# 10th EUROPEAN ZOO NUTRITION CONFERENCE

17-20th January 2019

EUROPEAN ASSOCIATION OF ZOOS AND AQUARIA
EAZA

Marwell Zoo

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## Foreword

Dear friends and colleagues,

We are delighted to present you the 10th European Zoo Nutrition Conference in Marwell Zoo, United Kingdom. Whilst the second ever nutrition conference was officially to be held at Marwell Zoo back in 2001, this had to be cancelled at the last minute due to foot and mouth, so Marwell is very happy to have this opportunity to host again under (hopefully) better circumstances!

The organising committee is pleased to present you a wide range of talks and posters, varying from the role of Vitamin D, anteater nutrition, browse provision, nutrition as preventative medicine, faecal consistency, obesity management, effects of diet on behaviour and keeper opinions on feeding regimes.
Prior to the conference, the EAZA Academy in cooperation with the EAZA Nutrition Group (ENG), organised a workshop on January 17th entitled: Meat and fish quality, nutrition and feed presentation. In addition to this, during the conference we will have several workstations during the zoo visit on Saturday, where information will be presented on specific zoo animal nutrition items. After this there will be the opportunity to go on zoo tours which will be led by the keepers, or to explore the collection by yourself.

We gratefully acknowledge financial support via sponsorship by Arie Blok – Kasper Faunafood, Granovit Zoofeed, Kiezebrink International, Lucerne for Browsers – Itchen Valley, Mazuri Zoofoods, Saint Laurent SAS, Versele-Laga NV and Zooprofis. Their financial help, similar to their products, supported the ENG and present conference. We kindly invite you to visit their stand, talk to the representatives, consult their websites and listen to their sponsor pitches.

Preparing a conference takes a long time, a lot of conversations, both in person and digitally, as well as some manual labour. Many institutions and persons are involved: The organizing committee would like to thank the director of Marwell Zoo and the Marwell Hotel for hosting this conference. Many thanks to the EAZA Executive office and especially to Mirko Marseille for coordinating the financial part of the conference, processing registrations as well as for regularly updating the conference website and taking responsibility of announcements. And a big thank you to the organizing committee of this conference especially to Anouk Fens for coordinating sponsor communications and assisting with all general tasks, Joeke Nijboer for his efforts towards the workshop and Marcus Clauss for coordinating the program.

And finally, on behalf of the ENG I am pleased to welcome all delegates. We hope you will enjoy the conference!

Ollie Szyszka
Marwell Zoo
Chair EAZA Nutrition Group

Marwell Zoo, January 2019

**Organizing Committee**

Marcus Clauss, University of Zurich,

Anouk Fens, Apenheul Primate Park

Joeke Nijboer, Nijboer Consultancy

Ollie Szyszka, Marwell Zoo

**Acknowledgements to our sponsors**

We wish to recognize and thank the following companies which have contributed to the success of this 8th European Zoo Nutrition Conference:

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## Resource Index

**Mineral / Vitamin Supplements**



**Feeding equipment / Feeding enrichment tools**


ZOO
SPECIALTIES


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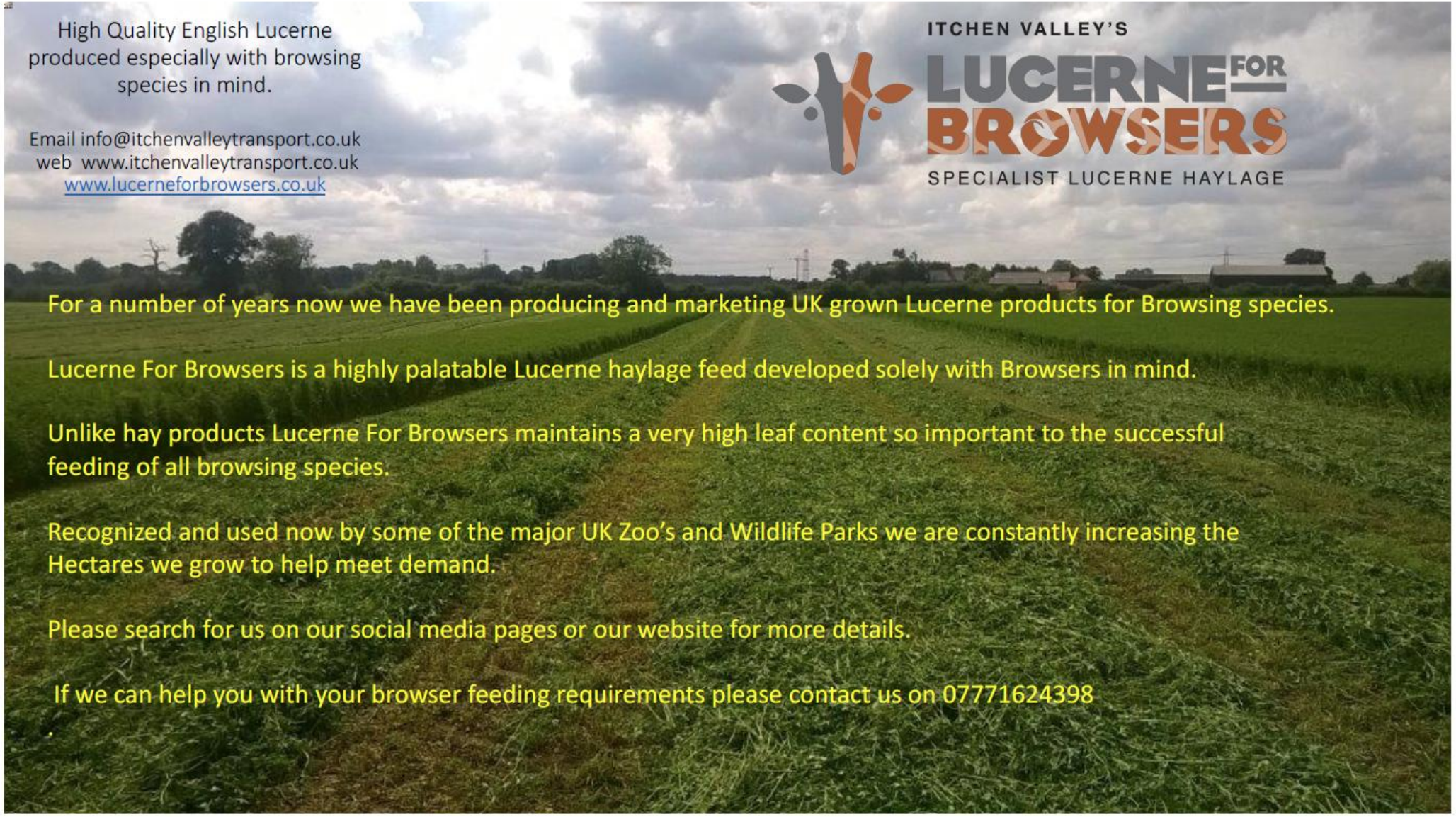
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**THURSDAY 17TH January**

EAZA Academy Workshop:
*Meat and Fish Quality, nutrition and feed presentation*
*Location: Marwell Zoo*
*Facilitators: Richard Chivers, Dr. Len Lipman, Dr. Annette Liesegang, Dr. Marcus Clauss*

08.15/30 Arrival at Marwell Zoo.
Train departures to collect delegates from the main entrance at 8.15 and 8.30

09.00 **General introduction** – *Marcus Clauss*

09.15 **Fish Quality; factors affecting quality and quality assessment** – *Richard Chivers*

10.15 **Practical element – assessing fish quality** – *Richard Chivers*

10.50 Break (to be rotated around the practical element)

11.20 **Meat Quality: Factors affecting quality and quality assessment** – *Len Lipman*

12.20 **Practical element – assessing meat quality** – *Len Lipman / Joeke Nijboer*

13.00 Lunch Break (to be rotated around the practical element)

13.45 **Meat and fish Nutrition** – *Annette Liesegang*

14.45 Break

15.20 **Feed presentation** – *Marcus Clauss*

16.20 Workshop finished

16.30 Train departures to collect delegates to the main entrance

European Zoo Nutirition Conference
18.00 Registration & Icebreaker – Marwell Hotel (until 20.00)

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08.00 Registration – Marwell Hotel

08.20 Zoo entry – Marwell Zoo

08.30 Welcome and organisation for zoo visit

09.00 Zoo visit - practical workstations
- Body condition scoring
- Conducting a diet change
- Forage sampling / interpreting analysis
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11.00 Break

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18:30 Conference Dinner – Marwell Hotel

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**POSTER PRESENTATIONS** **Page**

*Pitch II:*

**Vitamin D for Health in Humans & Animals – the Sunshine Superstar or just Media Hype!**

*Susan Lanham-New*[1]

[1]*Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, GU2 7XH. Surrey. UK*
*Correspondence email: s.lanham-new@surrey.ac.uk*

Vitamin D is a fundamentally critical nutrient that the humans and animals require to function properly. It plays an important role in musculoskeletal health due to its involvement in the regulation of calcium and phosphorus and should be considered a ‚One-Health' nutrient. Having a low level of vitamin D (called vitamin D deficiency) in the body is not good for a wide range of health outcomes - in humans these include risk of osteoporotic and stress fractures, risk of cardiovascular disease and some cancers, and lowering of capability of the immune system. Vitamin D is an unusual nutrient; it is not a *vital* - amine in the true sense of the word but rather a pro-hormone where the main source of vitamin D is ultra-violet exposure, not dietary intake. Humans and animals alike produce vitamin D in this way, with 7d-hydrocholesterol being critical in our production of vitamin D. Interesting, there are two forms of vitamin D – vitamin $D_2$ and vitamin $D_3$ – are both metabolised into 25-hydroxyvitamin D (25OHD) in the liver, which is the marker of vitamin D status. 25OHD is then transported to the kidney via the serum, bound to vitamin D binding protein (VDBP) where 25OHD is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which is the active form of vitamin D and which is involved in so many key health outcomes. Vitamin D deficiency is a real problem world-wide, especially amongst older people and ethnic minority groups and many animals have vitamin D deficiency. The new publication from the UK Government's Public Health England Department recommends that our vitamin D intake should be 10 mcg per day and this recommendation compares well (albeit lower) with other vitamin D proposals world-wide. Few countries however have a *specific* vitamin D policy for populations and there is no consensus on vitamin D requirements in animals and this is an area for further urgent research. Our collaborative work at Surrey with: 1) the food industry has been focused on how the food we eat can be fortified with vitamin D and whether the plant or animal source of vitamin D is the best form for increasing our vitamin D levels in the body; 2) with Ministry of Defence has shown how critical vitamin D is to health outcomes in different population groups and what potential vitamin D strategies can be for increasing intake; 3) with Universities worldwide to try to solve the global issue of vitamin D deficiency in a sustainable, effective and innovative way. Collaborative interfaces between academia, government and industry, including a number of LMICs are vital in our finding long-term solutions to this global issue of vitamin D deficiency and we need to urgently look at the approaches we have taken in humans and adapt these to the animal model.

*KEYWORDS: Vitamin D, UVB exposure, diet, deficiency, fracture*

**UVB and Vitamin D3: If all our animals need full spectrum lighting, how can we provide it?**

*Frances M Baines*

*UV Guide UK, Greenfield, School Lane, Govilon, Abergavenny, Monmouthshire, Wales, UK NP7 9NT*
*Correspondence email: fbaines@uvguide.co.uk*

Vitamin $D_3$ has a vital endocrine function - maintaining calcium homeostasis - but recent research has revealed it as a potent regulator of gene transcription, too, with particularly important effects on the immune system (Hossein-Nezhad and Holick, 2013). Very few natural diets contain more than traces of vitamin $D_3$; it seems likely that most wild vertebrates which live on the surface of the planet rely upon cutaneous synthesis, enabled by exposure to the UVB in natural daylight. Physiological and behavioural adaptations ensure that ambient light levels in the microhabitat are sufficient for optimal synthesis.

When zoos were first established towards the end of the 19$^{th}$ century, horrific losses due to "rickets" were common across all taxa imported from sunnier climes. It was discovered that adding vitamin $D_3$ to the diet was largely preventative. This method of supplementation, although un-natural, is still widely used today – despite the fact that we still know very little about the actual daily vitamin $D_3$ requirements of any exotic animals.

However, recent research has greatly expanded our knowledge of vitamin D physiology. The benefits of full spectrum light are now known to extend much further than the prevention of metabolic bone disease, and indeed, further than just the enabling of vitamin $D_3$ synthesis in the skin. It is already widely accepted that most, and possibly all, reptiles and amphibians kept in captivity benefit from species-appropriate levels of true full spectrum lighting, as a replacement for sunlight. However, the idea that all other animals – birds, mammals, even invertebrates - housed indoors might benefit from specialist lighting including UVB is still very new and controversial.

We now have extremely good, readily available, full spectrum lighting solutions which can provide UVB equivalent to that found in natural sunlight, plus good levels of visible light and short-wavelength infrared. Provision of effective lighting for almost anything, from a tiny dartfrog vivarium to a huge primate enclosure, is now possible. With metabolic bone disease still a serious problem across all taxa, the questions we need to answer now are "how much UV does this animal need?" and "how can we supply it?"

Species vary widely in their native microhabitats, and hence their lighting requirements in captivity. It is possible that a method devised for estimating suitable UV levels for reptiles (Baines et al., 2016) may prove useful, and facilitate trials of full spectrum lighting with other taxa. I conclude this presentation by reviewing the use of some full spectrum lighting systems for reptiles, amphibians, mammals and birds, considering the benefits of different lighting configurations, and illustrating their use in several UK zoos.

*KEYWORDS: Vitamin D3, UVB, Metabolic bone disease, MBD, UV, lighting*

*Hossein-Nezhad, A. & Holick, M. F. (2013) Vitamin D for health: a global perspective. In Mayo Clinic Proceedings 2013 Jul 1 (Vol. 88, No. 7, pp. 720-755). Elsevier.*

*Baines, F., Chattell, J., Dale, J., Garrick, D., Gill, I., Goetz, M., Skelton, T. & Swatman, M. (2016) How much UV-B does my reptile need? The UV-Tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity. Journal of Zoo and Aquarium Research 4, 42-63.*

**Factors associated with vitamin D status in primates**

*Geert P.J. Janssens[1], Romy van Noije[1], Andrea Brenes Soto[1], Christine Kaandorp[2]*

*[1]Faculty of Veterinary Medicine, Ghent University [2]GaiaZoo, Kerkrade, The Netherlands*
*Correspondence email: geert.janssens@ugent.be*

Although vitamin D has always been considered as an important vitamin, its main function has long been narrowed down to its role in calcium homeostasis. Yet, vitamin D serves many other life functions, such as immune competence and even mental health. Animals get their vitamin D either through dietary intake or via synthesis in the skin during UV-exposure. It is known that large inter-species differences exist in the ability to acquire vitamin D through either the dietary or the dermal route. When keeping primates in zoos in countries with low UV-exposure and/or behind glass, the exposure to natural UV-radiation may be too low to guarantee normal dermal synthesis. A way to overcome deficiency in such cases would be the dietary supplementation of vitamin D, but little is known about the efficacy of that route across primate species. Northern zoos regularly report cases of primates showing potential signs of vitamin D deficiency, especially in winter, such as depression and muscle pains.

In this study, 157 results from serum 25-hydroxy-vitamin $D_3$ (25-OH-$D_3$)values were collected from European zoos and African sanctuaries from data of clinical records, as analysed by various laboratories in the course of clinical evaluations of the individuals at their respective collections. From each individual, this information was gathered as well: species, skin colour (1=light, 2= intermediate, 3=dark), season of sampling, geographical location, and vitamin $D_3$ supplementation. The main species represented in the dataset were chimpanzees and gorillas, but primates from Old and New World were present. Data were analysed with ANOVA.

Remarkably, the serum 25-OH-$D_3$ concentrations were even lower in the animals receiving vitamin D supplements ($P$=0.002). This could be explained by the fact that only darker-skinned primates (score 2 or 3) were supplemented, whereas skin colour showed a strong association with vitamin D status: primates with pale skin (n=16) had serum concentrations of 109 ± 60 nmol/l, whereas this was (n=72) 62 ± 30 nmol/l for intermediate skin colour (N=72) and 39 ± 37 nmol/l for dark-skinned primates (n=52) ($P$<0.001). There was a strong confounding of light skin (score 1) with New world species, so that interpretation needs to be taken with care since those species usually get vitamin $D_3$ enriched pellets, but within Old World primates, skin colour was still associated with vitamin D status.

Independent of skin colour, summer and spring values were higher than those in winter ($P$<0.001). When comparing 25-OH-$D_3$ titres of the European chimpanzees with counterparts in African sanctuaries, the latter (n=14) had higher values (118±47 nmol/l) than those in European zoos (n=62; 65±29 nmol/l) ($P$<0.001).

Although a number of factors may have been confounded in the collected dataset, the results suggest that additional UV-radiation may be necessary to guarantee adequate vitamin D status in primates kept in zoos with low UV exposure.

*KEYWORDS: Chimpanzees – cholecalciferol – UV-exposure*

**Searching for potential nutritional causes of heart disease in apes - Micronutrient analysis in captive chimpanzees (*Pan troglodytes*)**

*Matyas Liptovszky[1], Sophie Moittie[1,2], Phillipa Dobbs[1], Kerstin Baiker[2], Kate White[2], Devan Raindi[3], Prof Wilhelm Stahl[4], Melissa Grant[3]*

*[1]Twycross Zoo, United Kingdom; [2]University of Nottingham, School of Veterinary Medicine and Science, United Kingdom; [3]University of Birmingham, School of Dentistry, United Kingdom; [4]Heinrich-Heine-Universität, Institute of Biochemistry and Molecular Biology I, Germany*
*Correspondence email: matyas.liptovszky@twycrosszoo.org*

Heart diseases are one of the most frequent causes of death in both great apes and humans, with a limited understanding of the causes. Though this can represent age related changes, it is known that myocardial fibrosis was found in zoo housed great apes as young as 10 years of age. Nutrition is a well-known factor in human cardiac disease, but hardly any knowledge has been gained so far about great apes in this regard. Also, significant differences have been identified between humans and great apes suffering from cardiac diseases. The aim of this study was to collect preliminary data regarding selected micronutrients, which might have an effect on heart health in captive chimpanzees.
Vitamin A (retinol) and selected carotenoids (lutein, zeaxanthin, cryptoxanthin, total lycopene, α-carotene, ß-carotene) were analysed in a group of 12 chimpanzees (n=12) housed in a UK zoo. Vitamin D (25-OH-vit$D_2$, 25-OH-vit$D_3$ and total 25-OH-vitD) was analysed in 22 chimpanzees (n=37); while vitamin E (alpha-tocopherol) and selenium were analysed in 18 animals (n=29). The animals were aged between 10-54 years. The above nutrients are known to affect cardiac health in humans or other animal species through various metabolic routes; therefore, they were selected for analysis as part of a European wide collaborative effort to study great ape cardiac diseases by the Ape Heart Project, based at Twycross Zoo.
Vitamin A was found in a range that overlapped with, but was much wider than, the human reference range. Vitamin E and selenium were also found to be within or close to the human reference range. Vitamin D showed a great variability, and significant differences were found between winter versus summer values, which is similar to previous studies in Northern and Western European human populations. Most abundant were the levels of lutein and zeaxanthin. The levels were lower than in the human reference range, but similar to previously published results in other primate species (Snodderly et al 1990). Lycopene, α- and β-carotene were only found in traces. Age was not associated with the levels of any of the above nutrients in this group.
Videan et al. (2009) suggested that due to the species difference in longevity, chimpanzees have decreased levels of antioxidants compared to humans of equal age, which might result in earlier increased oxidative stress and increased risk for cardiovascular diseases in chimpanzees. Our data support their findings. Vitamin D is also increasingly associated with cardiac and other diseases in humans and our results proved that at least some animals in some parts of the year are vitamin D deficient in a UK zoo if we would use the established human minimum levels. However it is important to emphasise that no normal levels of vitamins or carotenoids are established in wild great apes, therefore we need to be careful comparing these results. We encourage further research in this field to help better understand the effect of nutrition on great ape health. Studies in wild great apes would be of great value to establish normal ranges for these micronutrients, while regular screening of the captive population would give us comparative data regarding different husbandry and nutritional practices.

KEYWORDS: *chimpanzee, great ape, vitamins, carotenoids, heart disease*

*Snodderly et al. (1990) Plasma carotenoids of monkeys (Macaca fascicularis and Saimiri sciureus) fed a nonpurified diet. The Journal of nutrition, 120(12), pp.1663-1671.*

**Feeding practices in insectivorous mammal species**

*Kerry Hunt*

*University Centre Sparsholt*
*Correspondence email: kerry.hunt@sparsholt.ac.uk*

The diets of captive insectivorous mammal species have been of some research interest in recent years, however we are still lacking in knowledge when it comes to the nutritional needs of many of these species. So far, the only published research on wild diets for myrmecophage species is on tamandua (*Tamandua tetradactyla*) and this research is over 20 years old. This lack of knowledge on other myrmecophage species nutritional profiles has led to numerous nutrition focused health issues including diarrhoea, vitamin K deficiencies, obesity and diabetes.

Diets vary widely between collections from almost exclusively termant to those that produce their own mixture of meat, fruit and dog or cat biscuits. Whilst complete diets, like Termant, have been produced and are currently widely utilised in industry, these were originally developed for just one species and are now being applied to multiple other species with varying success. In recent years the nutritional profile of invertebrate species has been of increasing interest for human diets. This increasing knowledge base could be utilised in the development of more nutritionally suitable diets for captive wild animals, with average values for protein, fat, ash and nitrogen free extract being calculated for both termites and hymenoptera.

Diet sheets were provided by multiple collections keeping aardvarks *(Orycteropus afer)*. The dry matter nutrient content of these diets was analysed using both Zootrition software and some proximate analysis at the University Centre Sparsholt laboratories. One-sample t-test suggests that diets fed to captive aardvarks are significantly lower in protein ($t=-6.01$, $p=0.004$) than the termites. The diets were also significantly lower in fats than the average value of hymenoptera ($t=-19.75$, $p<0.001$).

Data were also collected on the diets of captive greater tenrecs (*Setifer setosus*, n=6) and lesser tenrecs (*Echinops telfairi*, n=47) housed across zoological collections and private collections. Most of these diets provided some invertebrates within the diet; however, the diets varied from 100% invertebrate based to invertebrates making up less that 25%. Other items listed as provided to these species include fruits and vegetables, cat food and raw meat. Findings showed a significant difference ($p<0.05$) in the nutritional composition of the diets between collections.

By summarizing the current literature on the nutritional profile of ant and termite species, as well as the current research into the nutritional requirements of several myrmecophage species, this research aims to evaluate the work so far and from this make recommendations as to the future research requirements to improve the welfare of captive insectivorous mammal species.

*KEYWORDS: Myrmecophage, insectivorous mammal, nutritional disorders, diet profiles*

**Nutritional Analysis on Captive Aardvarks and Separation Solution of Termant using Emulsifiers**

*Hannah Davies, Jo Bond, Kerry Hunt*

*University Centre Sparsholt*
*Correspondence email: kerry.hunt@sparsholt.ac.uk*

The aardvark (*Orycteropus afer)* is a myrmecophagous species. In the wild, termites make up the majority of the aardvark's diet. In addition, this species feeds on locusts, ants, small mammals and vegetable matter. In captivity, a complete diet called Termant is often used, which was developed to meet the nutritional requirements of captive myrmecophagous species. This diet is fed to giant anteaters (*Myrmecophaga tridactyla*), pangolins, armadillos and aardvarks. Whilst the diet has been marketed as a complete diet, issues documented with the use of this diet include softer faeces in captive ant-eating mammals, and a low water-holding capacity, so that after preparing a slurry, water rises to the top of the bowl and solids sediment. This study looked at the impact of various emulsifiers on the separation time of Termant as well as the nutritional effect of adding these ingredients.

Emulsifiers are thickening agents which are used daily in cooking a variety of foods, such as soups, sauces and desserts. Xanthan gum, guar gum and gelatine were used for this study. Xanthan gum is developed by fermenting corn sugar, which creates a microbial polysaccharide. Guar gum is created from guar beans. Gelatine is produced from animal collagen. Four feed mixtures were produced: (1) Termant alone, (2) Termant & xanthan gum, (3) Termant & guar gum, (4)Termant & gelatine, all at a ratio of 200:1. In addition to the four mixtures, four different ratios of water dilution were tested to determine the ideal amount needed for producing a stable Termant slurry.

A ratio of 200:1 Termant to emulsifier was utilised for each emulsifier, using 15 g of Termant with 0.075 g of emulsifier. Four different dilutions were also utilised to see whether this had an impact, these were 2 ml of water per gram of Termant, 2.125 ml/g, 2.25 ml/g and 2.5 ml/g. Time lapse cameras on a iPad were utilised to record the separation over a 6 hour period. The speed of separation was measured, as was the amount of separation in millimetres.

The results suggest there is a significant difference ($p<0.05$) in the amount of separation at every dilution with the use of the emulsifiers, with the best two mixtures being Termant & xanthan gum and Termant & guar gum at a dilution of 2 ml/g. Both of these mixtures also still met the nutritional requirements of captive aardvark. However, palatability tests need to be performed to see if these mixtures will be actually consumed, and their consequence for faeces consistency need to be assessed.

*KEYWORDS: Myrmecophage,* Aardvark, Termant, separation, emulsifiers

**Ten years of browse provision at ZSL London Zoo and ZSL Whipsnade Zoo: What have we learned and where are we going?**

*Sven Seiffert*

*Zoological Society of London*
*Correspondence email: Sven.Seiffert@zsl.org*

In 2018 the horticulture teams at ZSL supplied £150,000 worth of browse to the animal sections. While this didn't meet all ZSL London Zoo and ZSL Whipsnade Zoo's requirements, it nevertheless constitutes a considerable contribution to meeting animal welfare needs at both zoos. It also reflects a departure from the ad hoc delivery of browse in the past to using a more strategic and systematic approach that integrates browse provision into the ZSL horticulture department's operation.

Key starting points for making this transition were defining browse provision as a strategic aim, increasing and documenting the knowledge about plant toxicity and palatability in relation to specific animal species, and setting up a regular browse harvesting and supply schedule. Additional changes included adjusting the horticultural management of both sites, rethinking the traditional practice of winter tree management, establishing browse plantations and working with neighbouring landowners. One essential aspect throughout this process was the proactive collaboration with relevant stakeholders, such as ZSL's keepers, curators and nutritionists.

In addition to increasing the quantity and variety of delivered browse, the horticulture department implemented a system for measuring and recording its supplies. This makes it possible to evaluate the operation and, for example, establish the monetary value of supplies. The system also offers a suitable tool for assessing resource requirements.

Other browse provision related activities consisted of the sampling and nutrient analysis of more than 30 browse species in spring, summer and autumn over several years, the production of browse silage and the running of browse workshops for keepers.

While the browse provision at both zoos has made considerable progress, there is scope for improvements and further advances. Better feedback, for example, would plug existing knowledge gaps and enable a more targeted supply. And, the browse provision by the horticulture teams could be increased by making more resources available. Expanding the production of browse silage and exploring the use of a greater range of evergreen species to provide material out of season represent additional opportunities. Most importantly though, there is a need to quantify actual browse requirements so that current shortfalls and the necessary resources to address these can be tangibly identified. It would also be desirable to assess the long-term impact of browse provision on animal welfare in both zoos through further research.

*KEYWORDS: Browse, animal welfare, silage, nutrient analysis*

**The effects of frozen storage on the chemical composition, fermentability and palatability of temperate browse in zoos**

*Ellen van Herk[1], Martine Verheij[2], Anouk Fens[2], Thomas Bionda[2], Arun Idoe[2], Guido Bosch[1]*

*[1]Animal Nutrition Group, Wageningen University, The Netherlands, [2]Apenheul Primate Park, Apeldoorn, The Netherlands.*
*Correspondence email: edavanherk@gmail.com*

Fibrous plant material, such as leaves, bark, twigs and roots make up an important part of the *in situ* diet of browsers. In order to digest such a high-fibrous diet, a browser's digestive tract contains microbiota, in either the forestomach, the hindgut, or both. Offering only low amounts of fibre and/or high amounts of rapidly fermentable carbohydrates (primarily sugars) in zoo diets can lead to disturbances of the digestive system, causing for example acidosis or diarrhoea. Browse is, therefore, also an essential part of browsers' *ex situ* diet. In temperate climates, extra browse is collected and stored in freezers during summer, to cover the winter months. However, processes like ice crystal formation and enzymatic reactions are expected to have an effect on nutritional value and palatability of frozen browse. Gaining more insight in these aspects could improve zoo diet compositions over different seasons and help prevent health- and behaviour-related problems.
The goal of this project was to investigate how a freezing process influences the nutritional value and palatability of six browse species (oak *Quercus rubra*, willow *Salix alba*, lime *Tilia americana*, maple *Acer campestre*, elm *Ulmus minor* and hazel *Corylus avellana*) were harvested in spring, with three biological replicates each. Half of each replicate was stored in freezers (-20°C) for 6 months, while the other half was evaluated fresh. Evaluation was made for 1) nutritional value and 2) feed intake behaviour. The nutritional value of fresh and frozen browse was evaluated by chemical characterisation (dry matter, ash, nitrogen, crude fat, crude fibre, NDF, ADF, ADL, calcium and phosphorus) and by *in vitro* gas production using goat rumen fluid. At the time of writing, only the *in vitro* gas production of fresh leaves had been determined. For the *in vitro* gas production, OM contents were based on fixed ash contents of 10% and rough dry matter determinations per browse species. Feed intake behaviour was studied in two forestomach fermenting primate species (*Trachypithecus auratus*, *Semnopithecus entellus*), one hindgut fermenting primate species (*Alouatta seniculus*) and one ruminant deer species (*Pudu puda*). Studies were done in four periods: winter, thawed browse; spring, young, fresh browse; late summer, mature, fresh browse; autumn, both fresh and thawed browse. Using a cafeteria-style experiment, eating time and preference ranks were determined after 30 minutes event sampling during 4 days.
Results showed that *in vitro* gas production was highest for lime (307.7 ± 12.9 ml/g OM) and elm (301.0 ± 14.0 ml/g OM), and lower for willow (266.8 ± 4.1 ml/g OM), oak (233.8 ± 136 ml/g OM), maple (215.1 ± 12.6 ml/g OM) and hazel (187.6 ± 16.6 ml/g OM). Preliminary feed intake results of all four animal species show that differences in eating time and preference ranks were more pronounced when browse was fresh compared to the thawed state. Preference in the two langur species was highest for elm, and lowest for hazel, but opposite in the red howlers, who preferred hazel over other species. As *in situ* howlers consumed less mature (fibrous) leaves (12-38% of feeding time), compared to langurs (35-51% of feeding time), the fact that *ex situ* howlers preferred browse with the lowest fermentability was unexpected. Interestingly, eating time and preference rank of oak leaves were higher after the freezing process than when presented fresh.
Based on these first results, it is concluded that 1) browse species show large differences in fermentability, 2) a freezing process seems to relatively decrease palatability of some browse species, while increasing that of others, and 3) langurs, howlers and pudus show differences in browse preferences.

*KEYWORDS: browse, leaves, foraging time, preference, fermentation, forestomach, hindgut, ruminant, storage, freezing*

**Inconsistency with forage vitamin A and vitamin E analytics concerning for diet formulation**

*Kimberly Ange-van Heugten[1], Jordan Wood[1], Larry J Minter[2], Troy Tollefson[3]*

*[1]Department of Animal Science, North Carolina State University, [2]North Carolina Zoo, 4401 Zoo Pkwy, Asheboro, NC 27205 and [3]Mazuri® Exotic Animal Nutrition, PMI Nutrition, Land O' Lakes, Inc. St. Louis, MO, 63039*
*Correspondence email: kim_ange@ncsu.edu*

In order to increase activity and enhance animal welfare in human managed populations, there has been a push to increase forage and browse quantities within the diets of many exotic herbivorous species. Therefore, the North Carolina Zoo has recently implemented a program to add increased browse species, specifically within the elephant diet. To ensure diets still meet animal species specific nutrient recommendations, all browse and forages species have been measured for nutrient profiles to include in diet analyses software. As nutrient data from different laboratories were consulted for comparison purposes, concerns were identified with regard to inconsistencies among sources of vitamin A and vitamin E measurements within plants. Therefore, this research commenced by collecting representative samples of two species of hay (Alfalfa (*Medicago sativa*) and Timothy (*Phleum pratense*)) from the NC Zoo.

These samples were kept frozen at -20°C until being dried at 60°C, then ground in a Thomas Wiley Mill through a 2 mm screen. Processed samples were sent to four commercial and commonly utilized American laboratories and analysed for Vitamins A, E and carotenoids for comparison. Each sample of Alfalfa and Timothy were split into two collections and labelled as if there were two different samples of each hay to evaluate repeatability within the lab.

The forage species variation within individual lab ranged from 10 – 17 % for what we considered identical samples. However, variation among labs for Beta Carotene ranged more dramatically. Both Alfalfa and Timothy hays at Lab 1 were two times lower than Lab 2 and twenty times lower than Lab 3 while being listed as non-detectable for Lab 4. Even more alarming, Vitamin E at Lab 2 was 130 times greater than Labs 1, 3 and 4. All labs were contacted to confirm correct comparison of units across labs.

With such large variation in the measurements for vitamin A and vitamin E within our samples, it is a potential concern that some institutions may use incorrect analyses when formulating diets. This is particularly true with browse species that have not previously had these analytes determined. We realize that labs use different analytical techniques to obtain their results but such huge variations with results for these dietarily essential nutrients is alarming. Unless all diet formulations use results from the same lab with plant samples that are processed the same way (only fresh / only frozen / etc.) there seems to be doubt on whether recommendations for animal specific nutrient needs are being met.

*KEYWORDS: Vitamin A, Vitamin E, hay, nutrient analyses*

**Practical raptor nutrition - how, why, when it goes wrong and how to avoid the pitfalls**

*N.A.Forbes BVetMed Dip ECZM(avian) FRCVS*
*Correspondence email: neil.forbes2011@yahoo.co.uk*

Diseases of captive raptors related to the food they have consumed are many and varied. This paper high lights the basic principles of raptor nutrition, together with the risks associated with ingestion of foods of varying provenance. Feeding in special cicumstances will be addressed.

Nutrition must be provided so as to ensure freedom from 'from hunger, thirst by ready access to fresh water and a diet to maintain full health and vigour.' The risks associated with certain foods must be appreciated and assessed. Supplements, probiotics, vitamins and minerals may be necessary at times of stress and increased metabolic demand (growing, training, breeding, moulting, or following illness). Infectious diseases are less likely if mammalian rather than avian food is provided. Each raptor species has evolved to fill an ecological niche. In the absence of detailed nutritional data the dietary needs are most likely to be met by feeding a diet approximating to that which would be taken, under ideal conditions, in the wild.[1] The maintenance energy requirement of raptors is 110Kcal/kg $^{0.75}$/day.

Thus the daily intake of a 100g bird is 25% of its' bodyweight, a 700g bird 15% of its' bodyweight, 1200g bird 10.7% of bodyweight, 4000g 6.25% of bodyweight, 7000g bird 3.5% of bodyweight. Larger birds eat more food but require a significantly smaller percentage of their body mass as daily food intake. The consumption of a prey animal by a raptor involves the bird eating: casting (fur & feather), muscle, bone, viscera and the prey's gut content. In supplying food to captive birds, all these elements should be considered.

Foods available for feeding to captive raptors include day-old chick (doc), "grown-ons" (chickens or turkeys of several weeks old), quail, rabbit, various rodents, beef, venison, lamb and horsemeat. Over 50% of UK raptor keepers feed only day old chicks. Any diet limited to one source is likely to be unsatisfactory. There are marked inter species differences in nutritional requirement.

*Source and storage of food*: Wholesomeness, source, method of killing, freezing and storage must be appropriate. Raptor food species receiving ante mortem medication, must have an appropriate with drawl period. Once killed any food must be effectively frozen quickly to avoid any bacterial proliferation. Frozen food in transit must not defrost, frozen storage must not exceed 3 months. Foods should be defrosted (at 4-8$^{o}$C), to avoid bacterial proliferation. The potential risks of zoonotic infections should always be considered when handling raptors or their food.

*Contamination of foods*: this may involve toxins, bacteria, viruses, parasites and will be considered.

Common deficiencies and excesses will be discussed. Water provision, use of nutritional supplements, casting material and GIT obstructions, decreased GIT motility, 'sour crop', feeding in alternate situations, feeding prior to travel will all be discussed.

**Differences in digestive traits between two Old World vultures**

*Anika Daneel[1], Katherine Whitehouse-Tedd[2], Gerard Whitehouse-Tedd[3], Ellen S. Dierenfeld[2,4] & Geert P.J. Janssens[1]*

*[1]Faculty of Veterinary Medicine, Ghent University, Belgium; [2]School of Animal, Rural and Environmental Sciences, Nottingham Trent University., Southwell, UK; [3]Kalba Bird of Prey Centre, Sharjah, United Arab Emirates; [4]Ellen S. Dierenfeld, LLC, St. Louis, Missouri, USA*
*Correspondence email: geert.janssens@ugent.be*

Two Old World vulture species lappet-faced vultures (*Torgos tracheliotos*) (n=3) and griffon vultures (*Gyps fulvus*) (n=4) kept individually at Kalba Bird of Prey Centre, UAE, were compared to determine potential differences in feeding and digestive strategies. During the 4-day trial, both species received the same diet, comprising of a whole rat during the first three days and a one-time titanium dioxide ($TiO_2$) bolus hidden in half a chicken during the last day. The rat diet was used to determine feeding patterns, colour and consistency of the excreta, while the $TiO_2$ bolus was used to determine digesta passage according to Thielemans et al. (1978) by analysing Ti in the individual excreta in a 24h collection period.
It was observed that lappet-faced vultures consumed significantly more food than griffon vultures (85 g/kg$^{0.75}$/d compared to 38 g/kg$^{0.75}$/d; P=0.022) and fed less selectively, while griffon vultures left significantly more pieces such as skeleton and skin (P = 0.035), even when the amount offered was decreased. Lappet-faced vultures predominantly produced brown excreta (60 %; P<0.001), while griffon vultures predominantly produced green excreta (62 %; P=0.001). The consistency of excreta did not differ between the two species and both species mostly produced soft excreta. The concentrations of both N (P=0.005) and C (P=0.019) in the excreta of lappet-faced vultures (128 g/kg DM and 218 g/kg DM respectively) were considerably lower than for griffon vultures (178 g/kg DM and 280 g/kg DM respectively). Across species, there was a positive correlation between food intake and brown excreta, while the concentrations of N and C in the excreta were both negatively correlated with the production of green excreta. The mean retention time (21.4 ±1.5h for the griffon vultures versus 22.1 ±0.7h for the lappet-faced vultures) did not differ significantly between species (P=0.484), which could be due to incomplete sampling overnight.
In nature, lappet-faced vultures are frequently observed to feed on tougher carcass components, mostly consisting of tissues with a lower nutritional value, such as skin and tendons, which are harder to digest. This could possibly contribute to the ingestion of larger amounts of food including bones, and hence lower percentages of N and C in excreta by lappet-faced vultures due to the diluting effect of harder-to-digest matter such as bone (ash). In contrast, griffon vultures tend to start feeding earlier when arriving at a carcass, preferentially ingesting soft tissues, such as intestines and muscles, which could contribute to the ingestion of less food since these tissues are more easily digested. Since griffon vultures consume less food, the gastrointestinal tract is empty for longer periods of time which could explain green excreta, since this phenomenon is most likely associated with relatively larger bile proportions in an empty tract. Altogether, differences were observed between the two species concerning their feeding and digestion strategies. The findings emphasize the importance of keeping raptors on species-specific diets, which for lappet-faced vultures could be based on tougher tissues (such as bones containing tendons) while for griffon vultures a diet based on digestible tissues (such as muscles) would be more suitable.

*KEYWORDS: diet, excreta, griffon vulture, lappet-faced vulture, titanium dioxide*

**Considering the behavioural consequences of feeding regimes: a case study on lesser flamingos *(Phoeniconaias minor)***

*Paul Rose [1,2] and Laura Soole [3]*

*[1] Centre for Research in Animal Behaviour, Washington Singer Labs, University of Exeter, Perry Road, Exeter, Devon, EX4 4QG, UK; [2] WWT, Slimbridge Wetland Centre, Gloucestershire, GL2 7BT, UK; [3] University Centre Sparsholt, Sparsholt, Winchester, Hampshire, SO21 2NF, UK.*
*Correspondence email: p.rose@exeter.ac.uk*

Whilst it is becoming common-place to evaluate the nutritional components of zoo diets, less work is published on the behavioural effects of dietary preparation and presentation for the range of species housed in zoological collections. For specialised feeders with complex behavioural repertoires, the way in which food is presented may impact on appetitive foraging activities and social behaviours between individuals. Flamingos are an excellent example of a highly social species with a specialised feeding method that may be fed in a manner far-removed from the situation for which wild birds have evolved foraging adaptations. As plumage pigmentation depends upon dietary intake of carotenoids, and a study of free-ranging animals shows that foraging activities can be disrupted by aggression from other birds, we investigated the effect of three different methods of feeding a pelleted diet to a group of captive lesser flamingos *(Phoeniconaias minor)* -- bowl, feeding pool, outdoor feeding area -- on the time spent foraging and in aggressive behaviours.

Video footage of the birds' activities at different feeding stations from 2013 to 2015 was used to determine time spent aggressive at an individual and group level. A feeding bowl was predominantly used during 2013 with an indoor pool re-designed in 2014. Outdoor feeding occurred in all years during summer. Feeding method depended on weather and season, with bowls and indoor pools being used more in winter. Total number of seconds spent engaged in feeding and in aggression was recorded by continuous sampling. The colour of individual birds was also scored from one to four, with one being mainly white and four being mainly pink.

Chi-square analysis showed that total foraging was significantly different between feeding styles ($P < 0.001$). When feeding in their outdoor pool, filtering naturally, maximum foraging was twice as much as elsewhere, whilst aggression was half as much as other feeding methods. As such, we can reliably say that feeding method was a significant predictor of aggression ($P < 0.001$) along with group size ($P < 0.001$) -- 55 % of variation in group aggression was explained by foraging style. A Poisson regression shows a significant relationship for foraging ($P < 0.001$) and aggression ($P < 0.001$) with plumage colour of individual birds. Post-hoc testing confirms that paler flamingos spent more time foraging than brighter birds ($P < 0.002$) and brighter flamingos may be less aggressive than paler birds, but this only tends towards significance ($P = 0.06$).

This study enhances our understanding of how husbandry and species' biology impacts on captive behaviour and can help inform feeding method by providing data-based modifications to food presentation. In the case of flamingos, the implementation of spacious outdoor feeding areas has the potential to encourage natural foraging patterns by reducing excess aggression between birds when crowded in a smaller area, and can also improve flamingo welfare by improving flock social stability.

KEYWORDS: *flamingo, animal behaviour, animal welfare, food presentation*

**The past, present and future of Animal Nutrition at Walt Disney World**

*Shannon Livingston[1], Kathleen Sullivan[1], Scott Williams[1], Eduardo Valdes[1]*

*[1] Department of Animal Health, Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake, FL 32830, USA.*
*Correspondence email: Shannon.E.Livingston@Disney.com*

Disney's Animal Kingdom opened as the 4th and largest theme park at Walt Disney World Florida on Earth Day, 1998. The animal nutrition centre has always been part of Disney's Animal Kingdom, however originally functioned as the centralized location for the ordering and storage of animal food which was then prepared by the animal care teams. Shortly after the park opened, the animal nutrition centre, staffed by 6 people, was assigned all responsibilities for animal food production, including preparation and delivery of diets. An animal nutritionist was employed starting in 2001, assuming responsibility of nutrition from the veterinary team. The nutritionist partnered with a manufacturer of zoo animal feeds to create more appropriate options, many of which are commercially available today. The Disney IT team worked to create a diet software program that functioned with the existing animal inventory and medical records systems, allows for individual and group diet records and has the ability to create custom feeding groups for more efficient diet preparation. The program continues to evolve and now includes a nutrient calculator.

The animal nutrition team has grown to include 23 people, 5 of whom hold graduate degrees in the field of nutritional sciences. Responsibilities of the team have expanded and include the nutritional needs of all animals within the Animals, Science and Environment team and not just those at Animal Kingdom. The nutrition team works 7 days per week, 10 hours per day to create and deliver hundreds of balanced diets. Daily operations include 5 people working to prepare the animal diets, 2 people delivering all food items and diets throughout the animal areas and 1 person washing the reusable diet containers.

A browse farm has been part of the park since it opened, however management of the program transitioned from the Horticulture team to the animal nutrition centre in 2009. Browse is considered a critical diet ingredient for many animals and is offered throughout the year for browsing species such as the okapi (*Okapia johnstoni*), where browse makes up 18 to 25 % of the estimated dry matter intake (DMI), and black rhinos (*Biceros dicornis minor*) where it comprises over 30% of DMI.

The nutrition lab conducts regular quality control on all food items, with the data used to assess the nutrient content of the diets. Most samples are sent to commercial laboratories for proximate nutrient analysis, fibre and soluble carbohydrates, and select vitamins and minerals, although fatty and amino acids may also be tested. Hay varieties and fish are tested monthly using an in-house NIRS. Practicality-based studies to assess the diets and their impact on animal health are ongoing, and information gathered is shared within the zoo and scientific community. Recent studies have included investigations into the vitamin E status of African elephants (*Loxodonta africana*), Southern black rhinoceros and white rhinoceros (*Ceratotherium simum simum*); testing the impact of different levels of vitamin E supplementation, serum levels of vitamin E and the fecal excretion of vitamin E. These studies have resulted in adjustments to the supplementation of vitamin E and changes in the laboratories used to test serum vitamin E levels.

Disney's Animal Nutrition Centre aims to continue moving the zoo nutrition field forward, working for innovative feed development and proactively designing scientifically valid nutritional studies in relation to wellness. A goal is to be a leader within the global zoo nutrition community, working collaboratively to help ensure the survival of species for the next generation. Partnering with animal care and veterinary teams, the animal nutrition centre has a key role in maintaining the health and wellbeing of the animals at Disney's Animal Kingdom, Animal Kingdom Lodge and the Living Seas at Epcot. Healthy animals exhibiting natural behaviours can impact the millions of Disney guests and influence their feelings towards wildlife and wild places, hopefully fostering a true appreciation of the animals and their environment.

*KEYWORDS: Walt Disney World, animal nutrition, exotic animal nutrition research, wellness*

**Feeding the roar and ending extinction – how zoo nutrition serves San Diego Zoo Global**

*Andrea Fidgett*

*San Diego Zoo Global, P.O. Box 120551, San Diego, CA 92112-0551, USA*
*Correspondence email: afidgett@sandiegozoo.org*

San Diego Zoo Global (SDZG) is a not-for-profit organization headquartered in San Diego that operates the San Diego Zoo, the San Diego Zoo Safari Park, the San Diego Zoo Institute for Conservation Research, and the San Diego Zoo Global Wildlife Conservancy. SDZG is committed to saving species worldwide by uniting expertise in animal care and conservation science with dedication for inspiring passion for nature. According to zoo lore, Dr. Harry Wegeworth was inspired to start a zoo in San Diego when he and his brother were driving by animals left over from the 1915-16 Panama-California Exposition in Balboa Park and they heard a lion roar. The 100+ year history of the San Diego Zoo has many tales of perseverance and ingenuity when it came to meeting the needs of the animals in its care and in the early years, one of the greatest challenges was securing food for the animals. The Great Depression was another difficult time for the young Zoo, but it also brought opportunity through the Works Progress Administration (WPA). By 1937, WPA workers had constructed a commissary – also known as the Forage Warehouse - at the Zoo, which is still standing today. The Safari Park began as a supplementary breeding facility for the San Diego Zoo in 1972 and the current commissary is one of the original buildings.

A formalised animal feeding program was initiated in 1993, following the appointment of the Zoo's first dedicated animal nutritionist. Today, Nutritional Services sit within the Animal Health directorate and the team has grown to serve the specific needs at each of the zoo and safari park collections, encompassing more than 800 animal species and 7,500 individual animals. To ensure each animal thrives, Nutritional Services interface closely with Veterinary Services, Disease Investigations, Collection Husbandry and Science staff, Institute for Conservation Research colleagues, and San Diego Zoo Global conservation partners, providing scientific-based diets and leading the fight to end extinction through in situ and ex situ nutritional research. Ongoing investigations include studying cheetah gut microbiomes, understanding the nutritional aetiology of osteoarthropathy in snakes and formulating bulk milk-replacement options for a Kenyan elephant orphanage.

As with any reputable zoo, irrespective of size, the commitment to ensuring every animal is fed appropriately is beyond doubt. Nevertheless SDZG's current facilities have not evolved with the animal collection, nor do they fully embrace the emergence of nutritional science as significant component of zoo animal care and the complexities of diet management. Being a progressive zoo animal feeding program faced with designing future facilities, the challenge is not only to consider those aforementioned elements, and the practicalities of ingredient flow and work efficiency, but also biosecurity, emergency preparedness and food security. At time of writing, an outbreak of virulent Newcastle disease is confirmed in Southern California, posing a threat to avian collections and imposing tighter restrictions across all operations for contact with poultry products. Rurally situated, wild fires have encroached onto Safari Park property several times in its existence (including 2018) and with limited storage capacity, the site is reliant upon regular feed deliveries; extended road closures caused by natural disasters are acknowledged as a risk to the continuity of care.

This talk touches on the history, current challenges and future of zoo nutrition as it has served, and continues to serve San Diego Zoo Global.

*KEYWORDS: San Diego Zoo Global, Biosecurity, Emergency Preparedness*

**Body condition scoring of Hermann's tortoises (*Testudo hermanni*) and comparison to known objective body condition measurements**

*Angela Gimmel[1], Sabine Öfner[2], Annette Liesegang[1]*

*[1]Institute of Animal Nutrition, Vetsuisse Faculty, University of Zurich, Switzerland;*
*[2]Auffangstation für Reptilien, Munich e.V., Munich, Germany*
*Correspondence email: aliese@nutrivet.uzh.ch*

The body condition score (BCS) is a subjective, semi-quantitative method of evaluating body fat and musculature and is used in numerous species such as cats, dogs, poultry, horses and cattle. Recognizing obesity (or the contrary, emaciation) is very important in tortoises kept as pets or as part of a collection in zoos, because it gives an indication of the animals' health status. In tortoises, one subjective body condition score could be found for desert tortoises as well as different pre-existing objective body condition indices (BCI), which use different calculations that include body weight (BW) and straight carapace length (SCL). For example, the Jackson's ratio uses BW/SCL to predict body condition; another condition index uses the log (BW/predicted BW), where predicted BW = a x $SCL^b$. For this calculation a = - 3.024 and b = 2.684 was used for females and a = - 3.322 and b = 2.811 was used for males respectively, using published data for the Hermann's tortoises (*Testudo hermanni*) for the month of September. The different BCI must be applied with caution, because the information of body mass itself does not need to only relate to body condition but may represent for example eggs carried by a female.

34 Hermann's tortoises (24 males and 10 females), stationed at the "Auffangstation für Reptilien" in Munich (reptile rescue centre, RRC) were subject of this study. The sexes were kept in separate enclosures to prevent breeding. The enclosures were in a large greenhouse with natural earth flooring and natural vegetation. The tortoises were fed each day with different green forages and had free access to cuttlefish bones and the vegetation in the enclosure. Each tortoise had its BW and SCL measured. The examination took place in September, before hibernation. Manual palpation of the cervical vertebrae (sharp, easy palpable, palpable, palpable with pressure) and the tail vertebrae (sharp, easy palpable, palpable, palpable with pressure) were assigned to specific scores by three veterinarians. A BCS (mean of examiners' scores) was given to each tortoise according to the manual palpation. The BCS system was chosen to range from 1-5 in 0.25-point steps, with 2.5 considered as ideal BCS.

For the studied tortoises, BCS ranged from 1.25 to 2.75. Out of 34 tortoises, 1 tortoise was scored with a 1.25, 5 tortoises were scored with a 1.5, 5 tortoises with a 1.75, 5 tortoises with a 2, 8 tortoises with a 2.25, 7 tortoises with a 2.5, and 3 tortoises with a 2.75. The mean (± standard deviation) BW was 805 ± 305 g, the median SCL was 15.9 ± 5.6 cm. The BCS did neither correlate with the Jackson's ratio nor with the condition index (log (BW/predicted BW). The BCS scores were significantly higher in the females than in the males.

Out of 34 tortoises at the RRC, 24 were considered below ideal body condition, 8 had a Jackson's ratio below 4 (which was suggested to be too low for hibernation for animals of this body size) and 6 had a condition index of < -0.1, which is equivalent to an observed mass below 80% of the predicted mass. Most of the animals with a low BCS were males. This might be explained through a higher level of stress in the enclosure, as the males were sexually active and a high level of competition was evident. It is possible that the BCS and the BCI did not correlate in the studied population because no electronic callipers were used to measure SCL.

A BCS of a tortoise includes a manual palpation of the animal and thus gives the examining veterinarian additional information to the objectively measured or calculated index. Therefore, it is always preferable to use a BCS over a BCI.

*KEYWORDS: Hermann's tortoises, body condition score, Jackson ratio*

**Comparison of the nutritional content of the captive and wild diets of the Critically Endangered mountain chicken frog (*Leptodactylus fallax*) to improve its captive husbandry**

*Stephanie Jayson[1], Amanda Ferguson[1], Matthias Goetz[2], Andrew Routh[2], Benjamin Tapley[1], Luke Harding,[3] Christopher J. Michaels[1], Jeff Dawson[2]*

*[1] Zoological Society of London, Regent's Park, London, United Kingdom*
*[2] Durrell Wildlife Conservation Trust, Les Augrès Manor, La Profonde Rue, Trinity, Jersey, United Kingdom*
*[3] Paignton Zoo Environmental Park, Totnes Road, Paignton, Devon, United Kingdom*
*Correspondence email: amanda.ferguson@zsl.org*

It is vital to provide appropriate nutrition to maintain healthy populations in conservation breeding programmes. Knowledge of the wild diet of a species can be used to inform captive diet formulation. The nutritional content of the wild diet of the Critically Endangered mountain chicken frog (*Leptodactylus fallax*) is unknown, like that of most amphibians.
In this study, we analysed the nutritional content of food items that comprise 91% of the wild diet of *L. fallax*, by dry weight of food items, and all food items offered to captive *L. fallax* at ZSL London Zoo and Jersey Zoo. We subsequently compared the nutritional content of the wild diet and captive diet at ZSL London Zoo consumed by *L. fallax*. The captive diet at ZSL London Zoo, without dusting of nutritional supplements, was higher in gross energy and crude fat and lower in ash, calcium and calcium:phosphorus ratio than the wild diet.The majority of food items offered in the captive diets (commercially reared crickets *Gryllus* sp and beetle grubs *Pachnodus marginata*) had a high omega-6:omega-3 fatty acid ratio, only locusts (*Schistocerca gregaria)* had a low ratio. In contrast, invertebrates making up 68% of the wild diet (species from *Tettigoniidae, Gastropoda, Coleoptera, Decapoda, Oligochaeta and Arachnida)* had a low omega-6:omega-3 fatty acid ratio. <4.72:1. Dusting with nutritional supplements addressed the calcium deficit and increased vitamin A but did not alter the fatty acid profile.
We recommend a combination of modifications to better reflect the nutritional content of the wild diet, including attempts to sustainably culture invertebrate food items that have a higher calcium and lower crude fat content and testing of these items for palatability and digestibility; alteration of the rearing diet of invertebrates to reduce fat content and promote a lower omega 6:omega 3 fatty acid ratio; and, given the current reliance on commercially produced invertebrates, nutrient targeted gut loading and review of dusting protocol to ensure that a nutritionally appropriate supplement is used with effective delivery. Nutritional analysis of captive and wild diets is recommended for other species in conservation breeding programmes to improve captive husbandry and ultimately fitness.

*KEYWORDS: amphibian, nutrition, zoo, conservation breeding programme*

**Why should a frog choose a prey that is not that nutritious? Facts and some theories**

*Arturo Muñoz[1], Gabriel Callapa[2], Geert P. J. Janssens[1]*

*[1]Department of Nutrition, Genetics and Ethology, Ghent University, Belgium; [2]Natural History Museum Alcide d'Orbigny, Cochabamba, Bolivia*
*Correspondence email: arturo.munozsaravia@ugent.be*

Understanding the foraging strategies of an endangered species can render insights in how ecosystems may be affected by their loss. It can also provide very useful data for captive breeding programs that are already present in the Americas and some zoos in Europe. The Titicaca water frog (*Telmatobius culeus*) has very singular adaptations that allowed the species to succeed in extreme conditions of a high Andean lake. There is little information about its natural diet and no information about the nutrients in that diet. Present captive diets are often based on a limited number of diet items that are not natural to the frog, such as bloodworms (*Tubifex*).
We collected information about the availability of the potential prey items, and analysed them as well as the stomach content of 37 opportunistically collected individual *T. culeus*, providing detailed information about the use and preference of potential food resources. Nutrient composition was analysed for all potential prey items, and we used that information to try to explain why the frog is choosing certain prey items over others.
Of the stomach contents, 32 contained macroinvertebrates and fish with a total of 544 prey items. The most frequent items were the amphipod *Hyalella* spp., and two snails, *Heleobia* sp. and *Biomphalaria* sp., accounting for 77% of the total number of prey items, 63% in volume and 70% in mass. Snails (*Heleobia* sp. and *Biomphalaria* sp.) contained lower concentrations of energy and protein compared with other prey items available for the frog. *Hyalella* spp. were in the middle range of energy and protein, whereas fish, together with flatworms, leeches and Coleoptera, were at the top of the range.
The frequently consumed *Hyalella* spp. were the most abundant prey items. The snails were abundant in the gut content even though they were less abundant in the habitat and had low nutritional value. While this most likely is the cause of factors not assessed, in particular the hunting success for different prey, another potential reason for targeting snails might be their shells. The presence of shells in frog faeces suggest that the shells were at least not completely digested, so that calcium supply may not fully explain the preference for the snails; also because *Heleobia* sp. only contained low levels of calcium (0.3% versus 30% in *Biomphalaria* sp.). We therefore hypothesise that snails could help in breaking down the exoskeleton of *Hyalella*, similar to the grinding function of gizzard stones in birds. The abundance of prey items of low nutritional quality might be relevant for diets in captivity.

*KEYWORDS: Telmatobius, diet, snails, digestion*

**Survey of feeding practices and nutrition of captive Aldabra giant tortoise (*Geochelone gigantea*) populations in EAZA institutions**

*Daniel Harrold, Dr. Amy Plowman, & Luke Harding.*

*Whitley Wildlife Conservation Trust, Paignton Zoo Environmental Park, Devon, England.*
*Correspondence email: daniel.harrold@zsl.org*

Zoo animal nutrition is an ever-developing field within conservation biology research and is crucially important for the longevity of often-threatened taxa housed within institutions. Herpetological nutrition, at the genus or species level at least, remains in its infancy. *Ex-situ* collections should strive to formulate species-specific diets for as many reptile and amphibian taxa as possible to improve captive health and welfare. Aldabra giant tortoises (*Geochelone gigantea*) are common and charismatic reptiles kept in many European collections. There is sparse published literature on captive nutrition of these giants compared to the closely-related Galapagos tortoises (*Chelonoidis spp.*).

A dietary survey was sent to 44 EAZA institutions with *G. gigantea* registered in their living collections on ZIMS (Species360). Responses from 18 collections (41%) from seven nations were received, accounting for 92 tortoises. Survey questions ranged from population demographics to basic husbandry practices, which then focussed on modes of feeding, health, and breeding success. Quantitative data was plotted graphically to evaluate common trends.

Average population size was 4.84 individuals with a sex ratio of 1:1 and a mean age of 28.3 years (range 9 to 157 years). 78% of institutions report no current health problems. 61% of collections highlight a lack of mating and breeding, but this is unremarkable as 54% of individuals are under the age of 20 years and so are not sexually mature. 39% of collections do not vary their diet sheets seasonally; however, 28% reduce the amount of fruits and vegetables provided due to restrictions to access foodstuffs in winter months. Favoured feed items included domestic weeds, endive, tomato, carrot, and bell pepper with fennel, carrot, courgette, and leek commonly left uneaten. Over half (56%) confirmed the use of dietary supplementation with most common products used including Nutrobal, cuttlefish bones, and calcium carbonate powder.

The results demonstrate some clear disparity between feeding regimes, quantities, and feed items among collections, highlighting the need for increased collaboration and synthesis of currently implemented feeding practices. There were few physiological conditions noted which provides reassurance that these tortoises are adequately maintained with the provided diets. The poor fecundity rate and hatching success, along with large dietary variation within European collections, may mean this species will greatly benefit from published best practice guidelines. Increased data-sharing and collaboration between institutions should be promoted for this and other related species.

*KEYWORDS: Aldabra giant tortoise, Diet sheet, G. gigantea, Husbandry, Nutrition, Welfare*

**Fur, feathers and feeding poles – how cats feed**

*Andrew C. Kitchener[1] & Graham Law[2]*

*[1]Department of Natural Sciences, National Museums Scotland, Chambers Street, Edinburgh EH1 1JF, UK*
*[2]Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary & Life Sciences, Graham Kerr Building' University of Glasgow, Glasgow G12 8QQ*
*Correspondence email: a.kitchener@nms.ac.uk & Graham.Law@glasgow.ac.uk*

Good nutrition is essential for the health and well-being of captive and wild animals. The provision of prepared processed diets gives captive animals all the required nutrients to ensure a healthy body, but especially in the case of carnivorous mammals they result in a considerable behavioural deficit, which may lead to abnormal behaviours, such as stereotypies, and cause unintended skeletal and dental pathologies. In the wild the nutritional quality of prey animals will vary significantly with age and condition, but over a life time this should balance out to maintain good overall health. In this presentation we examine the behavioural deficit in captive cats caused by prepared diets and offer solutions as to how to overcome this through enrichment techniques that mimic the energy outlay and behaviours used in hunting to contribute to the physical and mental well-being of captive cats.

**Dual faecal consistency in captive tigers (*Panthera tigris*): more than just water content differences**

*Karla Esparza[1], Jörg M. Steiner[2], Linda Penfold[3], Lara Metrione[3], Heidi Bissell[4], Ellen S. Dierenfeld[5,6], Katherine Whitehouse-Tedd[6], Jonathan A. Lidbury[2], Jan S. Suchodolski[2] & Lisa Yon[1]*

*[1]School of Veterinary Medicine and Science, University of Nottingham; [2] Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University; [3]South-East Zoo Alliance for Reproduction and Conservation, USA; [4]SeaWorld Parks & Entertainment, USA; [5]Ellen S. Dierenfeld LLC, USA; [6]School of Animal, Rural and Environmental Sciences, Nottingham Trent University*
*Correspondence email: svxkce@nottingham.ac.uk*

Faecal consistency scores have been used as non-invasive indicators of gastrointestinal health in felids. Diet can have a considerable impact on faecal consistency. For example, whole prey or structurally complex diets have been associated with the presence of a dual faecal consistency (i.e., a single scat containing both firmer and softer consistency components) in several carnivores. However, double consistency faeces have not been previously reported in tigers (*Panthera tigris*). Similarly, reports on concentrations of biomarkers or hormone metabolites in faeces with dual faecal consistencies are absent.
In a randomised crossover study, eight captive tigers were fed either a Base Diet consisting of 100% minced horse meat, or an Experimental Diet comprising 80% minced horse meat and 20% whole degutted rabbit (daily total intake as offered) for an 8-week period, after which they were switched to the opposite diet. Two faecal inflammatory biomarkers (N-methylhistamine (NMH) and S100A12) and two faecal glucocorticoid metabolites (FGM: cortisol and corticosterone) were measured in scats with dual consistencies to evaluate if differences in concentrations occurred between consistencies within scats. Faecal samples were scored using the Felid Taxonomy Advisory Group 1-5 scale. When dual consistency scats were identified, separate aliquots were collected for each consistency, referred to as cons A (firmer) and cons B (softer). For FGM determination, faeces were collected every 3 days throughout the duration of the feeding trial, while for measurement of inflammatory biomarkers, samples were collected over three consecutive days in weeks 0, 8 and 16. Prior to analysis, faecal aliquots were freeze-dried to remove water content. Inflammatory biomarkers were measured using an in-house ELISA, and FGM were determined by polyclonal enzyme immunoassays. Statistical analysis was performed using the Wilcoxon signed-rank test, and effect size was calculated using Pearson's correlation coefficient.
Seventeen samples were analysed for inflammatory biomarkers, and 50 for FGM. NMH concentration levels did not differ significantly between cons A (Mdn = 129 ng/g, $Q_1$ 46, $Q_3$ 393) and cons B (Mdn = 163 ng/g, $Q_1$ 83, $Q_3$ 421; $p = 0.177$), and neither were SA100A12 concentrations (cons A Mdn = 6 µg/g, $Q_1$ 4, $Q_3$ 16; cons B Mdn = 52 µg/g, $Q_1$ 8, $Q_3$ 151; $p = 0.120$). However, cortisol concentration in cons B (Mdn = 105 ng/g, $Q_1$ 66, $Q_3$ 190) were significantly higher than in cons A (Mdn = 71 ng/g, $Q_1$ 42, $Q_3$ 90; $p < 0.001$). Similarly, corticosterone levels were significantly higher in cons B (Mdn = 206 ng/g, $Q_1$ 155, $Q_3$ 242) than cons A (Mdn = 169 ng/g, $Q_1$ 143, $Q_3$ 207; $p = 0.024$).
The lack of statistically significant differences between consistencies for inflammatory biomarkers should be interpreted with caution due to the wide variability of the data and small sample size. For FGM, the presence of higher concentrations of both metabolites in cons B may be influenced by retention time in the digestive tract. However, until the possible mechanisms behind the separation of digesta and presence of a dual faecal consistency in carnivores are elucidated, the potential biological significance of these results cannot be determined. The presence of dual consistency faeces could make it challenging to assess gastrointestinal health based exclusively on faecal scoring, and should be borne in mind by staff in charge of monitoring this parameter.

*KEYWORDS: Faecal consistency, faecal glucocorticoid metabolite, inflammatory biomarker, Panthera tigris, tiger, whole prey*

**Nutrient composition of diets consumed by wild European brown bears (*Ursus arctos arctos*): Insights for optimising current feeding strategies for captive bears**

*Leen Verbist[1], Guido Bosch[2], Annelies De Cuyper[3] & Sarah Depauw[1]*

*[1]Odisee University College, Agro- and Biotechnology, Sint-Niklaas, Belgium; [2]Animal Nutrition Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands; [3]Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*
*Correspondence email: leen.verbist@odisee.be*

Over the course of 19 years, the Bear Forest, a bear sanctuary initiated by Bears in Mind in Ouwehand Zoo Rhenen, has established their own Natural Feeding Program (NFP). Though NFP aims to simulate seasonal variations in energy and nutrient intake, data on nutrient composition of the natural diet of European brown bears are scarce.
To support and optimise the NFP, we reviewed the feeding ecology of European brown bears and estimated the nutrient profile of their diet in the wild. Diet composition was retrieved from ecological studies based on faecal volume of consumed dietary items and corrected for possible difference in digestion rates of these food items by using correction factors from literature. Nutrient composition of dietary items was retrieved from literature and food composition databases. Subsequently, data on diet and nutrient composition were combined to calculate the energy and macronutrient composition of wild European brown bears diets. Data were organised according to season and climate zones.
The seasonal variation in macronutrient profile is very divergent according to the climate zone were European brown bears live. The bears tend to consume more animal matter in spring, which is considered to be high-quality food, to meet their energy demands so they can recover from hibernation. This is reflected in a high protein (nutrients expressed on dry matter basis) content of approximately 50%-60% of the diet during spring and early summer. The latter, however, only applies to bears in Estonia, Norway, Slovakia and Sweden where the climate is humid continental and characterised by cold winters. Animals from Croatia and Greece, living in temperate climates characterized by hot summers and mild winters, cannot depend on high vertebrate intake and mainly eat green vegetation and soft mast (fleshy fruits), which results in diets containing only about 15% protein. Regardless of climatic region, protein is highest in spring and summer and reduces by half in autumn. Bears from Croatia and Greece mainly rely on high-lipid foods such as nuts and seeds during the hyperphagic stage in autumn. This results in diets containing up to 20% of fat, which is two to five times more than in summer. While dietary energy content in temperate climates characterized by hot summers and mild winters, consequently increases from 1600 kJ in spring towards 1900 kJ ME/100g in autumn, the exact opposite is observed in the humid continental climates characterized by cold winters. Bears occupying these climate zones have no access to high-lipid foods during the hyperphagic stage and compensate this by eating more soft mast. The nutrient profile of these hyperphagic diets is therefore relatively low in fat (10%) and high in nitrogen-free extract (NfE; up to 55%) in comparison with spring and summer (15-20% fat; 10-15% NfE). Despite the fact that brown bears in Croatia and Greece consume more fat in autumn, NfE is still the most abundant nutrient in their diet throughout the year (42% to 65%) due to the considerable consumption of green vegetation and/or soft mast in these regions.
Currently, nutritional guidelines for brown bears in captivity advise a gradual change from green vegetation and occasional meat in spring to more nutritious items such as fruits and bread during summer and an increase in nuts and fatty meat towards autumn. Comparing this to our data, based on the European brown bear only, these recommendations are merely in line with European brown bears living in temperate climates characterized by hot summers and mild winters. Further investigation is required to define whether the current feeding recommendations need to be adapted according to the climate from which the bears originate or rather to the climate they are housed in.
*KEYWORDS: European brown bear, wild diet, captive diet, macronutrient content, energy content, nutrient profile*

**Investigating the Feeding Hierarchy of Long Tailed Chinchillas and Degus within a Shared Enclosure**

*Jordan Stevenson[1] & Lauren Samet[2]*

*[1]Moulton College, University of Northampton, UK; [2]Animal Management & Welfare, University of Northampton, UK.*
*Correspondence email: lauren.samet@northampton.ac.uk*

Long-tailed chinchillas (*Chinchilla lanigera*) and degus (*Octodon degus*) are herbivorous rodent species often housed together in captive settings. This co-habitation can lead to inter-species dominance hierarchies alongside the intra-species expected hierarchical structures. Dominance behaviour is linked to aggression and other antagonistic social behaviours, which if chronic can be stressful to group individuals and impact welfare.

This study aimed to investigate whether there were hierarchies present between mixed sex populations of *Chinchilla lanigera* (n = 5) and *Octodon degu* (n = 12) were housed together at an institution, an whether this might impact feeding behaviours and food access. Direct observations were carried out over twenty days using four different conditions: Ranking dominance by scoring the first seven individuals that gained access to (1) one of two feed bowls placed on the floor (repeated at 15 feed times), (2) one of two feed bowls placed in an elevated position and the other next to a nesting area (repeated at 15 feed times) (observations for conditions 1 and 2 were made at morning feeds of a complementary muesli rodent diet between 9am and 10am), (3) food items when scatter fed (repeated at 15 feed times), and (4) measuring the time taken to ingest food and the order in which the type of fresh food was selected for when fresh feed was scatter fed (repeated at 15 feed times) (observations for conditions 3 and 4 were made during afternoon chopped fresh vegetable feeds between 2pm and 5pm).

Results suggested that under normal feeding conditions (1), degus showed a significant feeding dominance over chinchillas ($p<0.001$). However, when feeding bowl placement was altered (2), no significant hierarchy was found ($p=0.15$). This was likely due to degus and chinchillas showing preferences for, and dominance over, bowls placed in nesting location and in an elevated position, respectively ($p<0.001$). Species feeding order (recorded via the use of an ethogram) noted degus eating sooner than chinchillas under normal feeding conditions (1), supporting the presence of dominance. However, when scatter fed (3), the order of feeding revealed no such pattern, suggesting there was less scope for hierarchy under this feeding regime. Degus showed a preference to select carrot first from their fresh feeds, whilst chinchillas primarily selected cucumber (this preference did not change between conditions 1-3).

It must be noted that the diet fed to these groups at the time of the study was not optimal for the species involved, and this was raised upon presenting the results to the institution, alongside the recommendation of generally providing more bowls at feed times anyway. Time taken ingesting food decreased when food was scatter fed, which was thought to be related to an increased time spent foraging. The results indicated that thought over bowl positioning and inclusion of scatter feeds could improve chinchillas' access to a balanced diet within a mixed species enclosure. However, food preference when feeding mixed vegetables could still skew balance, therefore feeding a single type of fresh vegetable and rotating the type fed daily could be a practical solution to overcome this. The study's results led to the practical implementation of such measures to reduce dominance hierarchies and lower aggression at feed times within the exhibit.

*KEYWORDS: Degus, Chinchillas, feeding hierarchy, scatter feeding, food preference.*

**The importance of nutrition in the preventative medicine of geriatric zoo animals**

*Francis Cabana[1], Guillaume Douay[1], Abraham Mathew[2], Shangzhe Xie[2], Yaoprapa Mathura[2], Ali A. Ahmad[2], Yirui Heng[2], Charlene Yeong[2], Ellen Rasidi[2], Sonja Luz[2]*

*[1]Wildlife Nutrition Centre, Wildlife Reserves Singapore, 80 Mandai Lake Road, 729826, Singapore*
*[2]Conservation, Research and Veterinary Services, Wildlife Reserves Singapore, 80 Mandai Lake Road, 729826, Singapore*
*Correspondence email: francis.cabana@wrs.com.sg*

Nutrition is often overlooked by wildlife and domestic health practitioners such as veterinarians, unless nutrition is the cause of an observed pathology. In reality, nutrition is a powerful preventative health measure which should be considered for every animal under human care. Nutrition is linked to many health issues including but not limited to: obesity, heart disease, diabetes, gastro-intestinal disorders, dental disease, kidney and liver related illnesses, bone disease, skin and pelage issues, ocular disease, and others. Problems are compounded for geriatric animals when they live well beyond average longevity records in the wild. As they age, chronic health issues will commonly appear.

Every taxon has species-specific issues once they reach the geriatric stage, yet some seem to be common in all animals, such as arthritis, renal insufficiency, weight loss, increasing food selectivity, decreased digestive capacity and cataracts. Although it is unlikely that nutrition can completely prevent these health issues in aged animals, it may have the potential to delay the onset, or even decrease the severity of observed symptoms.

We began supplying bespoke geriatric supplements for every animal that has reached 75 % of their expected lifespan in captivity. We had 188 geriatric animals when this program began (47 % mammals, 45 % birds and 8% reptiles). Each animal was visually assessed for aging-associated diseases by a veterinarian before supplements were prescribed. Animals were scored on lameness, weight bearing, body condition and feather/fur condition. This scoring occurred once a month for four months. Three keepers scored the animal every two weeks for four months on another set of criteria. They indicated if there were any visible improvement (scored as a 1), no change (scored as a 2) or worsening (scored as a 3) for the following categories: rising from rest, limping, stiffness, aggression, running, walking, jumping, climbing, paying and activity level.

Preliminary results suggest that significant improvements of limping, stiffness and activity levels are achievable for artiodactyls, perissodactyls, raptors, primates and small carnivores through the use of these supplements. Rising from rest, weight bearing and lameness may be improved for artiodactyls and large carnivores. Coat condition may be significantly improved for birds, carnivores and perissodactyls.

Visible benefits were largely attributed to the addition of omega-3 fatty acids in their diets, as other supplements were aimed at preventing health issues that are not as obvious such as kidney and ocular conditions. For this reason, only the change of obvious clinical manifestations was measurable over four months. Through optimum nutrition at the geriatric life stage, we can keep our animals more comfortable and possibly resolve or delay a number of health issues.

*KEYWORDS: Senior, supplement, omega 3, limping, stiffness*

**Food for thought: Keeper opinions on welfare and nutritional perspectives of fruit-free feeding in Primates**

*Rebecca Hammerton[1], Kerry Hunt[1] & Lisa Riley[2]*

*[1]University Centre Sparsholt, Sparsholt, Winchester, Hampshire, United Kingdom, SO21 2NF; [2]Centre for Animal Welfare, University of Winchester, Sparkford Road, Winchester, SO22 4NR*
*Correspondence email: rlmhammerton@gmail.com*

Over recent years nutritional comparisons between wild and captive primate diets have demonstrated the need to re-evaluate zoo diets. Removal and reduction of fruit from captive primate diets to provide a more 'wild-type' nutritional composition (lower sugar, higher fibre) has produced several potential welfare benefits, including a reduction of obesity, a reduction in dental procedures and a reduction in aggressive and self-directed behaviours. As zoos shift great ape diets towards cultivated fruit-free feeding (hereafter fruit-free diet), this study aimed to ascertain keeper knowledge and opinions on fruit-free diets. Feeding regimes currently in use alongside the prevalence of abnormal behaviour and diet-related health conditions were also investigated.

Twenty great ape keepers from 11 collections (representing 61.1% of great ape collections in the British Isles) completed a questionnaire that requested both quantitative and qualitative data. Non-parametric statistical analyses were employed where appropriate alongside thematic analysis. Of the 20 great ape keepers, seven were feeding a fruit-free diet, seven were in the process of changing to a fruit-free diet and six were feeding a fruit containing diet. Keepers already feeding a fruit-free diet had significantly more self-reported knowledge on the trend of cultivated fruit-free diets than expected ($P = 0.001$). All great apes were fed multiple times a day (medium 5, range 3 – 9) and keepers used multiple feeding strategies (e.g. whole food, chopped food, simple enrichment etc) (median 8, range 4 – 8). Chopped food and complex enrichment was used by all keepers, whilst direct access to food (either in bowls or piles) were significantly lower than expected given the overall distribution of feeding strategies ($P = 0.001$). Fewer keepers feeding a fruit-free diet presented food in bowls, whilst more than expected presented food diced or used feeding enrichment ($P = 0.04$). Most keepers believed their apes' diet was not comparable to the wild diet (70% of keepers who fed fruit-free and 60% of keepers who included fruit in their apes diets). All keepers, irrespective of diet, agreed or strongly agreed that their apes were in good health, despite 85% of the surveyed keepers noting a health condition. A total of fifteen abnormal behaviours were noted across great ape species, with regurgitation/reingestion, regurgitation, and coprophagy reported significantly more than expected ($P = 0.045$) overall, regardless of diet type. When considering diet type, spinning ($P = 0.001$) and self-patting ($P = 0.001$) were noted to be significantly more prevalent (as noted by keepers) in great apes fed a fruit-free diet. In addition the frequency of spinning ($P = 0.009$) and self-patting ($P = 0.001$) (as reported by keepers) was also significantly higher in collections that fed a fruit-free diet compared to collections that fed a diet containing fruit. All other behaviours did not differ between the diet types; however, species differences were noted with a higher prevalence of abnormal behaviours reported in gorillas ($P < 0.001$) compared to chimpanzees and orangutans. No dietary factor was a significant predictor of abnormal behaviour or health conditions in great apes.

The results suggest keepers should be involved further in diet changes, with improvements to diet presentation and seasonality being noted as relevant small-scale changes. In addition, further studies of nutritional predictors of diet-related abnormal behaviour are required across collections keeping primates that feed fruit-free and fruit containing diets, and the question whether keepers feeding fruit-free diets are more sensitive to behavioural abnormalities should be further investigated.

*KEYWORDS: Great apes, keeper opinion, food presentation, abnormal behaviour, fruit-free diets.*

**Obesity management in equid species at Marwell Wildlife, including a case study on the presentation and treatment of laminitis in Przewalski Horses *(Equus ferus przewalskii)***

*Jackie Squires, Justine Shotton*

*Marwell Wildlife, Colden Common, Winchester, England*
*Correspondence email: jackiem@marwell.org.uk*

Obesity is defined as abnormal or excessive fat accumulation that may impair health, and is one of the most common clinical presentations in captive equids. The provision of diets of too high calorific value, usually accompanied by a lack of exercise, are the key contributing factors to this condition. Four species of equid at Marwell Wildlife are currently being managed for obesity. This study describes the initial stages in the removal of all concentrate feeds from Marwell's standard equid diets, with the overall aim of maintaining animals on forage supplemented with a complementary vitamin and mineral lick. These processes, along with changes to pasture management, are part of a wider plan to try and mirror natural fluctuations in dietary intake and composition.

Moving away from an often more traditional approach of static concentrate rations year round, diets are now adjusted accordingly to mimic seasonality. Provision of reduced concentrate rations has been implemented with diets to date decreased by 52%, 45% and 64% for Grevy's Zebra *(Equus grevyi)*, Hartmann's Mountain Zebra *(Equus zebra hartmannae)* and Somali Wild Ass *(Equus africanus somaliensis)* respectively. Furthermore, Somali Wild Ass have been transferred from a more traditional equid concentrate nut onto a suitable forage balancer designed to supplement the diets of overweight equids. All diet reductions were carried out on a gradual basis with forage provision maintained for *ad libitum* consumption throughout.

Assessment of individual body condition is the key factor that determines how successfully animals have adjusted to these dietary manipulations, in conjunction with overall health status. Dietary modifications are then implemented based on the needs of the majority of animals within a given species, with specific adjustments made to individuals' diets as necessary (e.g. stalling separately for feeding to allow the provision of individually tailored rations).

In addition to adjustment of concentrate rations, we aim to manage paddocks in ways designed to reflect natural grazing areas by limiting high-volume, high-quality forage consumption, and minimising intake of high sugar material which can predispose to laminitis outbreaks. Investigation into the nutritional content of paddock grass and grass hay forages is in progress with preliminary data received from laboratory analysis.

It is predicted that this overall approach will parallel known wild diet variations and help to instigate a more natural seasonal fluctuation in body condition. The importance of this can be supported by literature, which suggests that seasonal changes in physiological and behavioural parameters found in the Przewalski horse are evidently under endogenous control. This strategy is designed to prepare the animal well in advance for predictable seasonal changes of climate and of availability and quality of food.

Over the last two years we have had a number of cases of chronic and severe laminitis in the Przewalski horse group, particularly in overweight individuals. This seems to be related to pituitary *Pars intermedia* dysfunction, as well as animals with hoof overgrowth and above desired body condition score. Laminitis is multifactorial, but it is hoped that a more natural seasonal approach to dietary provision and the subsequently improved body condition will help to reduce hoof overgrowth, reduce pressure on laminae and help to reduce the incidence of clinical debilitating laminitis in this species.

Furthermore, ongoing collaboration between both keepers and veterinary staff & nutritionists is helping to streamline the perception of desirable body condition.

*KEYWORDS: Body condition, concentrate feeding, equid, laminitis, obesity, pasture management.*

**Impact of starch and sugar addition into the diet on roughage intake by addax and Reeves's muntjac**

*Marcin Przybyło[1], Sara Dander[1], Karolina Krawiec[1], Alina Kloska[2] & Paweł Górka[1]*

*[1]University of Agriculture in Krakow, Faculty of Animal Science, Department of Animal Nutrition and Dietetics, Poland; [2]Silesian Zoological Garden, Chorzow, Poland*
*Correspondence email: m.przybylo@ur.krakow.pl*

It is recommended that grains and fruits should be excluded from diets for captive ruminants. Indeed, high intake of starch and sugars can be considered as an important factor having negative impact on the gastrointestinal tract functioning in ruminants kept in zoological gardens. This, in turn, may lead to a reduced intake of structural feeds, e.g. roughages – preferred sources of nutrients for this group of zoo animals. However, although starch intake by ruminants in the natural diet is rather negligible, sugar content in both browses and grasses can vary greatly, which may lead to substantial intake of sugars. Moreover, impact of starch and sugar intake in the diet may affect browsers and grazers differently. Therefore, the aim of the study was to determine the effect of additional starch, sugar or both (starch and sugar) supplementation in the diet on feed intake by addax (*Addax nasomaculatus*) and Reeves's muntjac (*Muntiacus reevesi*) – grazer and browser feeding type ruminants representatives, respectively.

Two studies were conducted, both according to Latin square design (4 × 4). In Study 1, four adult females of addax and in Study 2, four adult males of muntjac were used. Animals had free access to meadow hay (addax; Study 1) or dehydrated chopped lucerne (muntjac; Study 2). Additionally animals were fed a basal diet consisted of small portion of wheat bran (diet A), wheat bran and wheat (diet B), wheat bran and sucrose (diet C) or wheat bran, wheat and sucrose (diet D). Ground wheat was used as a source of starch and sucrose was used as a source of sugar in the diet. The amounts of wheat and sucrose were set to account for 15% and 2% (respectively) of estimated dry matter (DM) for each species prior to the study, respectively. Feed intake was controlled daily.

In Study 1, there was no effect of sucrose supplementation on DM intake of hay by addax ($P > 0.10$), while the addition of wheat to the diet reduced DM intake of hay ($P < 0.01$). However, total DM intake was not affected ($P > 0.10$) by wheat or sucrose supplementation. In Study 2, sucrose supplementation tended to decrease ($P < 0.10$) DM intake of lucerne by muntjac, but did not affect total DM intake ($P > 0.10$). On the other hand, wheat supplementation decreased ($P < 0.01$) DM intake of lucerne ($P < 0.01$) and total DM intake ($P < 0.01$).

In summary, the decrease in roughage intake due to wheat supplementation was greater for muntjac than addax, whereas sugar intake, in general, had less (muntjac) or no (addax) impact on intake of roughages by these species of animals. Therefore, if high intake of roughages by captive ruminants is fundamental for gastrointestinal health, then grains (starch) supplementation should be limited as it may have more negative impact on roughage intake than fruits (sugars).

*KEYWORDS: sucrose, grain, antelope, deer, grazer, browser*

**An investigation into the effects of dietary alterations on the behaviour and daily food intake of giraffes at Paignton Zoo Environmental Park.**

*Louise Cox[1], Siobhan O'Regan[1,2], Daniel Harrold[1] & Amy Plowman[1]*

*[1]Whitley Wildlife Conservation Trust, Paignton Zoo, UK.*
*[2]Plymouth University, Plymouth, UK.*
*Correspondence email: louise.cox@paigntonzoo.org.uk*

Giraffes (*Giraffa* spp.) can be a challenging genus to maintain diets for in captivity. Sub-acute mortality in giraffes has been reported and inadequate diets have been suggested as a contributing factor. It is therefore important to ensure diets are appropriate in order to prevent this.

An investigation was conducted to determine the effect of diet modifications on behaviour (daily activity budget and anticipatory behaviour) and voluntary food intake in a group of Rothschild's giraffe (*Giraffa camelopardalis rothschildi*) at Paignton Zoo, UK. Feeding routines were recorded over five conditions; 1) baseline (with no change from the regular routine), 2) increased Dengie hifi provision (a mix of chopped straw, lucerne and molasses; from an average of 3.6 kg/day to an average of 9.3 kg/day), 3) increased browse provision and 4) a change of pellet from Dodson and Horrell ruminant cubes to Boskos pellet (slightly adjusting the amount given to account for energy differences between pellets). Condition 5 was an unpredictable feeding schedule where the time that the giraffes were given access into their inside area (directly before feeding in the afternoons) varied between 15:39 and 16:20 hours, whilst in the other conditions (except baseline) the average time was 15:59 hours. During the unpredictable feeding condition, browse and lucerne provision was also increased due to concerns that some of the giraffes may have been underweight. The daily intake of lucerne and browse fed in the outside paddock was monitored for the whole group, whilst intake from the morning, evening and overnight lucerne haynets were recorded separately for the female group and male. Daily activity and stereotypic behaviours were recorded throughout all conditions. Anticipatory behaviour was monitored in all conditions except for the baseline, for the hour before the giraffes were given inside access in the afternoon. Additionally, cameras were used overnight to record the time that the lucerne feed was finished.

Dietary drift was seen in all conditions, with large variation seen in the amounts of food offered daily, e.g. cabbage provision ranged from 3800 g to 9161 g over the study period. As a group, time spent feeding and ruminating remained similar throughout conditions 2, 3, 4 and 5. Overnight, the feeders (containing Lucerne) were cleared on average before 22:30 hours, during conditions 2, 3 and 4. During condition 5, the unpredictable feed condition, food remained in the feeders until the morning for 80% of study days, which may be due to the increased browse and lucerne provision. This finding suggests that offering additional food may provide additional opportunities for giraffes to feed throughout the night and the amounts provided during this condition are more appropriate for the groups' foraging needs. Close monitoring of the giraffe diet at Paignton Zoo is ongoing.

*KEYWORDS: behaviour, feeding regimes, intake study, Rothschild giraffes.*

**Weigh and see - the relationship of body mass and body condition scores in European zoo elephants**

*Christian Schiffmann[1,2], Stefan Hoby[3,4], Marcus Clauss[1] & Jean-Michel Hatt[1]*

*[1]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich; [2]Elefantenhof Platschow, Germany; [3]Zoologischer Garten Basel, Switzerland; [4]Tierpark Bern, Switzerland*
*Correspondence email: c.schiffmann.elephantproject@gmail.com*

Regular body mass (BM) monitoring plays a key role in preventative health care of zoo animals. In some species including African (*Loxodonta africana*) and Asian elephants (*Elephas maximus*), the process of weighing can be challenging and alternative methods such as visual body condition scoring (BCS) have been developed.
We investigated the temporal development of both parameters regarding correlation patterns between them, and their sensitivity in dependence of an elephant´s life stage, in the nearly complete current European zoo elephant populations. While BM is more sensitive in calves and juveniles under the age of 8 years, both BM and BCS are considered equal in adult elephants. In elephants of advanced age (> 40 years), BCS might be more sensitive in assessing the physical status. Independent of species and sex, juvenile zoo elephants grow in BM nearly linearly with age, and reach a higher BM at an earlier age compared to free-ranging and semi-captive populations in the countries of origin. The BCS typically remains constant during this life stage, seemingly unaffected by growth. In adult animals, breeding females have a lower BM and BCS than non-breeders, and BM and BCS typically indicate fluctuations in the same direction.
Regular body mass recording in zoo elephants is strongly recommended in order to enhance our knowledge on body mass development and allow the formulation of practical recommendations. BCS presents a valuable and simple tool for complementary monitoring of an elephant´s condition, especially in adult and geriatric individuals.

*KEYWORDS: Body mass, visual body condition scoring, zoo elephants, monitoring tool*

**Browse preference in bonobos**

*Sarah Depauw[1], Geert P.J. Janssens[2], Jeroen M.G. Stevens[3,4], Guido Bosch[5]*

*[1]Odisee University College, Agro- and Biotechnology, Sint-Niklaas, Belgium; [2]Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;[3]Behavioral Ecology and Ecophysiology Group, Department of Biology, Antwerp University, Wilrijk, Belgium [4]Centre for Research and Conservation, Royal Zoological Society of Antwerp, Antwerp, Belgium; [5] Animal Nutrition Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands*
*Correspondence email: sarah.depauw@odisee.be*

In captive great apes, the feeding of leaves, twigs and bark of bushes and trees (browse) is often regarded as an extra on top of the daily ration. However, in the wild, a considerable amount of their diet consists of browse. In the frugivorous bonobo, 41% of the natural diet consists of leaves, herbs and bark. These food sources are high in fibre, a nutrient that is often insufficiently provided in captivity. Lack in fibre can induce health problems such as diarrhoea, constipation, obesity and cardio-vascular disease. Of equal importance is the positive effect of browse on mental health by providing satiety, increasing natural feeding behaviour and prolonging the time spend on feeding. A group of 7 bonobos housed in Planckendael was mostly fed willow. To enhance the daily provision of browse, it was questioned whether other browse species could also be introduced. We therefore examined the acceptance and preference of 5 available browse species in this bonobo group: willow, hazel, alder, bamboo and linden. Each new browse species was fed for 3 consecutive days allowing the bonobos to adapt to the new feed sources. When browse was offered, the animals were filmed for 30 minutes and time spent eating was recorded. Subsequently, all browse species were offered simultaneously during 8 days. Browse was provided in equal amounts and randomly positioned at two different locations per plant species. For each individual, first choice of browse was recorded. Additionally, we registered every 3 minutes what each individual was eating within the first 30 minutes after offering the browse. When provided separately, all browse species were consumed. However, when offered simultaneously, bamboo was never consumed within the first 30 minutes. The first choice was clearly willow (93%) but this did not result in willow being the only preferred browse species in this group. We did not record a significant difference ($p$ = 0.922) in intake between willow and the newly introduced linden, in contrast with the other browse species. Interestingly, when fed separately, bonobos spent more time eating linden and willow (25 min) in comparison with the non-preferred browse species (varying between 8 and 17 min).

Defining preferred browse species in bonobo groups, and potentially in other species, may be of importance for enrichment programmes aiming at prolonging feeding time. There are many hypotheses to explain browse preference in primates. One suggests that animals select for a certain nutrient composition, which reflects their dietary needs. Although seasonal changes in nutrient composition of browse are important to consider and can possibly change preference, this study showed a remarkable resemblance in fibre fractions between the two preferred browse species willow and linden (approximately 41% NDF, 30% ADF and 17% ADL on a dry matter basis). In contrast, bamboo leaves had 71% NDF, 33% ADF and only 6% ADL. Alder consisted of 41 %NDF, which is comparable with willow and linden, but had lower ADF (22%) and ADL (12%). The fibre fractions in hazel (49% NDF, 36% ADF and 20% ADL) were al higher than in willow and linden. Protein levels however were not markedly different among species offered. This could indicate a preference for a specific texture of browse, determined by fibre content, the influence of secondary plant compounds, or - in the case of bamboo - high silica levels.

*KEYWORDS: bonobo, Pan paniscus*, *browse, preference*

**Willow silage: what works and what doesn't?**

*Sarah Depauw[1], Annick Boeykens[2], Jannes Van Houcke[2]*

*[1] Odisee University College, Agro- and Biotechnology, Sint-Niklaas, Belgium*
*[2] Odisee University College, School of Technology, Department Chemistry, Gent – Belgium*
*Correspondence email: sarah.depauw@odisee.be*

Wintertime is a major challenge in European zoos housing browsers. At that time, the supply of fresh browse is scarce and the use of alternatives such as dried, ensiled or frozen browse is not widespread. An inadequate supply of browse during winter not only increases the risk of health issues, such as ruminal subacute acidosis and hoof problems, but also impairs natural behaviour and thus mental health in browsers. The production of browse silage is fairly cheap and easy. However, zoos indicate their lack of confidence in this technique; partly due to the variation of protocols used among zoos. Also, time consumption is an issue. This study compared therefore existing ensiling protocols for willow silage and also evaluated lucerne as a plausible additive to optimise fermentation processes. Additionally, willow was wrapped in bales to explore the possibility of commercialisation of browse silage. Short rotation willow fields, intended for biomass, were used as willow source. In total, 2 tons of willow twigs (< 0.8 mm Ø, *S. burjatica x S. viminalis*) were manually harvested in the beginning of June 2018. The willow was pressed into 30 L polyethylene drums with a self-developed automatic press machine (based on Lahance et al. 2012). In comparison with willow ensiled without additive, three types of additives were investigated: (1) molasses (4% of DM of willow) and a homofermentative silage additive ($10^8$ CFU/kg fresh willow); (2) nitrogen (flushing drum with 2 bar, during 1,5 min) and (3) fresh lucerne at a rate of 40% lucerne-60% willow. The rest of the willow was wrapped in 300 kg round bales by a local lucerne farmer. An extra layer of plastic was used to avoid piercing. Per protocol, 3 drums were sampled at 9 weeks, 6 months and 9 months of storage in a cool and dry place. Each sample will be analysed for nutritive value (including NDF, ADF and ADL), pH, temperature and fermentation products such as SCFA. In addition, Plate Count Agar is used to determine the total count of aerobic bacteria, whereas MRS Agar and *Sabouraud Dextrose Agar* are applied to cultivate *Lactobacillus* spp. and total yeast and mold, respectively. Additionally, a full mycotoxin scan will be performed after 6 months of storage before the start of the feeding trials. The use of additives did not significantly influence the total number of aerobic and lactic acid bacteria after 9 weeks of ensiling. However, additive (3) showed a marked increase in lactic acid bacteria ($3,1 \times 10^6$ CFU $g^{-1}$) compared to the control ($4,5 \times 10^5$ CFU $g^{-1}$). All additives decreased the growth of yeast and mold in willow silage. Compared to the control, containing $1,0 \times 10^4$ CFU $g^{-1}$ of yeast and 45,4 CFU $g^{-1}$ of mold, there was respectively a reduction of 83% and 60% for additive (1); 94% and 100% for additive (2); and 100% and 100% for additive (3). When using drums, zoos regularly detect fungal growth at the top layer of ensiled browse. The addition of lucerne seems a promising alternative to prevent this problem. Further analyses will reveal whether the addition of fresh lucerne improves the quality of ensiled willow compared with currently used additives.

*KEYWORDS: browse, willow, silage, additives*

**Determining nitrogen (protein) content of varying species of browse and herbage fed to western lowland gorillas (*Gorilla gorilla*) and bornean orangutans (*Pongo pygmaeus*) at Twycross Zoo.**

*Lydia Warren[1], Matyas Liptovszky[2], Clare Ellis[2] and Gavin White[1].*

*[1]Division of Animal Sciences, University of Nottingham, Sutton Bonington Campus, Leicestershire.*
*[2]Twycross Zoo, East Midlands Zoological Society, Burton Road, Atherstone, Warwickshire.*
*Correspondence email: Lydia.warren@nethereage.co.uk*

A wide variation of browse species is often fed to captive animals. The diet of captive western lowland gorillas (*Gorilla gorilla*) and bornean orangutans (*Pongo pygmaeus*) at Twycross Zoo is partially comprised of browse. Little is known about variations in nutritional quality between browse species. There is also minimal research indicating whether nutritional quality of browse is affected by seasonality, and the extent to which this variation affects behaviour exhibited by captive animals. As such, this research aimed to better characterise the nutritional value of browse (and herbage from within the enclosures) offered to both primate species at Twycross Zoo, with a specific focus on determining protein (nitrogen) content. Samples of browse and herbage (representing 13 species) were collected on and off-site, over a 4-month period (March – June 2018). Samples were dried until a constant moisture was achieved, then analysed using a combustion (Dumas) method, in conjunction with a CHNS elemental analyser. The nitrogen (N) content (g/kg) of a sample was determined on a dry matter (DM) basis. Due to sample amounts and number of replicates, 7 browse and herbage species were selected for further analysis. Behavioural observations were also conducted over several days to determine if behaviour following browse provision altered, depending on the species of browse provided. Observations were refined into distinct behavioural categories directly relating to browse, and a time budget was generated. Results indicated no significant difference in N content (g/kg) between browse species ($P = 0.138$), although N content (g/kg) of Ash (*Fraxinus excelsior*) was higher than that of Hornbeam (*Carpinus betulus*) (39.0 vs. 20.4 g/kg, respectively), or of a Lucerne (*Medicago sativa*) and hay mix (17.3 g/kg). A significant difference in N content (g/kg) was present, depending on the month of sample collection ($P = 0.04$), with a significant linear increase in N content (g/kg) across the sample collection period, from March – May ($P = 0.002$). Browse location (on vs. off-site) did not significantly affect N content (g/kg) of samples ($P = 0.351$). Behaviour in relation to browse provision was shown to alter between observations, however, it is not clear if this was due to browse species given, or due to extraneous variables, such as temperature, number of visitors, or noise levels at the zoo. The study findings have highlighted that N content (g/kg) of browse offered to captive primates can vary significantly, depending upon the effect of seasonality. A longer period of browse collection and analysis (e.g. over 12 months) would be warranted to further investigate how N content of browse fluctuates with season.

*KEYWORDS: Gorillas, Orangutans, browse, nitrogen, behaviour*

**The effect of pellet size on the feeding behaviour of ring-tailed lemurs (*Lemur catta*) and red ruffed lemurs (*Varecia rubra*) at Paignton Zoo**

*Jisri van Vliet[1], Amy Plowman[2]*

*[1]Van Hall Larenstein, University of Applied Science, [2]Paignton Zoo, Whitley Wildlife Conservation Trust*
*Correspondence email: jisrivv@gmail.com*

Nutrition is an important aspect of animal husbandry. Besides supplying food that matches the requirements of the concerning species, food presentation can also have a large impact on the animals' welfare. Making it harder to obtain food stimulates foraging behaviour, randomizing feeding times can reduce stereotypies, and giving whole vegetables rather than chopped ones can reduce aggression. Due to the potential benefits of food presentation, more research on this aspect could improve animal husbandry.
For this study the effect of different size pellets on the behaviour of lemurs was examined. Two different species were used, an all-male group of nine ring-tailed lemurs (*Lemur catta*) and a breeding pair of red ruffed lemurs (*Varecia rubra*), both located at Paignton Zoo Environmental Park. Three different pellets were fed for three weeks each (Dodson & Horrel leaf-eater, DK Zoological leaf-eater small, DK Zoological leaf-eater large). The amount of both DK zoological pellets was reduced slightly in order to adjust energy provision. During the testing period, the time spent feeding and the number of agonistic behaviours was noted in order to evaluate the effect of the different pellets.
Repeated measures ANOVAs were used to test whether the different pellets affected the behaviour of the lemurs. The total amount of time spent feeding differed significantly for both species, with an especially large difference in the ring-tailed lemurs. For both species the leaf-eater small pellet from DK Zoological resulted in the longest feeding times. A significant difference in agonistic behaviour was found in the ring-tailed lemurs, with the leaf-eater large pellets from DK Zoological resulting in the least amount. In the red ruffed lemurs, the lowest amount of agonistic behaviour was found when feeding the leaf-eater large pellets as well; however, this was not significantly lower than the other two pellets.
The harder consistency yet small size of the leaf-eater small pellets from DK Zoological probably accounted for the highest feeding times. The amount of agonistic behaviour was greatly reduced as well compared to the DH leaf-eater pellet, on which the highest amount of agonistic behaviours was recorded. However, the lowest amount of agonistic behaviours was obtained by feeding the large pellets. This appeared to be mainly due to the option to hoard the larger pellets. This allowed the lemurs to withdraw to a preferred spot and eat the pellets there, reducing the number of conflicts over food.
The leaf-eater small pellets appear to be the best option with the longest feeding time and relatively low agonistic behaviours. However, when looking to reduce aggression within a group, the leaf-eater large pellets may be the preferable option. In conclusion, pellet size can effectively alter both the feeding time and aggression but also the way the animals will feed.

*KEYWORDS: lemur, pellet size, feeding time, aggression*

**Body mass fluctuations in adult zoo elephants - relationship with breeding status and molar progression**

*Christian Schiffmann[1,2], Stefan Hoby[3,4], Jean-Michel Hatt[1], Daryl Codron[5,6] & Marcus Clauss[1]*

*[1]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich; [2]Elefantenhof Platschow, Germany; [3]Zoologischer Garten Basel, Switzerland, [4]Tierpark Bern, Switzerland; [5]Florisbad Quaternary Research Department, National Museum, South Africa; [6]Centre for Environmental Management, University of the Free State, South Africa*
*Correspondence email: mclauss@vetclinics.uzh.ch*

Elephants do not replace deciduous teeth once with permanent teeth as most mammals, but replace a single cheek teeth per jaw-side five times in their lives in a process called molar progression. While this gradual process has been well-documented for the purpose of age determination, a less-considered possible side effect of this progression is that functional chewing surface fluctuates, being larger when two cheek teeth are both partially in use and smaller when only one cheek tooth is used fully.
We found that body mass of both breeding and non-breeding female zoo elephants (*Elephas maximus* and *Loxodonta africana*) shows a cyclic undulation with peaks separated by many years, which is therefore unrelated to reproduction or annual seasonality.
We propose variation in functional chewing surface, resulting chewing efficiency, and resulting increased food intake and/or digestive efficiency as the underlying cause. As elephants reproduce all year-round and thus are not synchronized in their molar progression pattern, climate-related fluctuations in resource availability are likely to mask this pattern in free-ranging animals. In contrast, it emerges under the comparatively constant zoo conditions, and illustrates the relevance of the dental apparatus for herbivorous mammals.
Over the lifetime of a zoo elephant, when assessing the animal's body condition and body mass, this cause of potentially natural cyclicity should be kept in mind.

*KEYWORDS: Elephant, body mass, growth, molar progression, breeding*

**Difference in prey preference as cause for difference in husbandry? The wolf versus the jackal, a literature review**

*Han Opsomer[1], Annelies De Cuyper[1], Marcus Clauss[2] & Geert P.J. Janssens[1]*

*[1]Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University [2]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich*
*Correspondence email: han.opsomer@gmail.com*

The wolf (*Canis lupus*) and the golden jackal (*Canis aureus*) show a great number of behavioural, morphological and physiological similarities. Both hunt prey, cache food, are found in similar habitats, can live in packs, and mark out territories. However, they show notable dissimilarities in their prey preference, hunting strategy, use of their habitat, social organisation and feeding behaviour.

Even though both the wolf and the jackal are known to hunt the same species, the wolf prefers large prey to small and medium sized animals, opposite to the jackal. The difference in preference explains why the wolf has adopted a feast-and-famine lifestyle compared to the frequent small meals which the jackal ingests. In addition, the wolf can be categorised as a true carnivore, facultatively specialising to hunt favoured prey, in contrast to the jackal which is a true omnivorous generalist. Striking is that this difference in diet appears to determine the other discrepancies between these two canids.

Consuming a wider variety of food sources, the jackal is more adaptable to different environments both in close proximity to, and remote from, human civilisation. As it mainly targets smaller prey, it is more hindered by impediments like bushes or snow during a hunt. The wolf on the other hand is more successful in forest areas and in strong winters, compared to open plains and no snow, as they are less obstructed than their large prey is. The difference in preference also explains why wolves are more selective hunters, which use a more pursuit driven strategy, opposed to the searching strategy used by jackals. As prey size increases, so does the risk of a takedown. Since the danger of a hunt is significantly smaller in jackals, there is no need for them to minimise their chances of getting severely injured. Similarly, the energy expenditure of a kill also increases with prey size. This, and the increased risk of injury, causes wolves to benefit more from pack hunting than jackals, which are generally found to search for food on their own even when living in packs. Remarkably, in times of scarcity, wolves and jackals are known to adapt their approach towards the one preferred by the other species, as a consequence of wolves going for smaller, and jackals for large prey.

The dissimilarities in feeding strategies imply differences in these captive canids' husbandry to implement a more natural feeding behaviour. This would appear easier for jackals. As they spend most of their energy on the search for a wide variety of food sources, a highly variable diet, hidden in the animals' enclosure in unpredictable places does mimic their normal free-ranging behaviour. For wolves on the other hand, it is difficult to recreate situations in captivity resembling the pursuit and subduing stages of a wild hunt. As a consequence, a major element of the wolf's behavioural repertoire and energy expenditure cannot be accounted for. Looking at other aspects of the wild wolf's feeding strategy, different possibilities to address this discrepancy might be suggested. A copious highly nutritious meal could be followed by a longer period of administering low value food sources like bones, hide and cartilage which in the wild would be cached for later consumption. Since free-ranging wolves can survive on smaller prey as well, another option is to implement a strategy similar to the one suggested for jackals. Further research is warranted to examine which approach would be more beneficial and if this would be applicable for other feast-and-famine carnivores.

*KEYWORDS: Body mass – Feeding strategy – Jackal (Canis aureus) – Wolf (Canis lupus)*

**Investigating nutritional provision and influence of dietary composition on behaviour in a commonly-housed parrot -- a multi-zoo study on blue-and-gold macaws *(Ara ararauna)***

*Paul Rose[1], Leah Phillipson[2], Elaine McIlroy[2] & Kerry Hunt[2]*

*[1] Centre for Research in Animal Behaviour, Washington Singer Labs, University of Exeter, Perry Road, Exeter, Devon, EX4 4QG, UK [2]University Centre Sparsholt, Sparsholt, Winchester, Hampshire, SO21 2NF, UK*
*Correspondence email: p.rose@sparsholt.ac.uk*

Nutritional analysis of zoo bird diets can be tricky due to the large number of ingredients that are often fed. Such “canteen style” diets can lead to nutritional and behavioural problems when individuals display high selectivity for favoured (but potentially nutritionally poor) items or when the mix of ingredients does not fully replicate a wild-type feeding strategy. Past research documents a range of behavioural problems in captive parrots, some of which show link to poor nutrition and limited foraging abilities. The aim of this research was to i) assess the nutritional profile of diets fed to birds across several UK zoos and ii) determine the potential influence of diet on key state behaviours of captive blue-and-gold macaws.
Nutritional data were collected from six UK zoos in 2015. Both nutritional and behavioural data were collected from four zoos in 2017. A total of 25 hours of behavioural data, collected via instantaneous focal sampling, per macaw was recorded.
We evaluated the behaviour and nutritional analyses for the zoos present in both years and compared diet composition across all zoos for both years. The nutritional content of seeds, pellets, fruits and vegetables was determined using a mix of proximate analysis and the Zootrition database. Across all zoos, diets differed widely in the amount of food provided per bird, as well as in the proportion of ingredients. Some zoos fed less than 5% seeds and nuts in the total mix, whereas other zoos fed nearly 70% seeds and nuts. Over 90% of the diet of one zoo was fresh fruits, greens and vegetables. Nutritional analyses of ingredients (not including cuttlefish) showed the majority of diets fed to be higher in phosphorous than calcium, but there was no consistency between zoos regarding levels of crude fibre, crude fat or crude protein. Analysis of key state behaviours shows feeding and foraging to be consistently low across the zoos studied (minimum 3% total time, maximum 12%). As all macaws were inactive for the highest proportion of their activity budgets (minimum 40%, maximum 57%), zoos should consider the impact of diet presentation and constitution on behavioural diversity in this species. Stereotypic behaviour was low in our sample, with macaws at only one zoo displaying abnormal behaviour for less than 6 minutes/hr of the observation period. This zoo fed a diet of a commercial seed-based parrot mix with a range of fruits (56% seed mix and 44% fruit) and was the only zoo to feather-clip its birds to prevent escape. As wild parrots have been documented as foraging for the majority of their daytime activity budgets and wild foods can require longer handling time pre-consumption, zoos should consider the presentation of diets to increase overall time spent feeding to positively impact behavioural diversity. Of the zoos where behavioural data were collected, only one provided food in an “enriching manner”, with all others providing food in metal bowls. We suggest future research into food handling and foraging activity to see how pelleted rations and seed mixes can be presented in a more novel or interesting way that may decrease time spent inactivity. Research that assesses intake and wastage would also be useful to provide zoos with evidence of how much to feed, at specific times of the year and to allow for changes in physiology and development to prevent wastages and save on costs.

*KEYWORDS: Macaw, animal behaviour, dietary survey*

**African elephant (*Loxodonta africana*) serum glucose, serum insulin and fecal glucocorticoid concentration fluctuations over a 14-month period with differences between two laboratories for serum glucose**

*Kimberly Ange-van Heugten[1], Jordan Wood[1], Troy Tollefson[2], Janine Brown[3] Larry J Minter[4]*

*[1]Department of Animal Science, North Carolina State University, [2]Mazuri® Exotic Animal Nutrition, PMI Nutrition, Land O' Lakes, Inc. St. Louis, MO, 63039, [3]Smithsonian Conservation Biology Institute, Front Royal, VA 22630 [4]North Carolina Zoo, 4401 Zoo Pkwy, Asheboro, NC 27205*
*Correspondence email: kim_ange@ncsu.edu*

Prolonged elevation of blood glucose has toxic effects on several body systems, and therefore needs to be carefully regulated. Insulin is the main regulator of blood glucose levels, but some individuals become insulin resistant with a decreased ability of cells to respond to insulin. A glucose-to-insulin ratio (G:I) can be used to determine the effects of glucose and/or insulin if fasting is not possible.
Poor reproductive function in African elephants *(Loxodonta africana)* has been associated with higher concentrations of insulin and a lower G:I. A low G:I has also been associated with decreased exercise, poor diet diversity and increased body condition scores. The objective of this study was to evaluate how serum glucose, serum insulin, G:I, and fecal glucocorticoids fluctuate over time in African elephants, and then to compare two laboratory evaluations of serum glucose concentrations (and resulting G:I differences).
Serum glucose, insulin, and fecal glucocorticoids in monthly samples from six African elephants at the NC Zoo were measured at the Smithsonian Conservation Biology Institute between February 2016 and April 2017. Additionally, glucose was run in house at the NC Zoo for laboratory comparison.
While both labs reported glucose ranges within the published reference levels, the two labs differed in their glucose measures by a range of 10.6 – 20.5%. Both labs reported males with higher glucose and G:I ratios than females (P=0.001) and when analysed by season, autumn glucose concentrations were higher than winter for both labs (P=0.001). Males had lower serum insulin levels. Additionally, the six individual animals differed with respect to their glucose and insulin concentrations.
The large differences between glucose concentrations measured by the two laboratories resulted in major G:I differences. Because the G:I can be used as predictive measures for reproductive and body condition health, it is important that the scientific community considers using the same lab techniques. The fecal glucocorticoid concentrations (153.9 ±32.33 (SD) ng/g) in the male and female elephants of this study were near the range, albeit higher, of a recently reported average for this species (107 ng/g). This difference may need to be further reviewed.

*KEYWORDS: glucose, insulin, African elephants, fecal glucocorticoids*

**The utility of dried blood spots for the assessment of avian Vitamin D3 status compared with plasma analysis**

*Jenny E. Jaffe[1], Amanda Ferguson[1] and Christopher J. Michaels[1]*

*[1]Zoological Society of London, London, NW1 4RY, United Kingdom*
*Correspondence email: amanda.ferguson@zsl.org*

There is a high incidence of metabolic bone disease (MBD) in many vertebrate species kept in captivity. Methods to prevent this disease include diet review and reformulation to balance the calcium, phosphorous and vitamin $D_3$ as well as ultraviolet-B (UVB) provision to stimulate endogenous production of vitamin $D_3$. To assess the success of these interventions in raising vitamin $D_3$ status in the blood, the most commonly used method is analysing a relatively large volume of liquid serum for vitamin $D_3$ (serum analysis). Dried blood spots (DBS) have been used widely in human samples to assess vitamin $D_3$. The small blood volume needed and potential for syringe free sampling in field situations made this an attractive method for use in small taxa or in remote locations. However, DBS has not been validated in non-human species and so its utility is unknown; nevertheless a small but growing literature providing reference values and husbandry recommendations relies on this unvalidated method.

We assessed the vitamin $D_3$ status of adult corncrakes (*Crex crex*) forming part of the conservation breeding population for this species in Britain using both DBS and 'gold standard' serum/plasma analysis. The serum/plasma analysis provided a reference range (mean ± standard deviation (range): 19.7 ± 6.5 (7.9-30.9) nmol/L for plasma vitamin $D_3$ in corncrakes, which we compare with ranges known for other avian taxa. We used the results to assess the utility of DBS as a diagnostic tool. We found that DBS gave significantly higher results than serum and although the two datasets were highly correlated, analysis of the residuals showed significant systematic and random bias in the analysis.

These data suggest that with the current analytical methods the DBS method is not good enough at predicting plasma vitamin $D_3$ and thus not yet a useful diagnostic tool for corncrakes. Therefore, DBS results for vitamin $D_3$ from non-human blood samples should be treated with caution until validation in a range of taxa can be undertaken.

*KEYWORDS: Vitamin $D_3$, dried blood spot, corncrake*

**Can herbal nutraceuticals benefit group housed rats used within zoos and exotic collections for school-group education?**

*Lauren Samet[1] & Wanda McCormick[2]*

*[1]University of Northampton, UK; [2]Anglia Ruskin University, UK*
*Correspondence email: lauren.samet@northampton.ac.uk*

Rats are commonly kept in social groups within enriched environments for students to get 'hands on' experience with an animal whilst visiting a zoo or exotic collection. The reason for their popularity relates to their size, trainability and domesticity, making them suitable for even inexperienced students to pet and handle. As with any animal a certain level of stress may be associated with handling from unfamiliar people; handler experience can also affect the experience of handling for the rat.

As part of a larger study investigating effects of herbal nutraceuticals on captive animal welfare, ten female rats from an exotic collection took part in four separate five-week feeding trials to ascertain whether feeding herbs renowned traditionally for their calming properties (Chamomile, Vervain, Lemon Balm and Skullcap) had an impact on anxiety-based behaviours and motor-coordination (to differentiate between sedative and anxiolytic impact). Acting as their own control, subjects (living together within an enriched enclosure) were fed the herb being trialed via suspension in a gelatin cube alongside their normal diet daily for a period of four weeks following a control week, within which their normal diet and a plain gelatin cube were fed daily. During week one and week five of the trials, subjects' behaviours were measured using a test battery comprising the open field test (OFT), elevated plus maze (EPM), a balance beam pole test (BBPT) and an ease of capture score.Methodology was designed from that commonly used in anxiety studies using laboratory rodents.

There were no significant results across the test battery that indicated a direct reduction in anxiety within the subjects. However, the lemon balm trial revealed a significant increase in percentage of time spent within the centre of the OFT ($p = 0.033$) (rodents tending to display periphery preference when nervous). This alone was not enough to determine whether a change in anxiety levels had taken place. The impact of habituation could not be underestimated when evaluating the results; however the OFT and EPM test designs were based around rodent preference to avoid open spaces in any situation. Anecdotally, social barbering behaviours appeared to decrease during Vervain feeding, noted by the regrowth of hair between the ears of subjects that were previously effected. The BBPT indicated no significant impacts on motor coordination, which indicated that at the amount fed daily there was little to no evidence of sedation (an ethical implication for long term use), something previously undetermined in similar studies.

Over the course of the trial period, ease of capture scores improved for four of the ten subjects, remained the same for three and deteriorated for one subject, with two not completing the entire trial period due to health reasons. Ease of capture and researcher observations highlighted a clear difference in bold / shy temperaments amongst the group. Herbs did not affect the scores in this instance, reinforcing their role as a complementary dietary supplement only and not as a substitution for alternative strategies to reduce stress or as a "miracle cure" for temperament.

The results provide a direction for future behavioural nutraceutical research. The trials indicated the amount of herbal supplement provided daily was of a safe level without any severe signs of compromise to motor function, but with the possibility of 'calming' dominance behaviours in one feeding regime. Future research should explore the use of a crossover study and a larger sample size to further distinguish habituation from herbal effects.

*KEYWORDS: Rats, anxiety, herbs, behaviour, nutraceutical*

**New developed soft feed pellets with insects for exotic animals**

*Marleen Vrij[1], Joeke Nijboer[2]*

*[1]NGN Products, Den Bosch, The Netherlands; [2]Nijboer Consultancy, Bergschenhoek, The Netherlands*
*Correspondence email: mclauss@vetclinics.uzh.ch*

Animals in the wild eat soft and squeezable products. In captivity, animals are provided hard and dry pellets. Complaints about the hardness of pellets triggered NGN to think about an alternative feed which is soft and easy to ingest. For that reason, NGN has developed an own process and recipe to imitate the texture of natural diets. Drying is a very expensive and energy consuming processing step. The soft feed is produced by a new developed process which omits the drying step. To keep the feed stable, a mixture of organic acids, minerals and herbs are added to the feed.
Fresh and wet ingredients are converted into soft pellets, with moisture levels between 20% and 35%, which make them soft and flexible. Typical commercial pelleted diets have a moisture level around 12%. The product can be stored at ambient temperatures and has a shelf life of nine months.
High moisture products contain water-soluble substances, which gives the feed a more distinct taste compared to dry feed. The amino acid and fatty acid composition of insects are generally indicated as nutritious and healthy and can be compared with fishmeal.
The use of alternative ingredients like fresh insects, herbs and algae make the feed very palatable for carnivore, insectivore and omnivore animals. Feeds can contain up to 50% insects, depending on the target animal for which the feed is made.
Feeding high moisture feed to pigs, rats, ruminants and other animals have shown to increase the efficiency of protein digestion; due to the high moisture, gastric enzymes are more likely to penetrate foodstuffs more easily than dry feed. Although such research has not been done with zoo animals, it is likely that moisture has similar effects in zoo animals as in domestic species.
Potential benefits of using high moisture feed are that young animals accept the feed more easily than dry and hard pellets. Recently, soft feeds with insects have been tested with squirrel monkeys in several zoos; the soft pellets were preferred above the dry pellets. Also bearded dragons consumed the new developed herbal soft pellets. Many literature sources back up the idea of higher feed intake and feed conversion with increased moisture levels in the feed.

*KEYWORDS: Soft pellets, insects and alternative ingredients, herbs*

**Devising a hippopotamus (*Hippopotamus amphibius*) handrearing formula**

*Barbara A. Henry[1], Michael L. Power[2], Michael T. Maslanka[3] & Jenny A. Nollman[1]*

*[1] Cincinnati Zoo & Botanical Garden, Cincinnati, OH, 45220, USA. [2]Smithsonian Conservation Biology Institute, Conservation Ecology Center, Nutrition Laboratory, National Zoological Park, 3001 Connecticut Ave., Washington, DC 20008, USA. [3]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA*
*Correspondence email: barbara.henry@cincinnatizoo.org*

In January 2017, the Cincinnati Zoo & Botanical Garden was challenged with handrearing a six-week premature hippopotamus (*Hippopotamus amphibius*). Gray (1959) published nutritional parameters from a hippo milked at eleven weeks of lactation.
Using the data from Gray (1959) as a base, a formula was devised. Due to training techniques established during pregnancy, milk could be expressed from the dam on days 1, 3, 8 and 9 before the dam appeared to end lactation. Samples were sent to the Milk Repository in the Department of Nutrition Science at the Smithsonian National Zoological Park and Conservation Biology Institute. The fat content of dam milk increased from day 1 to day 9, while the sugar, protein, and ash contents remained relatively stable. The mean values for dam's milk at days 8 and 9 were 84.5% water, 7.3% protein, 4.4% sugar, 3.0% fat and 0.7% ash with a calculated GE of 0.87 kcal/g.
Based on the nutrient values of these samples, the formula was adjusted to more closely mimic dam's milk. The formula contained: water, Zoologic® milk matrix 33/40 and 42/25 with a small percent of a 50% dextrose solution. Due to premature birth and digestive issues, the formula was offered initially at a diluted concentration and increased slowly ensuring consistent weight gain. The final formula was reached after three months of increasing the concentration of solids. The final formula which had minimal digestive issues and successfully maintained growth was 88.5% water, 4.7% protein, 3.0% sugar, 3.3% fat and 0.6% ash. As a proportion of solids, protein accounted for 40.5%, sugar 25.9%, fat 28.4% and ash 5.2%, consistent with our estimate's for dam's milk.

*KEYWORDS: Hippopotamus, handrearing, milk*

*Gray, R.F. 1959. Hippopotamus milk analysis. Int. Zoo. Yb, 1:46*

**Protocol for hand-rearing of flamingo chicks**

*Clare Wylie[1], Cathrine Sauer[1], Wayne Mcleod[1], Andrew Owen[1]& Mark Vercoe[1]*

*[1]Chester Zoo, North of England Zoological Society, United Kingdom*
*Correspondence email: c.wylie@chesterzoo.org*

In 2018 it was decided to take all Caribbean flamingo (*Phoenicopterus ruber*) eggs at Chester Zoo for hand-rearing. In previous years, hand-rearing diets used at the zoo had included ingredients like blended fish, shrimp, hard-boiled egg yolk, adult flamingo pellets and occasionally also baby cereal. These diets had been quite time consuming to prepare, messy and often needed to be adapted and changed as the chicks grew. Thus, it was decided to review our protocol for flamingo hand-rearing.

The composition of flamingo crop milk is highly variable and changes with the age of the chick, but contains on average 35% crude protein and 58% fat. To mimic this composition, a simple egg-based hand-rearing diet with 37% crude protein and 49% fat was developed and tested by Dierenfeld et al. (2009). It was decided to test this hand-rearing diet and recommended protocol. However, the recipe had to be altered slightly to accommodate differences in availability of ingredients; the main difference being the use of Avimix (Vetark Professional) instead of pure vitamin E, which could not be sourced. The same procedure as stated in the original study was used to make and store the diet. Some of the American institutions changed to using powdered egg during their hand-rearing as they found it less time consuming. However, powdered egg was not readily available in the UK, so fresh eggs were used throughout. In the original protocol, the recommendation was to have 5 feeds per day. However, due to practical considerations, this was reduced to 4 feeds daily; approximately at 07:30, 11:30, 15:00 and 19:00 hours. The diet was tube fed to chicks using soft flexible tubing, cut to suitable lengths for each individual bird, increasing in size as the chicks grew, and attached to plastic syringes. The number of feeds per day were gradually reduced as the chicks started to self-feed following introduction of the adult flamingo pellet at day 10-11. Floating pellets were always offered first thing in the morning prior to tube feeding to encourage the chicks to start self-feeding. The pellets were presented in bowls filled with water. The volume of tube food given varied between individual feeds depending on the level of crop fill as evaluated by the keeper during feeding.

In total, 14 healthy chicks were fed to weaning using this hand-rearing diet and feeding regime. The average age at weaning was 43 ± 5 days (range 32-5066 days), by which age the chicks had reached a body mass of 1145 ± 245g (range 667-1573g). On day 0, the chicks weighed 96.3 ± 9.1g (range 86.3-118.0g) and grew with an average daily rate of 24.6 ± 4.5 g/day (range: 18.1-33.8 g/day) which was equivalent to 6.1 ± 0.5% (range 5.3-7.0%) of their body mass. The feeding schedule was tailored to each individual chick depending on its growth and ability to self-feed, but across all chicks the average number of feeds was gradually reduced as follows: 4 feeds given on day 1-12, 3 feeds given on day 12-20, 2 feeds given on day 21-26 and 1 feed given from day 27 onwards to weaning.

Compared to the study by Dierenfeld et al. (2009), the Chester Zoo flamingo chicks grew slower (6.5-6.8 vs. 6.1% of BM found in this study). This was probably due to the fact that both total daily volume and the number of daily feeds were higher in the previous study. The chicks of the present study were self-feeding sufficiently to be weaned at an earlier age and at a lower body mass the in the previous study. Pellet intake in this and in the previous study were not quantified, but the early weaning age in this study may have been a result of offering pellets in the morning before the chicks were tube fed compared to offering pellets after tube feeding as was the schedule in the previous study. Overall, the altered version of the protocol used proved to be less time consuming than the original protocol, but still achieved satisfactory weight gain and survival of the chicks.

*KEYWORDS: hand-rearing diet, growth*

*Dierenfeld et al. (2009) A simple and effective egg-based hand-rearing diet for flamingos. In: Zoo Animal Nutrition IV. Filander Verlag Fürth, Germany, pp 37-46.*

***The challenges, opportunities and status of animal welfare research in zoos and aquaria***

*Sally Binding[1], Holly Farmer[2], Laura Krusin[2], Katherine A. Cronin[3]*

*[1]European Association of Zoos and Aquaria (EAZA) Executive Office, Amsterdam, the Netherlands; [2]Whitley Wildlife Conservation Trust / Paignton Zoo, UK; [3]Lincoln Park Zoo, USA*
*Correspondence email: sally.binding@eaza.net*

Research into the conditions that promote positive animal welfare is essential in order for institutions to be armed with the knowledge necessary to create environments in which animals thrive. In order to determine trends and gaps in the empirical information available regarding animal welfare in zoos and aquariums, we conducted a systematic literature review of the primary peer-reviewed journals publishing zoo-based and welfare-based research: *Journal of Zoo and Aquarium Research*, *Journal of Applied Animal Welfare Science*, *Applied Animal Behaviour Science*, and *Zoo Biology*.

The literature review spanned 2013-2017 and revealed that 18% of 2142 publications in these journals were zoo-based and welfare focused; behaviour was by far the dominant welfare indicator utilised. The dominant topics studied included enrichment, social conditions and enclosure design while understudied topics included the welfare of ambassador animals, and the welfare impacts of noise and lighting. Taxonomic representation in these publications was notably skewed, with 92% of studies being vertebrate-focused (great apes being the dominant taxa).

We consider these findings alongside several challenges and opportunities inherent to conducting empirical research in zoos and aquaria, and hope this work helps demonstrate both the available knowledge and areas for more research emphasis moving forward.

*KEYWORDS: Animal welfare, research, zoos, aquaria*

***EAZA Animal Welfare Working Group***

*Sally Binding[1], Graeme Dick[2], Lisa Holmes[3], Holly Farmer[4]*

*[1]European Association of Zoos and Aquaria (EAZA) Executive Office, Amsterdam, the Netherlands; [2]Longleat, UK; [3]Chester Zoo, UK; [4]Whitley Wildlife Conservation Trust / Paignton Zoo, UK*
*Correspondence email: sally.binding@eaza.net*

The EAZA Animal Welfare Working Group (AWWG)'s mission is to support and advise ex situ programmes, Taxon Advisory Groups (TAGs) and EAZA committees and working groups in animal welfare best practice through evidence-based, animal welfare science, to promote positive animal welfare throughout all EAZA institutions.
In support of the EAZA Strategy our projects are:

- Development of an Animal Welfare Assessments Library and assessment choice tool;
- Liaise with TAGs through animal welfare representatives to distribute species-specific, evidence-based research;
- Organisation of EAZA's first Animal Welfare Forum, 2020.

The AWWG has 11 Members and Official Advisors, spans seven countries and draws expertise from the zoological industry, the academic field and outreach education sectors. We are seeking engagement from the EAZA Membership to aid us in successfully meeting our objectives for the EAZA community.

*KEYWORDS: EAZA, Animal Welfare Working Group*