-dose) and 3, 9, 12, 24, 48, 72, 96, 120 and 144 hr post dose. Comparison of the two elephant species found (1) A significantly greater plasma concentration of endogenous α -TOH in African elephants relative to Asian elephants: 0.70 ± 0.03 vs 0.26 ± 0.03 nmol/L · kg at baseline, $P < 0.001$. (2) A greater plasma response to administration of the natural α -TOH vitamer in African elephants relative to Asian elephants: 0.20 ± 0.006 vs 0.011 ± 0.003 nmol/L · kg at 24 hr post-dose, $P < 0.001$. In addition, the area under the plasma concentration versus time curve for natural α -TOH was almost three times greater in African relative to Asian elephants, while no difference was found for synthetic α -TOH. (3) The rate of elimination of both tocopherol vitamers from the plasma pool was almost twice as fast in Asian elephants compared to African elephants. (4) The slower rate of loss of natural α -TOH compared to synthetic α -TOH in African elephants resulted in a significantly higher ratio of natural to synthetic α -TOH in plasma relative to the Asian elephants, $P < 0.01$. These observations suggest that both African and Asian elephants may have mechanisms to discriminate between natural and synthetic α -TOH during absorption and elimination, and that these mechanisms may not be equivalent in both species. We also found that natural α -tocopherol is more bioavailable than synthetic α -tocopherol in both species of elephants.

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Investigations on the influence of dietary cobalt supply on the vitamin B₁₂ status of dairy cows

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Introduction: Micro-organisms in the forestomachs of ruminants are able to synthesize vitamin B₁₂ (which is not present in feedstuffs of plant origin) in amounts which are sufficient to meet the animals' requirements. However, a prerequisite is a sufficient supply of cobalt (Co), which is the central atom of the vitamin B₁₂ molecule. As the recommendations for cobalt supply to dairy cows are mainly based on experiments which were carried out with sheep or beef cattle, the objective of this study was to analyse any effects of dietary cobalt supplementation on the vitamin B₁₂ status of dairy cows.

Methods: The experiments encompassed a total of 20 dairy cows of the German Holstein breed, which were allotted to 2 groups (treatments) with different cobalt concentrations in the ration:

Group 1: controls without Co supplementation; ≈ 0.1 mg Co/kg DM (\approx requirement)

Group 2: extra supply of 0.2 mg/kg DM; ≈ 0.3 mg Co/kg DM

The animals were fed 1 kg balancing concentrate without or with a cobalt supplement. Additionally a dairy concentrate (0.15 mg Co/kg DM) was given according to milk yield. Wilted grass-silage was offered ad libitum as roughage; average daily intake amounted to about 12 kg DM.

During the experiment blood samples were taken at the beginning and subsequently in four week intervals for vitamin B₁₂ analysis (chemilumineszenz technique). On experimental day 100, 200 and the day of calving samples were taken from the liver using the biopsy technique to determine the cobalt concentration by ICP-analysis. Dry matter intake from roughage and concentrates as well as milk yield were recorded daily, milk composition twice a week.

Results: In both groups serum vitamin B₁₂ concentrations increased during the course of lactation, without significant differences between groups which received a ration with an analysed cobalt content of 0.13 mg/kg DM (group 1) and 0.27 mg/kg DM (group 2).

In the course of lactation vitamin B₁₂ concentration in the liver decreased in both groups, but the concentration in group 2 showed a tendency towards higher levels on day 100 and 200. On the day of calving this difference proved to be significant (0.51 ± 0.06 mg vitamin B₁₂/kg WW versus 0.64 ± 0.06 mg vitamin B₁₂/kg WW).

Differences in feed intake, milk yield and milk composition due to the different cobalt supply were not detected.

Conclusion: From the results it can be concluded that a cobalt content in the ration of 0.13 mg Co/kg DM seems to be sufficient. This value is lower than the GfE (1) allowances of 0.20 mg Co/kg DM.

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Effect of supplemental ascorbic acid on T₃-induced heart failure syndrome and metabolic parameters of broiler chickens

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A total of 420 male broiler chickens were randomly divided and fed a basal diet with or without 1.5 ppm T₃ from day 1 (1) and with or without 500 ppm vitamin C added to the diet. All birds had *ad libitum* access to feed and water but the light and temperature programs were provided as standard. During the study, the incidence of right ventricular failure and ascites, body weight, feed intake, feed conversion ratios, haematocrit, plasma T₃ and T₄ levels were determined (2).

Dietary T₃ markedly increased heart failure and ascites. The incidence of ascites was clearly reduced (60 %) by the effect of 500 ppm vitamin C supplementation in the T₃ and control groups. Broiler chickens that received ascorbic acid without T₃ showed a tendency for higher body weight and lower feed conversion ratios compared to those birds fed without ascorbic acid (Table 1). The influence of T₃ and ascorbic acid on haematocrit value was not significant but those birds fed diet with ascorbic acid showed a tendency for lower haematocrit values. Dietary T₃ supplementation significantly increased plasma T₃ levels (P<0.0001) and decreased T₄ levels (P<0.0001). Ascorbic acid significantly decreased plasma T₃ concentrations in birds fed a T₃ supplemented diet (P<0.0001) but not in control birds while plasma T₄ levels were higher in birds received ascorbic acid in the diet compared to those fed a control diet (P<0.0001).

Table 1: The number of heart failure and ascitic chickens, body weight, feed intake, feed conversion ratios, haematocrit values, plasma T₃ and T₄ levels in broiler chickens given a control (Co) or a T₃ supplemented diet (T₃) with (As) or without 500 ppm ascorbic acid.

Parameters/treatment	NCo	AsCo	NT ₃	AsT ₃	P- value
Heart failure	3/120	0/120	21/120	6/120	-
Body weight (g)	2002 ± 24a	2046 ± 20ab	1604 ± 40b	1601 ± 21b	0.0001
Feed intake (G)	3636 ± 34a	3543 ± 42a	3103 ± 39 b	3136 ± 59b	0.0001
Feed conversion	1.82 ± 0.03ab	1.73 ± 0.01b	1.94 ± 0.02a	1.95 ± 0.06a	0.01
Haematocrit (%)	29 ± 0.4ab	28 ± 0.3b	31 ± 0.5a	30 ± 0.4ab	0.01
T ₃ (ng/ml)	2.5 ± 0.2c	2.6 ± 0.2c	7.2 ± 0.5a	4.6 ± 0.5b	0.0001
T ₄ (ng/ml)	13 ± 0.8a	10 ± 0.8b	1.5 ± 0.1c	1.5 ± 0.1c	0.0001

There are several studies describing that ascorbic acid has a beneficial effect on heart failure syndrome and ascites by scavenging on the oxygen-derived free radicals (3) but in present study most remarkably ascorbic acid declined ascites incidence by reducing of hyperthyroidism and as a consequence the metabolic disorders. This finding confirms the earlier report when broiler chickens were reared in low environmental temperature (2).

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The influence of an increased cobalt supply on microbial vitamin B₁₂ synthesis in the rumen of dairy cows

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Introduction: In contrast to most mammals, ruminants are not dependent on a dietary vitamin B₁₂ supply, because micro-organisms in the forestomachs of ruminants can use cobalt to synthesize vitamin B₁₂ in amounts meeting ruminants' requirements. As it is known from the literature that the extent of microbial vitamin B₁₂ synthesis depends on the amount of cobalt (Co) provided with the ration (SMITH and MARSTON, 1970), the following investigations were designed to test the effect of an elevated dietary Co supply on the amount of vitamin B₁₂ reaching the duodenum.

Methods: 5 lactating cows ("German Holstein", av. body weight 678 ± 45 kg, av. milk yield 19.6 ± 3.7 kg) with a ruminal cannula and a T-cannula in the proximal duodenum were allotted to an incomplete cross-over. They were fed a ration of wilted grass-silage (10 kg DM), 1 kg balancing concentrate with or without cobalt supplement and additionally 3 kg dairy concentrate (ration: in DM 23.7% crude fiber, 21.5 % crude protein). With the exception of cobalt, minerals were fed according to the GfE in both groups.

The experiment comprised 2 periods of 21 days each, in which subsequently the unsupplemented or the supplemented ration was fed. After 14 days of adaptation the duodenal chyme was sampled applying a method published by ROHR et al. (1979). The cobalt concentrations in feedstuffs and digesta were analysed using the ICP-technique (ICP-OES; Fa. Spectro); vitamin B₁₂ concentrations in the digesta were determined by ELISA (RIDASCREEN[®] Vitamin B₁₂).

Results: In the supplemented ration a cobalt content of 0.29 mg/kg DM was analysed in comparison to the unsupplemented one (Co content of 0.17 mg/kg DM). Results show significantly higher amounts of vitamin B₁₂ reaching the duodenum for the supplemented group (8.63 ± 2.22 mg vitamin B₁₂/day) as compared to the controls (3.67 ± 0.69 mg vitamin B₁₂/day), although the individual levels vary considerably.

Conclusion: Feeding 0.29 mg Co/kg DM resulted in higher amounts of vitamin B₁₂ in the duodenal digesta. But further research is necessary to study if this affects the vitamin B₁₂ concentration in the serum, liver and milk of cows in any way.

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The effect of cobalt supply to pregnant cows on the vitamin B₁₂ status of their calves

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Introduction: Ruminants are able to synthesize vitamin B₁₂ in their forestomachs, but preruminant calves are dependent on a dietary supply. Because of indications from the literature that dietary cobalt increases vitamin B₁₂ concentration in ewes' colostrum and that there is an increase of vitamin B₁₂ in lambs' serum after ingestion of colostrum (HALPIN and CAPLE, 1982), the following investigation with dairy cows and their calves was designed to test the influence of oral cobalt supply to cows on the vitamin B₁₂ status of the calves.

Methods: The experiment was conducted with a total of 20 lactating dairy cows of the German Holstein breed. The average milk yield amounted to 32.5 kg FCM at the start of the experiment (70th day of lactation), which lasted 280 days. 10 animals each were allotted to the following cobalt treatments:

Group 1: without Co supplementation (0.13 mg Co/kg DM in the ration ≈ near to the recommended requirement)

Group 2: with an extra oral Co supply (0.27 mg Co/kg DM in the ration)

Milk yield was recorded daily and milk composition twice a week. Milk samples for vitamin B₁₂ analysis (chemilumineszenz) were taken at the beginning of the experiment, before drying off (day 220) and on the day of calving.

Additionally, venous blood samples of calves were taken directly after birth and on the following 4 days for vitamin B₁₂ determination in the serum.

Results: Vitamin B₁₂ concentrations in the colostrum were detected to be 4 to 6 times higher as compared to milk. Colostrum showed a tendency towards a higher vitamin B₁₂ concentration (21.0 ± 8.4 versus 16.7 ± 11.9 ng/ml) due to cobalt supplementation, but the results failed to reach significance. Dietary cobalt levels did not affect vitamin B₁₂ concentration in milk. Only a marginal increase was detected in the course of lactation from 3.77 ± 0.96 to 4.75 ± 3.05 ng vitamin B₁₂/ml for controls and from 3.66 ± 1.03 to 4.44 ± 0.96 ng vitamin B₁₂/ml for the supplemented cows. Differences in milk yield and milk composition (fat, protein and lactose) due to cobalt supplementation were not detected.

Extra cobalt supplementation to the ration of pregnant cows did not result in increased vitamin B₁₂ levels in the serum of their calves before they received colostrum. After the intake of colostrum the vitamin B₁₂ concentration in the serum of calves increased in both groups (342 ± 162 versus 361 ± 186 pg/ml) and decreased afterwards.

Conclusion: From the results it can be concluded that a cobalt content of 0.27 mg Co/kg DM in the ration of pregnant cows (higher than the recommended allowance of 0.10 mg/kg DM) does not effect the vitamin B₁₂ status of their calves.

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lipids

Chairperson : Olav Oftedal (USA)

Individual variation in pre-hibernation polyunsaturated fatty acid intake and its effect on over-winter survival by golden-mantled ground squirrels, *Spermophilus lateralis*

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The results of several controlled laboratory studies suggest that use, depth, and duration of torpor bouts in the golden-mantled ground squirrel (*Spermophilus lateralis*) are influenced by the intake of polyunsaturated fatty acids (PUFAs). However, it remains unclear if these nutritional limitations are similar for free-ranging animals. The goals of the study were as follows: 1) describe individual patterns of body condition and intake of PUFAs during the fattening period, 2) describe patterns of crude lipid and PUFA composition of plant species consumed by ground squirrels, and 3) determine if there are correlations between body condition, intake of PUFA, and over-winter survival. Animals were trapped in the White Mountains of California throughout the active season. Body condition was determined using TOBEC and blood samples were collected to determine plasma levels of PUFAs. Diet was determined by fecal analysis and behavioral observation. Plants matching those found in the diet were collected to determine fat and fatty acid composition. Lean mass of ground squirrels remained constant during the fattening period but body mass and percent body fat increased, with both lean mass and percent body fat varying between individual squirrels. Variation in body mass among juveniles was substantial, with the body mass of juvenile females being greater than that of juvenile males. Linoleic (18:2) and α -linolenic acid (18:3) were the primary PUFAs identified in plasma samples. There was a small but significant reduction in intake of 18:2 (40.9 \pm 1.9%) by adults just prior to the onset of hibernation. Circulating levels of 18:3 (4.3 \pm 0.9%) was low in most cases and absent from the plasma of all adults remaining active into September. 18:2 (44.1 \pm 6.9%) was the predominate PUFA consumed by juveniles, whereas 18:3 (0.9 \pm 0.6%) was largely absent from the diet that continue to fatten into September. These trends were not surprising since 18:2 is most abundant in seeds that were available throughout the sampling period, whereas 18:3 is an important component of chlorophyll and thus is expected to be largely unavailable in late summer and early fall as plants senesce. Intake of both 18:2 and 18:3 was greater for adult females than males, which may be associated the reduced time available for fattening in females. There were no gender differences in plasma PUFAs for juveniles. There were no significant differences in plasma PUFAs between individuals, thus any affect of PUFA intake on over-winter survival will more likely be associated with net accumulation of PUFAs and total body fat rather than concentration of PUFAs consumed. The relative concentrations of 18:2 and 18:3 in the plant most commonly consumed by ground squirrels, *Ivesia lycopodioides*, differed substantially from the relative concentrations of these fatty acids in ground squirrel plasma. Squirrels largely select for the reproductive structures of the plants, which had greater concentrations of crude lipid (7.9 \pm 5.3% DM) and both 18:2 (6.3 \pm 3.2 mg/g lipid) and 18:3 (11.7 \pm 5.3 mg/g lipid) than leaves and stems. Concentrations of 18:3 were greater than 18:2 for all parts of the plant examined, whereas concentrations of plasma 18:2 were much greater than that of 18:3. These results suggest notable intake of other items with much higher concentrations of 18:2 and lower 18:3 or plasma concentrations of 18:2 and 18:3 do not directly reflect the proportions of these PUFA's in the diet. The effect to PUFA intake over-winter survival was determined in June 2002 and will be discussed at the Symposium.

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Effect of a sub-maintenance, low-fat diet on body composition in Steller sea lions

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The decline of Steller sea lions (*Eumetopias jubatus*) in Alaska may be linked to an increased reliance on prey with low fat content, and/or periodic inadequate food intake. However, no studies have documented the effects of a sub-maintenance, low-fat diet on sea lion health or condition (percent body fat). We tested whether a sub-maintenance, low-fat diet of Atka mackerel induced greater decreases in body condition compared to a high-fat herring diet. Two juvenile female Steller sea lions (4 years old) were alternately maintained on isocaloric (total net energy/day) diets of Atka mackerel or herring. The animals were fed a sub-maintenance amount of fish, sufficient to lose a maximum of 15% of initial body mass over the 30 d trial period. As predicted for isocaloric diets, although substantial changes in body mass occurred ($-9.8 \pm 2.1\%$) there were no differences due to food type. However, total body lipids decreased an average of 6.0 kg while on an Atka mackerel diet versus 1.0 kg while eating herring. Correspondingly, lipid loss accounted for a greater proportion of the total mass loss when fed a diet of Atka mackerel (43%) than when fed herring (10%). Certain blood parameters changed in a predictable fashion when the animals were losing body mass, and some only changed on one of the diets. These parameters may prove useful for judging the nutritional status of wild sea lions. The preliminary results of our experiment indicate that a low-fat diet may compound the impact of low food availability on sea lion health.

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Do cats need arachidonic acid in the diet for reproduction?

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There is debate whether cats need arachidonic acid (AA) in the diet for reproduction. Rivers et al. (1975) suggested that cats could not convert linoleic acid (LA) to AA, and cats and lions lacked a Δ^6 desaturase. Sinclair et al. (1981) showed the presence of 20:3n-9 in the plasma of cats given an essential fatty acid-deficient (EFA-deficient) diet based on hydrogenated beef fat (HBF) and suggested an alternate pathway for AA synthesis using Δ^5 and Δ^8 desaturases. MacDonald et al. (1983) also reported that cats given HBF diet had low concentrations of 20:3n9 in plasma, and cats given a safflower oil (SO) diet had higher AA in plasma than cats given the HBF diet, but similar to cats given a SO-tuna oil diet. Male cats given the HBF diet had degeneration of the testes, but the testes of males with SO in the diet were histologically normal, but actual reproductive ability was not tested. Queens given AA-free diets with or without LA did not bear live kittens. When AA was given some of the queens completed a normal pregnancy. The authors concluded that LA met the needs for spermatogenesis, but AA was necessary for female reproduction. Pawlosky et al. (1994) demonstrated Δ^6 desaturase activity existed in cats, and the liver and brain cooperate to produce 22:6n-3 and 22:5n-6 when no preformed C20 or C22 precursors were in the diet. Pawlosky and Salem (1996) gave 3 groups of queens diets containing either 1% or 3% corn oil (CO), or 1% CO +AA. There was a high incidence of congenital defects in the kittens from the 1% CO group, 100% viability in the other 2 groups. As the diet containing 3% CO without AA supported reproduction, dietary factors other than AA appeared to be involved. Pawlosky et al (1997) gave corn oil based-diets to queens and was able to maintain 20:4n-6 concentrations in the developing brain and retina of kittens, but only those diets containing 22:6n-3 could support high concentrations of docosahexanoic acid in these tissues. Low concentrations of 22:5n-6 in the brains of CO-diet kittens suggested that kittens have a low biosynthetic capacity to produce this fatty acid and 22:6n-3. Electroretinograms of the kittens at 8 wk of age showed adverse changes in the kittens whose maternal diets were devoid of AA and C22:6n-3.

Our observations. We gave cats AA-free diets based on partially hydrogenated vegetable fat (% FA: C14:0, 0.2; C16:0, 12.9; C18:0, 11.4; C18:1, 43.3; C18:2, 29.0; C18:3, 2.2). Five male cats that received the vegetable fat diet alone for >2.5 years, were exposed to 12 queens which resulted a mean \pm sem of 5.25 ± 0.52 kittens/litter indicating that dietary AA was not necessary for reproduction in toms. Four queens that had the same dietary treatments as the toms came into estrus, mated and gained body weight, but only one successful pregnancy was completed with 3 normal kittens. The other 3 queens had 3, 1 and 1 pregnancies, but produced no viable kittens. Queens ate the majority of these kittens, but the parts found indicated the pregnancy went to full term. Our results support the conclusions of Pawlosky that reproduction in queens may be impaired not by lack of AA, but long chain n-3 fatty acids.

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Milk fat synthesis and draw down on body fat in the muskox (*Ovibos moschatus*)

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For ruminants both food supply and body condition can influence milk production and offspring growth and survival. However, there are few studies on the relative balance between *de novo* synthesis of lipid by the mammary gland from food energy and draw down (i.e. mobilization) of lipid from body fat to meet milk energy output. Since milk energy output scales allometrically with maternal body weight, it would appear that meeting energy requirements of the offspring is under strong adaptive regulation. For ruminants in Arctic systems food availability and quality as well as body fat reserves can vary annually, and often both can be scarce at parturition. Since body fat is used at higher efficiency than food metabolizable energy for both milk-lipid synthesis and also as the energy source to drive milk-protein synthesis, body fat mobilization may be more tightly regulated before food becomes dependably available. We measured milk intake by muskox calves with the $^3\text{HOH}/^2\text{HOH}$ technique (Holleman et al., 1975), determined *in vivo* maternal body fat from the body water pool size (White et al., 1989) and assayed [^3H] in milk lipids to determine the fraction of milk fat derived from the body water pool: a measure of *de novo* synthesis by the mammary gland (Assumptions: [H] in milk fat = 0.132 g/g, 53 % of H in newly synthesized fatty acid was derived from body water, Jungas 1968). All cows were grazing, and a high plane (HP) treatment involved supplementation with a high protein (18 %CP) pelleted ration given to half the group. For both high and low plane (LP) combined, mean specific activity ratio [^3H]fat-H/ [^3H]water-H was 0.39, indicating 74 % of milk lipid was synthesized *de novo* (range 57 % at wk 1-2 to 85% at wk 22). Initial output of milk fat was 150 g/d; it peaked on wk 4 at 170 g/d and declined linearly to <70 g/d at wk 22. Natural weaning occurred at approx. wk 32, but extended lactation into a 2nd y was frequently observed. Based on HP-LP differences, supplementary feed stimulated output of milk fat until wk 4-6 but was without effect at and after wk 8 of lactation. During wks 1-2 intake of the supplement was associated with a higher lipid mobilization (20 g/d) than *de novo* synthesis (5 g/d), but by wk 4 the reverse was noted; *de novo* synthesis (21g/d) exceeded lipid mobilization (9 g/d). By wk 8 no effect on *de novo* synthesis or mobilization was attributable to the supplement. An energy balance sheet for mammary lipid dynamics suggests that to drive increases in *de novo* synthesis of milk fat at 4 wk lactation, daily intake of high quality food would need to increase by 0.63 kg ($14 \text{ g/kg}^{0.75}$ for a muskox weighing 160 kg) over maintenance intake. The results lend theory to reports for sheep, reindeer and caribou that a green flush of food at or just after the time of parturition results in a higher rate of gain in offspring nursing from fat rather than thin females. It remains to be discovered if the change in importance between *de novo* synthesis and lipid mobilization that occurs during the 1st 4 wks of lactation is regulated or if it is responding stoichiometrically to current nutritional influences.

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Measuring nutrient intakes of free-ranging animals

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I describe a method for obtaining quantitative estimates of the intakes of nutrients, toxins, or other food components during foraging by free-ranging animals. I consider food components not only in the conventional sense of chemical compounds, such as riboflavin or oxalic acid, or classes of components, such as proteins or lipids, but also in a more general sense to include those properties--such as energy content, cost, foraging time, predation risk, and so forth--whose 'intakes' are linear functions either of the amounts of various foods that are ingested or of the amounts of time required to do so.

Intakes are estimated either as the mean amount of a given component taken in per day (the "daily intake") or as the rate of its intake as a function of elapsed time in foraging bouts devoted to it (the "food-specific intake rate"). To estimate mean daily intake of a nutrient or other food component, a factorial method is used, in which the number of grams of a given food component that are obtained from each food is obtained from the product of that food's mean values for its time budget, unit intake rate, unit mass, and composition. In turn, each of these factors is broken down into smaller factors that can be estimated from data that are obtainable under field conditions.

As byproducts of this method, one also obtains estimates of mean values for other salient characteristics of foraging, such as the fraction of potential feeding time spent eating a given food, as well as the number of units or of grams of that food consumed per day or per meal on that food, or per elapsed minute in bouts of feeding on it.

The applicability of this method has been demonstrated in several recent studies of foraging behavior in the baboons of Amboseli National Park, Kenya; for further details, see Altmann (1991, 1998), Altmann & Shopland (2002).

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Different dietary fat sources on broiler performance

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The purpose of the present experiment was to compare the effects of dietary fish oil (South Caspian Sprat oil (*Clupeonella grimmi*)) and poultry fat supplementation on growth performance of broiler chickens. One thousand 1-d-old broiler chicks from a commercial line (Hybro breed) were obtained from a local hatchery. Broiler chicks randomly distributed into five experimental groups (five treatment \times five replication) with 40 birds/replicate). Chicks were given one of the five experimental diets containing 3 or 6% South Caspian Sprat oil, 3 or 6% poultry fat and a control group with no fat diet. Wood shavings were used as litter and the lighting regimen provided 23 h of light per 24 h throughout the rearing period. Water and food were provided ad libitum. Temperature was set at 34°C during the first wk and gradually reduced by 2°C per wk down to 22°C. Broiler chickens were fed a basal diet containing 3000 kcal ME/Kg and 19% crude protein. The control diet was supplied for all experimental groups from the 1st day till two wk of age, but from 2 wk of age onwards, the treatment groups received the supplemented diets. At 2, 4 and 6 wk of age, live body weight was obtained on a pen basis, and feed intake per pen was recorded for the previous biweekly period. Mortality was recorded daily. At 6 wk of age, 10 birds of each experimental group were killed by cutting the jugular vein and abdominal fat were dissected and weighed. At 2 wk of age, broilers fed control diet during the starter period (1 to 2 wk) no significant differences in weight gain were found between experimental groups. At 6 wk of age, broilers fed poultry fat treated diet, realized the highest body weight gain, but these was significant ($P < 0.05$) only in comparison with birds fed diet with 6% fish oil. During the starter period (1 to 2 wk) no significant differences in feed intake were found between experimental groups. But cumulative feed intake averaged lower ($P < 0.05$) for chickens fed diet with 6% fish oil compared to other dietary groups. Cumulative feed conversion (kg feed intake per kg body weight gain) did not differ among treatments. Abdominal fat pad weight were significantly ($P < 0.05$) increased in chickens fed diet treated with poultry fat. In conclusion, the results of this experiment indicate that broiler diet supplemented with 3% fish oil (*Clupeonella grimmi*) is associated with a reduction in abdominal fat deposition and also had no adverse effect on growth performance. Furthermore, using moderate amount of fish oil in broiler diet may causes a change in the fatty acid composition of deposited fat in favor of unsaturated fatty acids.

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Fatty acid composition of plasma and red cells in a group of captive asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants

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When wild animals are brought into or kept in captivity, the result is generally a reduction in the content of polyunsaturated fatty acids (PUFA) and an increase in the n-6:n-3 fatty acid ratio. In theory, these shifts could have consequences on a variety of body functions. We investigated, for the first time, the FA composition of plasma and red blood cells of captive elephants, using a group of four Asian and two African female, adult animals. All animals originated from the same facility and received the same diet.

A comparison with data on free-ranging or camp elephants showed that the captive animals had lower proportions of PUFA, and for several lipid fractions a higher n-6:n-3 ratio. The difference in PUFA content seemed to be less drastic in African than in Asian elephants, suggesting that free-ranging African elephants might face situations of marginal PUFA supply, as previously proposed in the literature. The pattern found by other researchers in a small sample of elephant spermatozoa, that captive Asian elephants tend to have lower levels of n-3 and total unsaturated FA, prevailed in our small sample as well. In our case, differences due to different dietary regimes are unlikely. Reported differences in digestive strategy between the elephant species might account for this observed discrepancy.

Among the potential consequences of a low PUFA provision, the viability of sperm for artificial insemination practices, warrants particular attention in the future.

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Case report:**Effects of extruded linseed supplementation on the milk fatty acids pattern of dairy ewes**

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Conjugated linoleic acids (CLA), having positive human health effects, are specifically synthesised by ruminant. Numerous studies have demonstrated that cow's milk could be the most important source of CLA. Depending on the type of feed ingested, cow's milk can also be a valuable supplier of omega-3 (ω 3) fatty acids for human. To our knowledge, similar information on milk composition of dairy sheep is very scarce.

The aim of the experiment described there was to evaluate the influence of extruded linseed added to the diet on the milk fatty acids pattern of dairy ewes, particularly on CLA and ω 3 concentrations.

The experiment, divided into two periods of three weeks, was conducted on a 80 dairy ewes flock splitted in two comparable groups (23 Belgian Milk Scheep and 17 Lacaune). Each period included 16 days of adaptation to the diet followed by 5 days of milk tank sampling. 10% of the concentrate included in the experimental diet (E) was composed by extruded linseed. This feedstuff was replaced by copra cake in the control diet (C). After the first period, diets were permuted between groups. Whatever the diet, each sheep received daily 2.2 kg of concentrate and had a free access to hay.

Extruded linseed supplementation to ewes induced significant increases of CLA, ω 3, monounsaturated fatty acids and polyunsaturated fatty acids concentrations in sheep milk, respectively + 98% (1.17 vs 0.59)*, + 147% (1.51 vs 0.61)*, +32% (23.55 vs 17.90)* and +86% (5.97 vs 3.22)*. Concomitantly, we observed a 11%-decrease in saturated fatty acids levels (70.47 vs 78.88)* and a 54%-increase in the stearic acid concentration (10.22 vs 6.65)*. A 39%-reduction in the ω 6/ ω 3 ratio (1.81 vs 2.96) was also observed. Similarly to cow's milk, the major isomer of CLA in the sheep milk was *9-cis, 11 trans* (81.49 and 84.92% of total CLA for E and C, respectively). For all fatty acids measured, including CLA, differences between E and C were highly significant ($p < 0.05$).

Daily milk productions were not statistically different between diets ($p = 0.166$) and reached respectively 2.00 and 2.05 kg of milk/ewe/day for E and C. Diets had no effect on milk fat content ($p = 0.822$), which reached respectively 6.42 and 6.46 g/100 g of milk for E and C. Nevertheless, E induced a higher milk protein content than C (5.36 vs 5.20 g/100 of milk). These observations are in contradiction with results of previous studies showing a reduction in milk fat (Griinari et al., 1998 ; Chouinard et al., 1999) and in milk protein with high polyunsaturated fatty acids in cow diet (Focant et al., 1998).

This experiment was carried out under a project financed by « le Ministre Wallon de l'Agriculture et de la Ruralité, Direction Générale de l'Agriculture ».

We deeply appreciate the contribution and helpfulness of Pierre Artoisenet for flock management.

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* Data were expressed as % of total fatty acids. Comparisons were made between E and C diets.

minerals

Chairperson : Geert Janssens (Belgium)

Black tea consumption, iron status and risk for cardiovascular disease

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Several studies have demonstrated that black tea consumption decreases iron absorption. Tea consumption also has been inconsistently associated with a reduced risk for cardiovascular disease in human epidemiologic research. These observations on risk for cardiovascular disease also may be linked to iron status, or to other mechanisms. Despite the studies investigating the effect of tea on iron absorption, there are few data on the effect of tea on measures of iron status. As part of a dietary intervention study, 15 individuals (*Homo sapiens*) were fed a diet of fixed composition and three beverages: 1) black tea, 2) flavored water with caffeine (to match the caffeine content of the tea) and 3) flavored water without caffeine. The placebo beverages were similar to the tea with respect to color and taste. Each individual consumed five daily servings of each treatment beverage during three separate three-week periods, in a randomized crossover design study. The effects tea on iron status and blood lipids was measured at the end of each period. There was no effect of treatment on concentration ceruloplasmin or transferrin, and no effect on red blood cell count, white blood cell count, erythrocyte indices, hematocrit, and differential blood count. However, consumption of tea resulted in a 10.3 percent decrease in LDL cholesterol compared to placebo beverage having caffeine at the same concentration as in the tea. Triacylglyceride and HDL cholesterol were not different among treatments.

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The role of ferritin in iron storage in birds

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Iron overload is a condition which is found commonly in certain species of birds, such as mynahs and toucans (for a review see Cork, 2000). Species susceptible to developing iron overload generally have a diet consisting predominantly of insects and/or fruits, which are food sources with a low iron content (4). Given these facts the storage problem is likely to have a species broad genetic background, in which the regulation of the iron intake in the proximal intestine, where iron is normally absorbed and transferred to the body plays a central role. The possibility of an altered modulation and/or regulation of iron absorption, by a so-called 'mucosal block' was proposed and later confirmed in experimental research (2,3).

One of the proteins or iron responsive elements (IREs) suggested to function in the mucosal block is ferritin (ftn). As an iron storage protein ftn and its degradation product hemosiderin function as the most important iron binders in liver cells. The aim of this study was to find quantitative and/or qualitative differences in ftn in intestinal mucosa cells and liver in different species that could lead to a better understanding of the role of ftn in iron storage in birds.

In this study ftn, Fe, iron binding capacity (IBC) and total IBC were quantified in intestinal mucosa and liver of the following avian species: chicken (*G. domesticus*), turtledove (*Str. d. decaocto*), hill mynah (*G. religiosa*) and river mynah (*Acridotheres t. tristis*). Liver ftn was isolated in the same species as well as in pigeon (*Columba livia*), a sulphur-breasted toucan (*Ramphastos vitellinus*) and a common trumpeter (*Psophia crepitans*, fam. Psophiidae). These ftns were characterized for quantity ($\mu\text{g}/\text{mg}$ protein), molecular weight (mw in kDa), ratio and mw of the L- and H-subunits and purity (%).

The results show significant differences between ftns of birds in amount, iron binding characteristics, mw's and in composition of ftn (H/L ratio), both in liver and mucosal cells. The most important conclusions to be drawn from our results are 1. There is no difference in the amount of ftn ($\mu\text{g}/\text{mg}$ protein) in the intestinal mucosa cells between the doves and mynah species. The chicken has a very high amount of ftn in the mucosal cells. 2. Toucan, trumpeter and hill mynah show high amounts of ftn in the liver compared to chicken, dove and pigeon, but the river mynah had less than 5% of the amount in the hill mynah. 3. The TIBC of ftn in mynahs is twice the capacity of dove and chicken both in mucosa and liver cells. The average Fe saturation of ftn in the mynah livers was 71-74%, in dove and chicken 41-42%. In the intestinal mucosal cells the saturation was not different (average 58-75%). 4. There was a large difference in mw of liver ftns between chicken, dove, pigeon (369,390,393 kDa) and hill mynah, toucan, trumpeter (492, 494, 471 kDa). This is a reflection of the difference in H/L ratio (0.33, 0.25, 0.33) and (0.10, 0.08, 0.10), respectively. In the mucosa cells the H/L ratio was 5.0, 3.0 for chicken and dove and 1.5, 0.35 for river- and hill mynah

The possible role of ftn in iron storage will be discussed in the context of earlier research (3).

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Biotin is the first limiting nutrient for the growth of salmonella in chickens and iron is second limiting.

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In mammals, iron is considered to be the “first limiting” nutrient for the growth of most pathogenic microbes in body fluids due to its tight chelation by transferrin and lactoferrin. Birds lack the mammalian functional equivalent of lactoferrin and previous results in chickens indicated that the addition of iron did not impair the bacteriostatic properties of serum. It is possible that other nutrients might become limiting for growth of pathogens in avian body fluids. Avidin has an extraordinary high dissociation constant for biotin of 10^{-15} and is found in birds, but not mammals. This protein has the highest non-covalent binding affinity found in nature. Plasma biotin concentrations are markedly elevated during the acute phase response of chickens to bacterial challenges. Biotin is required for the growth of many microbial pathogens of birds, including species of *Candida*, *Listeria*, *Staphylococcus*, *Clostridia*, *Streptococcus*, *Salmonella*, *E. coli*, and eucaryotic parasites. To evaluate the role of avidin in limiting the growth of the chicken pathogen *Salmonella typhimurium* by withholding biotin a series of in vitro and in vivo experiments were conducted. Macrophages isolated from the peritoneal cavity and stimulated with *S. typhimurium* LPS expressed avidin mRNA and their conditioned medium inhibited the growth of *S. typhimurium*. This inhibition was reversed by the addition of biotin. Serum from chickens undergoing an acute phase response to *S. typhimurium* LPS inhibited *S. typhimurium* growth and this bacteriostatic property was reversed by biotin. The addition of iron did not increase the proliferation of *S. typhimurium* in chicken acute phase serum in the absence of biotin. However, iron significantly increased proliferation in the presence of biotin. These results indicate that biotin is the first limiting nutrient for the growth of *S. typhimurium* in the blood of chickens and iron may be second limiting.

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A process-based model to estimate air emissions from animal feeding operations

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Over recent decades in the United States, more livestock production has occurred on fewer farms. Between 1982 and 1997, animal feeding operations (AFOs) decreased by 51 percent while livestock production increased 10 percent because of increasing production on these fewer farms. During this time, geographic concentration of livestock production also has occurred. This type of geographic concentration has placed increasing pressure upon the U.S. Environmental Protection Agency (EPA) to regulate air emissions from AFOs because of odor and health concerns.

In 2002, the U.S. National Research Council (NRC) convened a *Committee on Air Emissions from Animal Feeding Operations* to conduct a scientific assessment for estimating air emissions of various pollutants (hydrogen sulfide, ammonia, odor, volatile organic compounds, particulate matter, nitric oxide, and nitrous oxide) from AFOs. The study included a review of the model farm construct proposed by EPA to estimate air emissions at individual AFOs. The committee suggested a process-based model farm approach that builds upon EPA's model farm construct using mathematical modeling and experimental data to simulate conversion and transfer of reactants and products through the farm enterprise. This process-based model farm approach would incorporate mathematical modeling to represent the interactions among the system components.

In this paper, the process-based model farm approach recommended by the NRC committee was examined utilizing data from a case-study dairy farm in New York State with a whole-farm nitrogen mass balance. The EPA model farm construct estimated nitrogen air emissions to be 22 300 kg per year while the process-based model farm approach predicted 30 100 kg. A sensitivity analysis was performed with the process-based model examining effects of precision feeding, liquid manure storage covering, land application with soil incorporation, and combinations of all three.

Feeding amino acids has been shown to decrease nitrogen excretion from dairy cattle up to 26,0 percent. With simulating use of this technology on the case study farm utilizing the process-based model farm approach, nitrogen air emissions were predicted to decrease by 22,3 percent. Covering liquid manure storage system can reduce ammonia emissions by 80 percent for swine effluent. If equally applicable to dairy effluent, predicted nitrogen air emissions for the case study farm were reduced by 16,4 percent. However, nitrogen leaching under this scenario would increase by 1 300 kg per year. Various combinations of these technologies were predicted to reduce nitrogen air emissions from the case study farm by up to 74,5 percent.

Model farms may be a useful device for describing groups of AFOs with similar production and management practices by a single well-defined farm. A process-based model farm approach (NRC, 2002) builds on EPA's model farm construct by using mathematical modeling, experimental data, and mass balance constraints to simulate conversion and transfer of reactants and products through the farm enterprise. Incorporation of water quality models into a process-based approach may provide the potential water quality impacts of different air quality management practices.

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Dietary considerations for iron storage disease in birds and implications for high vitamin A contents of formulated bird foods.

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Iron storage disease (ISD) is problematic for many frugivorous and insectivorous birds maintained on commercially formulated foods. High dietary iron has been implicated as a possible cause in the promotion of iron storage with recommendations for dietary levels of iron below 100 mg/kg, preferably below 25 mg/kg. Various other factors have been considered in the development of this disease, including genetic predisposition, immunological stress, viruses and nutrition. Nutritional aspects include enhancement of iron uptake by high levels of vitamin C and saturated fats, and an increase in lipid peroxidation by low levels of vitamin E (exacerbated by an excess of vitamin A).

While plant products contain provitamin A in the form of carotenoids (the conversion of which is regulated by the body) and vitamin A levels are negligible in insects, commercial foods for psittacines are generally high in vitamin A (Kaytee, 12 IU/g; Mazuri, 8.33 IU/g; Nutribird, 12 IU/g; Pretty Bird, 17 IU/g, Roudybush, 8.75 IU/g) when compared with poultry requirements of 1.5-4.0 IU/g, with few exceptions (HBD International, 1.44 IU/g). Studies of psittacines maintained on diets low in preformed vitamin A, indicate a significant increase in productivity and a decrease in the incidence of ISD in these birds. Some softbill aviculturalists also use cheap dog food low in preformed vitamin A, without any obvious negative affects.

In contrast to vitamin A, the presence of carotenoids in microsomal membranes partially inhibits the loss of α -tocopherol, especially during the late phase of oxidative stress, with β -carotene decreasing phospholipid hydroperoxide production. However, astaxanthin is two-fold more effective than β -carotene at inhibiting the production of lipid peroxidases and β -carotene also increases the absorption of iron. Canthaxanthin is another carotenoid compound that is supplemented to promote feather pigmentation in birds such as flamingos and scarlet ibis. However, canthaxanthin alters tocopherol status and decreases glutathione peroxidase, which functions together with the peptide glutathione to protect cells against the destructive effects of hydrogen peroxide. Dietary supplementation of canthaxanthin in flamingos may be implicated in the development of ISD.

It is possible that the high levels of vitamin A (retinyl palmitate esters) in these commercial products are contributing to iron storage disease in birds and replacement of vitamin A with specific carotenoids may be warranted. Given the differences in action of carotenoids such as β -carotene, astaxanthin and canthaxanthin, it is crucial that dietary requirements of birds for specific carotenoids are identified. It is evident that further research on this topic is required.

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Observations regarding the capacity of selenium to intensify the activity of vitamin E in chickens encephalomalacia

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Encephalomalacia is a disease with a controversy about its etiopathogenesis that still exists at present.

In order to establish whether Sodium Selenite is efficient or not in controlling encephalomalacia, we decided to follow several aspects of its efficiency insisting on the capacity to intensify vitamin E activity.

The study developed in an „*industrial type*” holding for poultry breeding where encephalomalacia involves 5%.

The diagnosis was established considering clinical, paraclinical and morphopathological investigations. The curative value of Sodium Selenite was searched in comparison with vitamin E and AD E vitamins curative value. The investigations were carried-out on 5 lots of chickens presenting incipient clinical manifestations of encephalomalacia considered reversible.

Analysing the results obtained we draw the conclusion that the curative value of Sodium Selenite is reduced, being only 11,5%.

Sodium Selenite has the capacity to intensify the activity of vitamin E and AD E vitamins; its curative value is negligible when it is used by itself.

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Diet and diet-related diseases in captive short-tailed leaf-nosed bats (*Carollia perspicillata*)

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The short-tailed leaf-nosed bat (*Carollia perspicillata*) is a small frugivorous bat whose home range stretches from southern Mexico to north eastern Brazil. It is also found on some of the Caribbean islands. In 1995 a small colony of approximately 50 individuals was established in Artis Zoo. Although there was reproductive success in this colony, mortality prevented the population from growing. Post mortem results from the Department of Veterinary Pathology of Utrecht University revealed insufficient calcification in the bones. Therefore the calcium content of the diet was increased. This seemed to be successful but a new problem occurred; post mortem examinations conducted after the diet change showed iron accumulation in liver cells. Since the main health problems seemed to be diet related, a study into the captive nutrition of *Carollia perspicillata* was started.

The nutritional content of the existing Artis diet was calculated with Zootrition™ dietary management software. At the same time, samples from the diet were analysed for crude protein, crude fat, crude fibre, calcium and phosphorous in a certified laboratory. The iron content of the diet was also analysed. To obtain reference values, an extensive survey was conducted among other zoos with these specific bats. In addition, literature on in-situ nutrition of *Carollia perspicillata* and nutrition of specialised frugivorous species was reviewed.

Results demonstrated a high iron content in the Artis diet. Data from Zootrition™ calculated an iron content of 139mg/kg dry matter. The laboratory results showed an even higher content of 177-mg/kg dry matter. Both figures are high above the recommended values proposed by Schoemaker and Beynen (2001) in their study on the diet composition of commercial diets for the highly frugivorous mynah birds (*Gracula spp.*). However the most important observation in this study was the fact that calcium and mineral supplements together with concentrates contributed more than 80 % to the total iron content of the diet in Artis. Similar results were found at other zoos that contributed to the survey.

Based on these findings, it is essential to check carefully the potential iron contribution of supplements and concentrates in diets for frugivorous animals. In this particular case the supposedly problem-solving calcium supplement caused a new diet related problem.

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