Optimizing the nutrition of captive Eurasian otters (*Lutra lutra*)

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Katrin A. Ruff

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Abbreviations

AD	apparent digestibility
A/C	quotient of allantoin to creatinine
AM/C	quotient of ammonium to creatinine
BM	body mass
BMR	basal metabolic rate
Ca	calcium
Cr_2O_3	chromium oxide
d	day
DE	digestible energy
DM	dry matter
g	gram
GE	gross energy
IU	international unit
K	potassium
kg	kilogram
kJ	kilojoules
ME	metabolizable energy
mg	milligram
min	minutes
mmol/l	millimole per litre
µmol/l	micromole per litre
MTT	mean transit time
Na	sodium
NE	net energy
nm	nanometre
n.s.	not significant
P	phosphorus
U/A	quotient of uric acid to allantoin
U/C	quotient of uric acid to creatinine
W	watt
Zn	zinc

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INTRODUCTION

Introduction

The Eurasian otter (*Lutra lutra*, Linné, 1758), a member of the Lutrinae, belongs to the family of the Mustelidae within the order Carnivora (MASON and MACDONALD 1986). The Lutrinae are included in the suborder of the Fissipedia, but are all semi-aquatic. *Lutra lutra* lives in fresh or salt waters, rivers, streams, lakes, marshes and along sea coasts (KRUUK 1995). Many morphological adaptations help them to forage efficiently in aquatic habitats (CHANIN 1993).

Although the species is often called "fish otter", it is not specialised exclusively on fish. Otters forage opportunistically on various prey species as obligate carnivores in water as well as on land. Beside fish, the prey spectrum contains amphibians, crayfish, reptiles, birds, small mammals, insects and molluscs (MASON and MACDONALD 1986). The composition of the prey species varies with availability and seasons. By looking at the consumed biomass, fish is in many habitats the most important food source with a preference for the slower-moving species due to a better hunting success on them (KRUUK 1995). Often the smaller individuals within a species seem to be preferred, but supposedly because they are more in number and easy prey. The common prey fish length is reported from 3 to 20 cm (ADRIAN and DELIBES 1987, HANSEN and JACOBSEN 1992, KNOLLSEISEN 1995, HOFMANN and BUTZECK 1992). Prey choice was also described for *Lutra lutra*. GEIDEZIS (1999) showed a species specific selection in a commercially-used pond area against carp although carp was available in huge amounts and is easy to hunt.

Otters forage actively, searching for and pursuing their prey, never lying in wait for it. Under water, they swim in all directions to find fish and forage under stones, amongst weed beds and roots in order to flush out prey (REUTHER 1993). Otters use sight and touch for foraging. Because most otters are nocturnal and the water is often turbid, the tactile sense seems to be the most important. They have large vibrissae that manage to detect prey without visual help (ERLINGE 1968). Water-birds are seized by swimming right under them and dragging them below the surface. Once captured, small prey is often already eaten at the water surface but larger fish which are more difficult to handle, are eaten on land with the help of the fore legs (CARSS et al. 1990). Mostly the prey is ingested as whole, consuming bones or fur of small mammals like mice completely. Exceptions are species with parts that can hurt the otter

like the palatal teeth of pike and poisonous or inedible parts, like the skin of toads, are left beside (RUFF 2003).

Otters are normally diving for around 30 seconds but dive time can reach up to 5 minutes going in depth down to 14 m (KRUUK 1995, FESTETICS 1982). Even in cold water, the body temperature is maintained around 38°C, resulting in a high metabolic rate (CHANIN 1993, KRUUK 1995). For thermal insulation otters rely almost entirely on their fur and air which is trapped inside the under-fur; they have no insulating subcutaneous fat layers (KRUUK 1995).

The Eurasian otter is polyoestrous and females can have young at any time of the year (KRUUK 1995). Both sexes usually become sexually mature at about two years of age (CHANIN 1993). The mean age of otters in the wild is expected in Scotland to be 2.7 years, on Shetland islands 3.1 years (KRUUK 1995). Captive otters can reach an age up to 20 years, although the mean age is 4.2 years (MELISSEN 2000).

With the beginning of the 19th century, the Eurasian otter inhabited the whole of Europe, large parts of Asia and even the northern part of Africa (FOSTER-TURLEY et al. 1990, NEL and SOMERS 1998). With the severe decline of Eurasian otter populations mainly in European countries over the past five decades, the species is now classified as highly endangered (MASON and MACDONALD 1986, REUTHER 2004).

Due to the endangered status, *Lutra lutra* is often kept in captivity. To foster captive reproduction attempts in European zoos, the species is part of the European Endangered Species Programme (EEP) of the European Association of Zoos and Aquaria (EAZA). But *Lutra lutra* is also maintained in many non-European countries all over the world from Russia to Kuwait. The successful reproduction of otters in keeping institutions is important to have an ex-situ population available which is at least self-supporting. This prevents zoos and wildlife parks to remove otters from the wild. It also enables them to provide animals for reintroduction programmes which are currently conducted e.g. in the Netherlands. Additionally, reproduction success will facilitate more zoos to keep otters what will result in a wider community that is familiar with the species and will become aware of the need of conserving the otter and its habitats (MELISSEN 2000). Furthermore, otters are attractive zoo animals because of their agility in the water and on land as well as the maintaining of play instinct till old-age (REUTHER 1991).

For a successful breeding of the species in captivity, the keeping conditions have to be optimal. But there are still problems in the husbandry of this species. The breeding success is not optimal. While numerous institutions have no reproduction success although pairs are kept, some institutions breed without problems; the reasons for these differences being unclear. The demand for otters exceeds the number available (REUTHER 1984, MELISSEN 2000).
Often health problems occur in captive Eurasian otters. The studbook data show that internal causes of death of captive otters are, besides perinatal problems, infections and diseases of the respiratory system, mainly diseases of the kidney and urinary system, digestive system and of the liver and biliary system (MELISSEN 2000). Deficiencies of calcium and vitamin A, E and B were reported (DUPLAIX-HALL 1972, TSCHIRCH 1978, AULERICH et al. 1995). Several diseases indicate nutritional causes and disorders.

Adequate nutrition is an essential for optimal husbandry. Improper feeding can severely affect health and well-being of captive animals (HATT, 2000). Improved nutrition has often positive effects on longevity, disease prevention and resistance, growth and reproduction (RATCLIFFE 1963, DIERENFELD 1997).
Over 46 nutrients have been identified as essential to the health of humans and other vertebrates. Besides basic nutrients like e.g. proteins that are essential to all vertebrates, requirements are species specific. When these nutrients are not supplied in appropriate amounts and forms, decreased disease resistance and a variety of nutrient-specific pathological conditions may result (ULLREY 1993, ALLEN and MONTALI 1995). Marginal nutrient intakes are apt to be manifest first as increased susceptibility to disease, reduced fertility, lower neonatal viability, suboptimal milk production, and retarded rates of growth (OFTEDAL and ALLEN 1998).
Human research nowadays shows, how often nutrition and health are closely linked (NAG 2001).The importance of optimal nutrition was not only recognized in humans but also in animals. Nevertheless, nutritional studies are rare for zoo animals, especially for carnivores. For domestic and laboratory animals, quantitative nutritional research has been established for most nutrients (NRC 1982, 1994, 1995, 2000, 2006, 2007). The need for nutrition research in livestock industries stems largely from the need for cost-effective production of milk, meat, eggs or pelt. For pet food industries the pet owners` satisfaction and their interest in well-being and longevity of their animals is the driving force for nutrition research. In the sector of competitive sports, e.g. horse-racing, many studies to improve nutrition and hence

performance were done. Thus economic factors have driven research in domestic animal nutrition; no such factors exist to encourage studies of nutritional requirements of zoo animals (ALLEN and MONTALI 1995). Nowadays, captive propagation of wild and exotic animals is becoming increasingly important for the survival of many species (NAG 2001). For providing optimal husbandry conditions, nutrition will become more important in this sector. To lose animals due to improper feeding is not acceptable from both, an ethical and a conservation standpoint (HATT 2000).

A reason for the relatively low number of nutritional studies for zoo animals is the difficulty to perform trials with quantitative collections of urine and feces. The animals are normally kept in large semi-natural enclosures for the presentation to the public. It is almost impossible to collect feces quantifiably from those enclosures. A quantitative collection of urine is impossible in normal zoo enclosures with natural ground. Laboratory conditions are needed for those trials. The direct determination of a requirement for specific nutrients is a lengthy process and involves feeding diets with different concentrations of a particular nutrient. Animal growth, reproductive effort, organ and tissue responses, etc. are then assessed for treatment effects (ALLEN and MONTALI 1995). The experimentally produced deficiencies or toxicities which are necessary for those studies to receive the maximum and minimum requirements of an animal can not be conducted with highly endangered species like Eurasian otters because of the high risk to loose animals with these trials. The large sample size needed to implement requirements is even more impossible to receive (ALLEN and MONTALI 1995). Beside the danger of handling larger carnivores, another problem is that wild animals often react with stress in trials were a direct contact to humans is necessary or they have to be maintained in small enclosures or metabolism cages. Animals have to be trained and accustomed to these conditions in advance which is very time-consuming.

The aim of a proper diet formulation for a certain species is to provide a nutritionally balanced diet. Of importance is also the palatability of the feed. A high palatability of all ingredients or the mixture ensures that the feed amount is reliably consumed by the animal (NAG 2001). But also practical demands have to be considered by formulating a diet ensuring that recommendations for species are implemented in the keeping institutions. All feed ingredients must be easy to purchase, good to store and may not be too expensive (NAG 2001,

HATT 2000). Furthermore, diets for zoo animals should stimulate natural feeding behaviours (NAG 2001).

The nutritional requirements of *Lutra lutra* are unknown. Beside energetic studies, no research was done for the Eurasian otter concerning adequate nutrition in captivity. Dietary management recommendations for other Lutrinae exist for Asian small-clawed otters (*Aonyx cinereus*) and North American River otters (*Lontra canadensis*). Both are based on data derived from so-called model species, mainly the domestic cat, farm mink and fox (MASLANKA and CRISSEY 1998, CRISSEY and REED-SMITH 2001).
Nutrition of *Lutra lutra* in zoos is based on tradition. This is quite usual for dietary management in captive wild animals due to the lack of scientifically based data. Diets of keeping institutions which maintain a species apparently successfully over longer periods are adopted from other keepers, even though the diets might often be suboptimal (OFTEDAL and ALLEN 1998). Animals may adapt to substandard diets for prolonged periods as long as additional nutrient demands such as those associated with growth, reproduction, or disease do not arise. Some zoo animals manage to survive for many years on these suboptimal diets, so they become commonplace and accepted (OFTEDAL and ALLEN 1998). The first successful otter keepers for *Lutra lutra* were WAYRE (1980) and PECHLANER (1980). Each of them managed to maintain otters for several years and additionally had reproduction success. Hence in the seventies, most otter keepers adopted their compositions or mixed the recommendations both gave (REUTHER 1991).
To date modifications of these diets do still exist. The today wide spread supplemental feeding of vegetable material like carrots, oats or bran is derived from these former diets without having any hints on the needs or utility value of plant material for the animals (REUTHER 1991, MELISSEN 2000). The diets reported by REUTHER (1991) of various institutions show a compound of feed ingredients which were used. Many of these ingredients, like beef heart, beef rumen or beef liver were from species an otter could never prey on in the wild.

In general, zoo diets are rarely adapted to the food in the natural habitat. Although it is not necessary to use the prey spectrum of the wild habitat in zoo diets, the nutrient levels should meet the requirements of the animal. To ensure an optimal supply with nutrients for the otter, zoos have to design substitute diets based on the knowledge of the specific requirements of the species. These, however, lack completely to date for *Lutra lutra* (HATT 2000).

Because trials with experimentally produced deficiencies or toxicities can not be conducted with Eurasian otters, nutritional basic data have to be investigated and indirect methods have to be used for being able to calculate a specific diet for the species that meets the requirements. Additionally to these "basic modules" which are constraining essentials for calculating diets, special species specific problems like nutrition-related diseases have to be implicated for being able to provide a comprehensive dietary management.

One of the most important basic modules for being able to calculate adequate diets is the knowledge of energy requirements of a species. The body needs energy for all features of life. Hence, an adequate supply with energy is an absolute essential for husbandry. Energy intake can vary with sex, age, reproductive status, individual, season, ambient temperature, etc. (KLEIBER 1967). To investigate the energy requirement of an otter, trials measuring the energy intake under ad libitum regimen with standard diet and numerous animals over several years are needed. But the energy ingested with feed can not be completely utilized from the body, losses through feces (10-25% of ingested energy), urine (ca. 5% of ingested energy) and heat combustion occur (MEYER and ZENTEK 2001).
Neither energy intake under ad libitum regimen nor utilisation efficiency of energy of different diets have been determined for *Lutra lutra* up to now. Therefore one part of the study (Chapter 2) was to investigate the intake digestible energy (DE) of captive Eurasian otters over a period of several years. To facilitate diet calculation on a DE basis for keeping institutions, apparent digestibility (AD) of energy was determined using different diets, typical for otter husbandry.

For the formulation of suitable diets for captive otters, not only the knowledge of utilization efficiency of energy is important but also the digestive efficiency of nutrients. In dietary recommendations for *Lutra lutra* often only feed amounts without a specification of used ingredients are reported without considering that feed stuffs can be of different value for the otter due to varying digestion efficiency (REUTHER 1991). The macro nutrients protein and fat are playing a major role within the nutrients for carnivores because they have no need for carbohydrates (NRC 1982, ROBBINS 1993). For measuring digestibilities, quantitative feces collections are needed which are difficult to conduct in normal zoo enclosures (see above). Markers provide a method for indirect quantification of digestibility without collecting the feces at large. Appropriate markers must be selected for each species because of physiologi-

cal variations in mammals due to their dietary adaptations (BERNARD et al. 1995). Cr_2O_3 is one of the mostly used markers for testing digestibility, but has not been extensively studied for non-domestic species (GUDMUNDSSON et al. 1998). Ideally, using the collection or marker method should result in an identical estimation of the AD but differences between the methods are quite common (MROZ et al. 1996).
Because no studies on digestibility were conducted to date with *Lutra lutra*, there are no tests about markers normally used for digestibility trials. Hence, another part of the study (Chapter 3) was to investigate the validity of the marker Cr_2O_3 for *Lutra lutra* as well as to determine the digestion efficiency of otters ingesting different diets, typical for otter husbandry, to facilitate diet calculations for keeping institutions.

Because species specific maximum and minimum requirement trials can not be conducted with the highly endangered Eurasian otter due to keeping situation and animal welfare constraints, an indirect method for obtaining requirements is necessary. One possibility is to search for model animals which seem to be comparable to the species and are well researched. Using domestic animals as models to adopt the recommendations for non-domestic species is common (DIERENFELD 1996). But with wildlife and exotics, species specific differences are becoming apparent and limitations of domestic animal models have to be identified. Therefore the species of interest must be tested towards their comparability.
The mink as a semi-aquatic carnivorous mustelid is well researched due to the maintaining of animals for pelt industries. Mink studies cover diverse basic data like digestibility coefficients, retention times and energy demands as well as nutrient recommendations (SINCLAIR et al. 1962, LOESCHKE 1959, GLEM-HANSEN 1980). For a comparison of the mink and the otter, data must be obtained with identical study design allowing a comparison of the species. Therefore, another part (Chapter 4) of the study was to investigate comparability of both species through measuring digestive efficiencies as well as gut passage times with the same trial design to evaluate the mink as a model species for dietary recommendations for the Eurasian otter.

Another indirect method to obtain hints on nutrient intakes of a species without the need of metabolic studies is to consider natural feeding ecology. Presuming the unproven assumption of diet optimality in free-ranging animals as the optimal foraging theory implicates, nutrient requirements can be as first approximate derived by analysing the natural in-situ diet (DIER-

ENFELD 1994, KREBS et al. 1978). Studies covering different habitats, seasonal changes and exact species specific analyses are a precondition for high-quality assessment of nutrient intakes with this method (DIERENFELD 1994). These basic studies have not yet been performed with *Lutra lutra* although numerous studies from the wild are existing. Hence, this indirect method was used in the study (Chapter 5) by comparing in-situ diets of *Lutra lutra* with zoo diets in order to give suggestions helping to optimize the ex-situ dietary management.

For nutritional studies often urine trials are necessary. Dietary influence on urinary concentrations of various analytes is often a problem for metabolic studies working with urine. Urinalyses are necessary for e.g. research investigating causes and risk factors concerning urolithiasis. Values of minerals, metabolites, amino acids and further organic substances in the urine are needed to detect abnormalities. Studies in Asian small-clawed otters investigating the formation of renal calculi found dietary influences on urinary analytes (PETRINI and TRECHSEL 1996, CALLE 1988, CALLE and ROBINSON 1985). Quantitative urine samples have never been collected and analysed for *Lutra lutra*. As a basis for further investigations, especially for urolithiasis research studies (see below), the dietary influence on various analytes was tested (Chapter 6).

A special species specific problem in otter husbandry is the occurrence of renal calculi, mainly in Eurasian and Asian small-clawed otters. For both, hints are pointing towards a relation with nutrition (PETRINI and TRECHSEL 1996, CALLE 1988, WEBER 2001). In Asian small-clawed otters renal calculi often occur in the captive population (66.1%), data from the wild are not existing (CALLE 1988). In the Eurasian otter, urolithiasis was found in up to 23.4% of the wild population (WEBER 2001). In the captive population it can be as much as 69.2%, indicating problems in husbandry (WEBER 2001, KEYMER et al. 1981). Whereas in Asian otters commonly calcium oxalate stones were found, Eurasian otters mainly form ammonium urate calculi (69.6%) (WEBER 2001).
In mammals, uric acid calculi occur in many species but only in low percentages of occurrence. The main components in dogs and cats is by far struvite (70.3% of feline calculi, 49.6% of canine calculi), followed by calcium oxalate (10.3% of feline calculi, 31.4% of canine calculi). Ammonium urate stones are reported in felines reaching 4.0%, in canines 6.8% (OSBORNE et al. 1989, OSBORNE et al. 1999).

An exception in dogs are Dalmatians in which this type of stone is typical. Studies report ammonium urate to be the main component (75-100%) in Dalmatians (CASE et al. 1993).
Ammonium urate calculi can be formed in urine which is oversaturated with urate and ammonium ions, promoted through at urinary pH above 5.75 (THORNHILL 1980). Normally the uric acid excretion in mammals is low because it is converted by the enzyme uricase to allantoin (THORNHILL 1980). Exceptions, however, are humans, apes and Dalmatian dogs, where the final product of purine metabolism is uric acid (KEELER 1940). In humans, uricase is virtually absent whereas in Dalmatians it is synthesized but the specific transport system for uric acid in the liver cell membranes has minimal capacity. But not all Dalmatian dogs with high urinary uric acid concentrations are urolith formers, the reason is as yet unknown (GIESECKE et al. 1985). Nutrition is an important factor in the formation of urate calculi in Dalmatians as well as in humans with respect to the purine and ammonium intake and urinary pH (HESSE and BACH 1982).
For *Lutra lutra*, the pathogenesis of stone formation is unknown. Dietary influences have not been tested. For being able to provide a comprehensive dietary management those important problems in husbandry with such a large impact on the captive population like renal calculi in *Lutra lutra*, have to be included in nutritional studies. Hence, metabolic trials were conducted in this study (Chapter 7) to assess risk factors for the formation of urate calculi as well as to test possible dietary influences on the disease.

The aim of this study was to conduct nutritional trials with the species to investigate basis data on nutrition and the nutrition-related disease of urolithiasis to provide information to improve the captive dietary management.

2

ENERGY INTAKE AND DIGESTIVE EFFICIENCY OF CAPTIVE EURASIAN OTTERS (*LUTRA LUTRA*)

Katrin Ruff[1], Hans-Heinrich Krüger[1], Stephan Steinlechner[2], and Jürgen Zentek[3]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany

[2]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

[3]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany

submitted

Abstract

An adequate energy supply is essential for animal husbandry in captivity. For optimizing the keeping of the endangered Eurasian otter (*Lutra lutra*) we measured digestible energy (DE) intake of captive otters and determined the apparent digestibility (AD) of energy for various diets to facilitate diet calculations for keeping institutions. DE intake was measured in 14 otters over periods of several years using a diet consisting of fish, chicken, beef rumen and heart under ad libitum regimen. AD was determined in nine captive Eurasian otters with 8 diets using the marker chromium oxide. The otters were adapted to the appropriate diet for 5 days, followed by a feces collection period of another 5 days. Proximate analyses were performed on the diet and feces to determine AD of energy. The mean AD of energy of all diets was 81 %, differing between diets with a range of 68 % for chicken diet to 86 % for beef rumen. The DE intake was on average 721 kJ/ kg body mass $(BM)^{0.75}$/d. Seasonal differences occurred with lower intake rates in summer (691 kJ/kg $BM^{0.75}$/d) than in winter (750 kJ/kg $BM^{0.75}$/d). Females had a higher DE intake (738 kJ/kg $BM^{0.75}$ /d) than males (698 kJ/kg $BM^{0.75}$/d).

Energy intake in otters was high in comparison to other carnivores. AD of energy differed between diets and should be considered in the ration formulation for *Lutra lutra* in captivity.

Introduction

Metabolic rates of mustelids are high in comparison to other mammals. The basal metabolic rate (BMR) in mustelids, weighing 1 kg and more, is approximately 20% higher (BMR in kJ per day(d)= 354 x $BM^{0.78}$; BM: body mass in kg) than expected from the mammalian standard curve with BMR in kJ/d= 293 x $BM^{0.75}$ (IVERSEN 1972, KLEIBER 1967). PFEIFFER and CULIK (1998) measured a BMR for the Eurasian otter exceeding the values predicted from the standard curve by 94%. After their calculation, an active captive otter of 6 kg body weight needs a supply of digestible energy (DE) of 707 kJ/kg $BM^{0.75}$ /d. Energy demands calculated for a sleeping otter were 401 kJ/kg $BM^{0.75}$/d (PFEIFFER 1996).

Because of the endangered status of the species in many parts of its distribution area, the Eurasian otter is often kept in captivity. For successful husbandry optimal nutrition is an essential. Therefore, the adequate supply with energy is obligatory. A zoo survey conducted in

2004 among 36 otter keeping institutions spread over Eurasia showed that the gross energy (GE) content of the feed (1747 to 2979 kJ/100g in dry matter) as well as the fed GE amount per animal per day (417 to 3062 kJ/ kg $BM^{0.75}$/ d) is highly variable between institutions. These differences are not explainable through varying keeping facilities nor climate zones, e.g. Moscow and Helsinki are comparable in temperature (year temperature average 4.8 and 4.4°C), but the zoos differ considerably in their energy allowances given on average to the otters (GE: 1024 to 4899 kJ/kg $BM^{0.75}$/ d). The energy supply of some institutions is just barely beyond the requirements of a sleeping otter (PFEIFFER 1996), not covering the energy needed for activity. The survey also turned out that feeding amounts in keeping institutions are often adapted according to the behaviour of the otters: energy amounts are boosted with increased aggressive behaviour and decrease when the otters can hardly be lured out of dens for feeding times. This regimen can be problematic e.g. due to subjective evaluation of the otters´ behaviour from different keepers. MELISSEN (2000) reports on large seasonal changes in the energy intake of Eurasian otters which in addition makes adequate energy supply difficult.

But for calculating feed rations the intake of gross energy (GE: amount of energy contained in food) is not sufficient. The knowledge of the efficiency of feed digestibility of different diets is obligatory because ingested GE can not be completely utilized by the body. For digestible energy (DE) the losses through feces and for metabolizable energy (ME) the losses through urine have to be subtracted from GE. Losses through voided combustible gases are small in carnivorous monogastric species and can be neglected. Loss resulting from metabolic energy transformation is differing heavily with activity of the individual. The net energy (NE) is the energy which is available for the organism (MEYER and ZENTEK 2001).

Up to now no research was done on feed utilization in the Eurasian otter and energy intake was not measured under ad libitum regimen and with known apparent digestibility (AD) coefficients of the used feed ingredients. The aim of this study was to investigate the DE intake of captive Eurasian otters over a period of several years. ADs of energy were determined using different diets, typical for otter husbandry, to facilitate diet calculation on a DE basis for keeping institutions.

Materials and methods

Animals

Energy intake trials were conducted with 4 adult otters (1 male, 3 female) over a 2-year-period from 2004 to 2005 and 10 adult otters within differing periods (male 1: 1990-2001, male 2: 1994-2003, male 3: 1987-1996, male 4: 1990-1996, male 5: 1997-2000; female 1: 1989-2003, female 2:1986-1998, female 3:1985-1995, female 4:1988-1995, female 5:1999-2004). These differing periods (below called "x-year-periods") lasted between 4 and 15 years. Female 1 had cubs in three of the years during the period of data collection, female 3 in two years. For these females data are excluded starting 8 weeks before pregnancy and lasting till the pups were weaned and separated from the female. DE was performed with 9 adult otters (4 male, 5 female). All were in good health and not under medical treatment. The otters were housed individually in semi-natural enclosures.

Diets

The diet for measuring energy intake over the 2-year period consisted to 30 % of bream, 30 % of day-old chicken, 30% of beef rumen and 10% of beef heart. The ingredients were equal for the x-year-period, but offered with different percentages over the years.

Eight diets were used for digestibility trials. Ingredients of diets 1-4 are shown in Table 1. Diet 5 consisted of herring, diet 6 of day-old-chicken, diet 7 of beef heart and diet 8 of beef rumen. The proximate nutrient analysis is shown in Table 2. Four feed ingredients were received deep frozen (herring, rat, guinea pigs and rabbits), the rest was purchased directly from the abattoir and processed within one day. Before a trial started, all ingredients were minced with a meat grinder (perforated plate with holes of 0.5 cm diameter) to assure that the marker was distributed homogenously. All prey animals were minced in whole, only the furs of the rabbits and guinea pigs as well as the feathers of chicken had been removed. After mincing, the feed mash was thoroughly mixed to assure that all nutrients were dispersed uniformly. For every otter the feed required for maintenance was calculated after MELISSEN (2000), due to the lack of other data, and deep frozen in plastic bags at – 40°C. The diets were defrosted overnight before feeding. Chromium oxide (Cr_2O_3) was used as a marker in this study for calculating energy digestibility. The concentration of chromium oxide in the feed was 0.2 % of fresh weight. Just before feeding the diet was mixed again and the marker

was thoroughly stirred in. Water was supplied ad libitum. Every otter was accustomed for two weeks to accept the minced feed before the trials started.

Table 1. Composition of diets (%)

	Diet			
Ingredient	1	2	3	4
Day-old-chicks	25	15		12.5
Bream	30	10	50	
Roach			50	
Herring				
Trout				31.25
Ground beef		65		6.25
Rumen	40			
Cattle heart	2.5	2		6.25
Cattle liver	2.5			
Chicken				12.5
Rabbit				6.25
Mouse				6.25
Rat				6.25
Guinea pig				6.25
Oat flakes		3		
Wheat bran		2		
Carrot		2		6.25
Yeast (fresh)		1		

Table 2. Analysis of nutrient composition (% dry matter basis) of the diets

	Diet							
	1	2	3	4	5	6	7	8
Dry matter*	26.7	34.3	26.5	27.5	20.2	21.0	37.8	48.4
Crude ash	7.1	4.1	15.2	8.6	11.8	5.2	12.1	22.8
Crude fiber	0.8	3.2	0.2	0.9	0.4	1.6	0.7	2.1
Crude protein	57.0	51.1	63.9	58.2	38.8	51.7	56.4	36.3
Crude fat	30.5	36.5	14.7	24.7	9.5	13.3	30.9	38.9
Energy (J/g)	23.7	25.5	20.0	23.2	21.8	21.2	30.7	14.5

* % of fresh matter

Experimental procedure

For energy intake trials, the whole feed for one day was put in the enclosure in a special box to prevent that feed was eaten by mice, rats or birds. Every day feed remnants were weighted back. Feed amounts were adjusted according to requirements on a daily basis. Animals were weight every six month during the 2-year-period, for the x-year-period weighting was irregular.

For digestibility trials feces were collected. To adapt the otters to the particular test diets minced chicks were fed with their diet before the trial with 12.5% of the new diet mixed in on the first day, 37.5% on the second, 50% on the third, 62.5% on the fourth, 75% on the fifth, and 87.5% on the sixth day. From the seventh they received 100% of the new diet for 5 days. Thereafter the sampling period started for 5 days.

Sample handling and analysis

The fecal samples were collected three times per day, pooled and frozen at – 40°C. Feces and diets were freeze-dried and subjected to a Weende analysis (VDLUFA 2003). Energy content was measured with bomb calorimetry. Chromium oxide was determined by atomic absorption spectrophotometry (WILLIAMS et al. 1962).

Apparent digestibility (AD) was calculated by the following formula (MAYNARD et al. 1969):

AD % = 100 – 100 x ((% chromium in diet / % chromium in feces) x (% nutrient in feces / % nutrient in diet)). DE was calculated from the GE intake, the calculated feces excretion and the energy content of the feces.

Statistics

Digestibility coefficients were compared by a one-factorial analysis of variance (ANOVA), followed by a Students t-test (ENGEL 1997). For testing seasonal differences of energy intake and sexes, a paired t-test was used. The significance level was set at $P < 0.05$.

Results

During the collection periods the animals maintained their body mass within +- 200 g per week. BM of males was 7.8 kg on average, of females 5.9 kg. All animals appeared healthy and were behaving normally.

Energy intake

Average DE intake of the otters was 721 kJ/kg $BM^{0.75}$/ d throughout the year (Table 3). Seasonal differences in energy intake in winter (October to March) and summer (April to September) were seen when comparing all data of animals and periods. Significantly less DE was ingested in summer (691 kJ/ kg $BM^{0.75}$/d) than in winter (750 kJ/ kg $BM^{0.75}$/d) with a difference of 7.9 %.

Table 3. Digestible energy intake in kJ/kg $BM^{0.75}$/d

	winter	summer	average
all otters	750[1]	691[1]	721
Male	729[1]	666[1]	698[2]
Female	766[1]	710[1]	738[2]
2-year-	754	724	739
x-year-	749	678	713

[1] significant difference between seasons; [2] significant difference between sexes

By comparing the different periods (Table 3 and Figure 1), the x-year-period had significant seasonal differences of 9.5 % between summer and winter with an average of 713 kJ/kg $BM^{0.75}$/d. The highest value (797 kJ/kg $BM^{0.75}$/d) was in December, the lowest in July (648 kJ/kg $BM^{0.75}$/d). The 2-year-period had a mean energy intake of 740 kJ/kg $BM^{0.75}$/d with a not significant seasonal difference of 3.9 % (highest value in January: 785 kJ/kg $BM^{0.75}$/d / lowest value in April: 701 kJ/kg $BM^{0.75}$/d).

Sexes differed significantly in DE intake, males ingested 698 and females 738 kJ/kg $BM^{0.75}$/d for all animals and periods. The difference in intake of the sexes is also reflected in seasonal variation. Males had a higher intake in winter (729 kJ/kg $BM^{0.75}$ /d) than in summer (666 kJ/kg $BM^{0.75}$/d), equally to females (winter: 766 kJ/kg $BM^{0.75}$/d; summer: 710 kJ/kg $BM^{0.75}$/d). Higher energy intakes of females were found for the 2-year and the x-year-period.

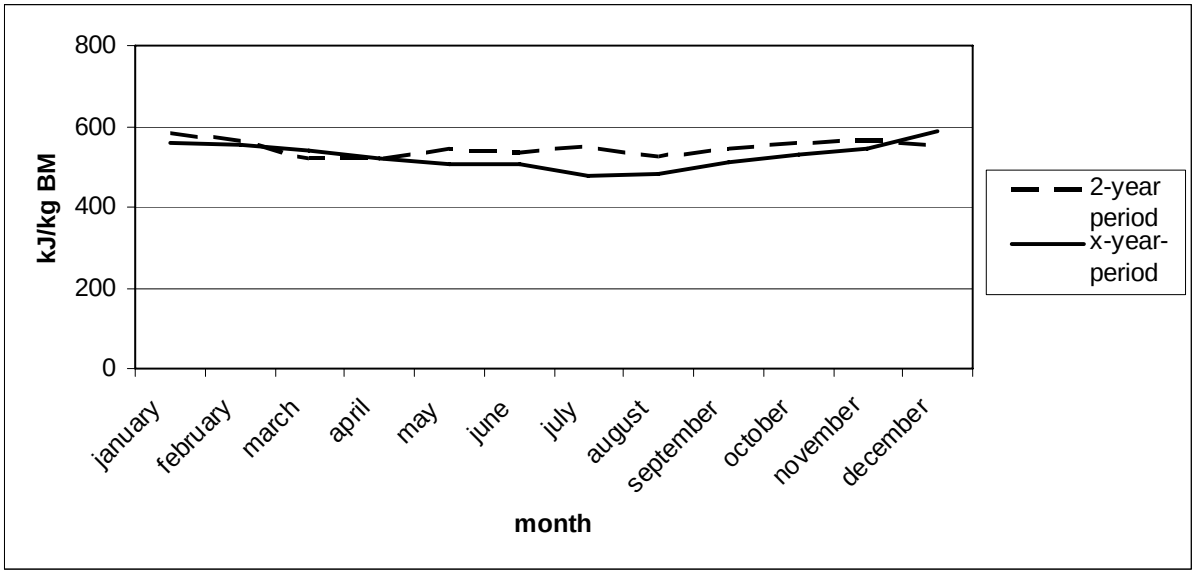

Figure 1. Digestible energy intake in kJ/kg BM for the 2-year period 2004 – 2005 (n=4) and the x-year-period (n=10)

Digestive efficiency

Apparent energy digestibilities of the different diets are presented in Table 4. The ADs of energy varied among diets (for significance see Table 4). The highest AD of energy was found in diet 3 (85.6 %), followed by diet 4 (83.7 %), diet 7 (83.2%), diet 5 (82.6 %), diet 2 (81.6 %) and diet 1 (79.9 %). The lowest AD coefficients of energy occurred while feeding diet 8 (72.2 %) and diet 6 (68.0 %).

Table 4. Apparent energy digestibilities (AD) in %[1]

	Diet								
	1	2	3	4	5	6	7	8	mean
AD %	79.9[a]	81.6[b]	85.6[b.c]	83.7[c]	82.6[b.c]	68.0	83.2[b]	72.2[a]	80.9
±	4.1	5.6	2.4	2.2	2.1	5.6	4.2	5.3	6.3

± standard error

[1] means with different superscripts differ ($p < 0.05$)

Discussion

The average DE intake of 720 kJ/kg $BM^{0.75}$/d is in accordance with literature data. The DE intake was more than twice as high as could be expected from the mammalian standard curve of KLEIBER (1967). Two studies measured the energy intake of *Lutra lutra* using differing methods; both have been limited in sample size or data sampling period. PFEIFFER (1996) calculated the time-energy-budget through measuring BMR, energetic costs and time budgets. Some animals were the same otters we used for the x-year-period in the same enclosures (female 1, 4; male 1, 3, 4) under the same feeding regimen. PFEIFFER (1996) calculated the DE requirement of an otter (6 kg BM) to be 707 kJ /kg $BM^{0.75}$/d, ranging only marginally below our measurements. MELISSEN (2000) measured a GE intake of 944 kJ/kg $BM^{0.75}$/d with a comparable method as we used. The diet in his study consisted of 66.6 % mackerel and 33.3 % chicken. By predicting the same AD like we had in our otters for this diet (DE coefficient of 77), the DE intake was with 717 kJ/kg $BM^{0.75}$/d essentially the same as our findings.

The energy intakes of *Lutra lutra* are higher then values of comparable terrestrial mammals, e.g. for cats (5.5-6 kg BM) an ME requirement of 176 kJ/kg BM/d and for dogs (5 kg BM) of 280-370 kJ/kg BM/d was reported, DE intake of bobcats was 194 kJ/kg BM/d (EARLE and SMITH 1991, MEYER and ZENTEK 2001, POWERS et al. 1989).

Energy is required for the BMR (energy expenditure of an animal in muscular and psychic repose, in a thermoneutral environment in a postabsorptive state) to maintain body weight. Further energy is needed for thermoregulation, locomotion, reproduction, etc. (ROBBINS 1993). IRVING (1973) expected semi-aquatic mammals to be in general high in BMR, but data of beavers are in accordance with the standard curve of terrestrial mammals (PFEIFFER 1996, ALLERS 1995, SCHMID-NIELSEN 1990). IVERSEN (1972) found high BMRs in many mustelid species, for both, terrestrial (weasel, polecat) and semi-aquatic species (mink, otter). ESTES (1989) presumed the high BMR to be an attribute of all Mustelidae. Most mustelids have an elongated body shape, inclusive *Lutra lutra*. Due to a greater surface area this long and thin shape is energetically not efficient i.e. higher thermoregulatory costs compensating heat losses. BROWN and LASIEWSKI (1972) presumed that energetic costs of the shape are more than compensated through an increased ability to obtain prey. For a semi-aquatic mustelid like the otter the heat loss is not only a problem on land but especially in water. The thermal conductivity of water is 25 fold higher than of air (SCHMID-NIELSEN

1990). The good insulation of otters through its dense fur keeping an air film between skin and guard hairs helps to reduce heat loss to a certain degree (WEISEL et al. 2005). But not only the thermoregulation is a problem in water but also transport costs are high for the otter due to its locomotion. Otters are adapted as semi-aquatic mustelids to swimming (on the surface) and diving as well as walking on land, hence neither of the modi is optimized. So the otter has a high energy requirement like our results indicate for captive animals (PFEIFFER 1996). It has to be stated that activity of our animals is expected to be high for a captive otter due to the large, semi-natural enclosures, but will probably not reach activity levels of wild otters.

MELISSEN (2000) found a seasonal change of energy intake with an increase in winter and a decrease in summer. Between summer and winter a difference of 24.6 % was measured. We found in the x-year-period a seasonal difference of 9.5 %, in the 2-year-period no significant differences occurred. The large discrepancy between both studies can not originate from different climates. MELISSEN (2000) measured only a one-year-period with two otters which were kept together without an ad libitum feeding regimen. But the study indicates that the seasonal differences can be more pronounced compared to this study. This could be mainly the case when otters are kept in climates with rough winters. Wild carnivores in seasonally cold environments periodically are confronted with high thermoregulatory costs accompanied by feed deprivation. Patterns of adaptation to these challenges include good thermal insulation and energy storage. As a non-hibernator, the Eurasian otter is active during the whole year. In contrast to other species of the Mustelidae, *Lutra lutra* does not store subcutaneous fat for the winter, as the badger or polecat do (SOMMER et al. 2005, KRUUK and PARISH 1982, KORHONEN and HARRI 1986). The otter seems to have no adaptations towards feed scarcity in winter. This is also reflected by its ability to reproduce the whole year, also in regions with cold winters, and data of good body condition indices found in necropsy of wild otters during winter and summer time (SOMMER et al. 2005). The very dense fur of the otter helps to minimize the thermoregulatory costs through insulation on land as well as in water (KRUUK 1995). Due to all indices, smaller seasonal changes in energy intake in comparison to other mustelids are expected for *Lutra lutra* when prey is not scarce what fits to our results of captive otters.

The higher energy intake of females is in accordance with other mustelids with sexual dimorphism in body mass (MOORS 1977). Males were 24.4 % heavier in BM than females, energy

intake of females was 6% higher than that of males. The relationship between energy requirements, BMR and body mass for mammals has been discussed by KLEIBER (1967), MCNAB (1970) and POCZOPKO (1971) showing that the metabolic rate per unit body mass decreases with increasing weight, but not linear.

Digestibility trials are difficult to perform in otters. The animals are normally kept in large enclosures were a total collection of feces is rarely possible. In this study, animals were kept in large, semi-natural enclosures with soil ground, making quantitative collection impossible. Thus the marker chromium oxide (Cr_2O_3) was used which provides a method for indirect quantification of digestibility without collecting the total feces. Cr_2O_3 is the most commonly used marker for digestibility trials in captive zoo carnivores (BARBOZA et al. 1994, BARBIERS et al. 1982, MORRIS et al. 1974). In studies comparing the marker Cr_2O_3 with total collection method in various species, an underestimation of the AD occurs. This has to be kept in mind when comparing our data to total collection studies (HILL et al. 1996, BARBIERS et al. 1982).

The mean energy AD of all diets was 80.9 %. Compared to data of other carnivores, the values are quite low. A problem in AD comparison between species is the use of various fee ingredients in the studies. In digestibility trials concerning the carnivores mink (LOESCHKE 1959), fox (AHLSTROM and SKREDE 1998), dog (BURROWS et al. 1982) and domestic cat (KIENZLE 1994) commercial dry or canned diets were mainly used. These processed diets can be more digestible than test diets made from fresh items. Therefore, mainly data of studies conducted with whole prey, where available, are discussed below. But also whole prey diets differ in energy AD, as our study showed, having significant differences between most of the diets. For exact interspecies comparisons, trials using comparable feed ingredients have to be conducted.

Within the Lutrinae, two studies measured energy ADs. For North American river otters fed cat, feline and polar bear commercial diet, AD (86.7 %) was higher than our values (WHITE et al. 2006). Various diets fed to sea otters (clams, abalone, crabs and squid) had the same AD (80.9 %) as for *Lutra lutra* with our diets. In comparison to other Mustelidae, the digestive efficiency of Eurasian otters seems lower. For black-footed ferrets fed mice an energy AD of 93.4 % was reported (HELLINGA et al. 1997). Fishers fed whole prey (hare, deer, vole, shrew, mice) had values of AD of 88.8 % (DAVISON et al. 1978) in mink fed commer-

cial diets (partly containing vegetable fractions) AD of energy was found from 72 to 94% (ROBERTS and KIRK 1964, ALLEN et al. 1964, CHWALIBOG et al. 1979).

By comparing the mean ADs of energy of Eurasian otters with other carnivorous species beside the Mustelidae, the data of otters seem to be lower. Means for apparent energy digestibility are reported for the non-domestic Felidae lion, tiger, leopard and puma fed with pure horse or beef meat from 91.8 to 98.1 % (BARBIERS et al. 1972, MILLS 1980, HACKENBURGER and ATKINSON 1983). The otter AD for beef meat based diet (diet 2) was 81.6 %. For herring diet fed to walruses the digestibility of energy was reported to be 93.1 % (FISHER et al. 1992), fed to Steller sea lions 95.4 % (ROSE and TRITES 2000) and to monk seals 96.1 % (GOODMAN-LOWE et al. 1999). The otter values for herring were lower (82.6 %).

The lowest ADs in this study occurred while feeding diet 6 (day-old-chicken) and 8 (rumen). Chickens were minced in whole. The feathers of poultry are in general hard to digest because of the structure of keratin which is difficult to access for digestive enzymes (MEYER and ZENTEK 2001, DUST et al. 2005, MURRAY et al. 1998). Parts of the minced chicken feet could be detected visually in feces which were obviously completely undigested. The quality of protein in rumen is also less than in meat (MEYER and ZENTEK 2001).

The otter has short passage rates (for fish diet 186 – 195 min) in comparison to other carnivores (JURISCH and GEIDEZIS 1997, LIBOIS et al. 1991). A fast passage rate through the small intestine can lead to a reduced digestibility (PEACHEY et al. 2000). This could be a reason for the low ADs found for *Lutra lutra*.

Conclusion

For the dietary management in zoos it can be assumed that AD of energy differed between diets and should be considered in the ration formulation for *Lutra lutra* in captivity. The energy demands of the species seem to be higher than that of other carnivores.

3

DIGESTIVE EFFICIENCY IN EURASIAN OTTERS (*LUTRA LUTRA*) AND INVESTIGATION ON CHROMIUM OXIDE AS MARKER

Katrin Ruff[1], Hans-Heinrich Krüger[1], Stephan Steinlechner[2], and Jürgen Zentek[3]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany

[2]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

[3]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany

submitted

Abstract

The Eurasian otter (*Lutra lutra*) is severely endangered in the wild. Concerted efforts have been made to maintain and breed this species in captivity, and adequate nutrition is essential for reproduction. For the formulation of suitable diets for captive otters, the knowledge of their digestive efficiency is important. Therefore, the apparent digestibility coefficients of nine captive Eurasian otters were determined with five different diets. The experiments were conducted using the marker chromium oxide. Because the marker has not been validated for Lutrinae, trials were carried out with five otters and three diets comparing apparent digestibility coefficients (AD) using the total fecal collection method versus the method with Cr_2O_3. In both experiments, the otters were adapted to the appropriate diet for 5 days, followed by a feces collection period of another 5 days. Proximate analyses were performed on the diet and feces to determine apparent digestibility of dry matter, crude fat, crude protein and crude fiber. The ADs evaluated with the marker method were significantly lower than with the total fecal collection. The underestimation of AD with the marker method was 5 % for dry matter, 2 % for crude protein and 3 % for fat, for fiber is was 14 %. The mean AD of dry matter was 77 %, of crude fiber 57 %, protein 84 % and fat 85%. The ADs differed between diets with a range of 75 to 86 % for dry matter, 36 to 75 % for crude fiber, 79 to 88 % for crude protein and 70 to 96 % for crude fat.

Based on these results, the marker Cr_2O_3 for *Lutra lutra* can be used for estimating the AD of dry matter, crude protein and crude fat considering an underestimation of ADs for the marker method. A lower digestive efficiency of otters seems to occur in comparison to other carnivores. This has to be taken into account when dietary recommendations are given for otters.

Introduction

The Eurasian otter (*Lutra lutra*) is often kept in captivity. It is a species which is severely endangered in the wild (REUTHER 2004) and therefore part of the European Endangered Species Programme (EEP) of the European Association of Zoos and Aquaria (EAZA). Beside that, otters are attractive zoo animals because of their agility in the water and on land as well as the maintaining of play instinct till old-age. But there are still problems in the husbandry of this species with low breeding success and nutrition-related diseases, e.g. a high

number of the zoo population is affected by urolithiasis (KEYMER 1981). Improper feeding can severely affect health and well-being (HATT 2000).

Otters are obligate carnivores. Their principle prey in the wild is fish, but crayfish, amphibians, insects, birds, small mammals and molluscs can be of regional and seasonal importance (GEIDEZIS 1999). The typical zoo diet is rarely comparable to that in their natural habitats. A zoo survey conducted in 2004 showed that feed composition does not correspond to their prey in the wild (see Chapter 5). Within the fish species the most fed is herring which is not within the prey spectrum of an otter. To ensure an optimal supply with nutrients, zoos have to design substitute diets based on a detailed basic knowledge of the specific requirements of the species (HATT 2000). Therefore, the knowledge of the efficiency of feed digestibility of different diets is a basic premise. Up to now no research was done with the Eurasian otter and data on feed utilization are lacking.

The aim of this study was to investigate the digestion efficiency of otters eating different diets, typical for otter husbandry. Because no studies were conducted to date with *Lutra lutra*, there are no tests about markers normally used for digestibility trials. Hence the validity of the marker Cr_2O_3, which is often used in trials with carnivores (UDEN 1980, BARBIERS 1982, FENTON and FENTON 1979), was tested for *Lutra lutra* by comparing the digestibility coefficients using the direct method (total fecal collection) and the indirect method (with chromium oxide).

Materials and methods

Animals

Digestibility trials were performed with 9 adult otters (4 males, 5 females). The comparative tests with the marker and the collection method were conducted with 4 otters (1 male, 3 females). All were in good health and not under medical treatment. The otters were housed individually in semi-natural enclosures.

Diets

Five diets were used for the digestibility trials (Table 1), three of them also for the marker tests (diet 1, 2, 4).

Table 1. Composition of diets (%)

	Diet				
Ingredient	1	2	3	4	5
Day-old-chicks	25	15		12.5	
Bream	30	10	50		
Roach			50		
Herring					100
Trout				31.25	
Ground beef		65		6.25	
Rumen	40				
Cattle heart	2.5	2		6.25	
Cattle liver	2.5				
Chicken				12.5	
Rabbit				6.25	
Mouse				6.25	
Rat				6.25	
Guinea pig				6.25	
Oat flakes		3			
Wheat bran		2			
Carrot		2		6.25	
Yeast (fresh)		1			

The proximate nutrient analysis is shown in Table 2. Data of crude fiber, crude protein and crude fat are measured in dry matter. The dry matter was measured referring to the fresh weight. Diet 2 was supplemented with cereals with high fiber contents and had a crude fiber content of 3.2 % in dry matter, all other diets had between 0.2 to 0.9 % crude fiber.

Table 2. Analysis of nutrient composition (% dry matter basis) of the diets

	Diet				
Diet fraction	1	2	3	4	5
Dry matter*	26.7	34.3	26.5	27.5	20.2
Crude ash	7.1	4.1	15.2	8.6	11.8
Crude fiber	0.8	3.2	0.2	0.9	0.4
Crude protein	57	51.1	63.9	58.2	38.8
Crude fat	30.5	36.5	14.7	24.7	9.5

* % of fresh matter

The diet with the lowest protein content was diet 1 consisting of herring with 38.8 % crude protein. The rest of the diets provided protein levels from 51 to 64 %. The fat content varied in a wide range from 9.5 (herring) to 36.5% (diet 2) between the diets.

Four feed ingredients were received deep frozen (herring, rat, guinea pigs and rabbits), the rest was purchased directly from the abattoir and processed within one day. Before a trial started, all ingredients were minced with a meat grinder (perforated plate with holes of 0.5 cm diameter) to assure that the marker was distributed homogenously. All prey animals were minced in whole, only the furs of the rabbits and guinea pigs as well as the feathers of chicken had been removed. After mincing, the feed mash was thoroughly mixed to assure that all nutrients were dispersed uniformly. For every otter the feed required for maintenance was calculated after MELISSEN (2000) and weighed to 1g accuracy and deep frozen in plastic bags at – 40°C. The diets were defrosted one night before feeding. Just before feeding the diet was mixed again and the marker was thoroughly stirred in. The concentration of chromium oxide in the feed was 0.2 % of fresh weight. Water was supplied ad libitum. Every otter was accustomed for two weeks to accept the minced feed before the trials started.

Experimental procedure

The first trials were carried out to test the validity of the marker chromium oxide (Cr_2O_3). Therefore the apparent digestibility coefficients were obtained through total fecal collection and through the calculation with the marker. To adapt the otters to the particular test diets minced chicks were fed together with their diet before the trial with 12.5% of the new diet mixed in on the first day, 37.5% on the second, 50% on the third, 62.5% on the fourth, 75% on the fifth, and 87.5% on the sixth day. From the seventh day on they received 100% of the new diet for 5 days. Thereafter the sampling period started for 5 days. For this period the otters were housed in metabolism cages to ensure a total collection of feces. Every metabolism cage (3.5 x 2.1 m) had a wire mesh (5 x 5 mm) at the bottom which allowed the urine to pass. The four otters were chosen for this trial because they did not show stress behaviour in the cages. They were accustomed for several days to the cages before the trials started.

For the digestibility trials the procedure was the same except that the otters stayed in their enclosures during the sampling period. The animals were kept in large semi-natural enclosures. The feces could therefore not be collected in total and the calculation was made with the marker method.

Sample handling and analysis

The feces samples were collected three times per day, pooled and frozen at – 40°C. Feces and diets were freeze-dried and subjected to a Weende analysis (VDLUFA 2003). Chromium oxide was determined by atomic absorption spectrophotometry (WILLIAMS et al. 1962).

Apparent digestibility (AD) was calculated by the following formula (MAYNARD et al. 1969):

AD % = ((diet, g x % nutrient in diet) – (feces, g x % nutrient in feces) / (diet, g x % nutrient in diet)) x 100.

Apparent digestibility by the marker method was calculated by the following formula (MAYNARD et al. 1969):

AD % = 100 – 100 x ((% chromium in diet / % chromium in feces) x (% nutrient in feces / % nutrient in diet))

Statistics

Data were subjected to a multifactorial analysis of variance for repeated measures (Programme: SPSS 14.0). The standard errors were calculated and significance of differences between the direct and the indirect method was distinguished with the paired t-test (SACHS 1992). Digestibility coefficients were compared by a one-factorial analysis of variance ANOVA, followed by a Students t-test (ENGEL 1997). The significance level was set at $P < 0.05$.

Results

Marker test trials

The AD for dry matter varied within the marker test trials from 81 to 89 % for the collection method and 79 to 83 % for the marker method. Crude fat determined by the direct method had a range of AD from 89 to 90 %, by the indirect method from 85 to 87 %. The collection method showed ADs for crude protein from 88 to 90 % and the marker method from 86 to 87 %. The results for the AD of fiber had a higher variability. The values from the direct method ranged from 51 to 84 %, those from the indirect method from 36 to 82 %. ADs calculated

with the indirect method were in general lower than those with the direct method (Table 3, $p < 0.05$). The dry matter apparent digestibility was underestimated with the indirect method by 4.7 %, fiber by 14 %, fat by 3.4 % and protein by 2.2 % in comparison to the collection method ($p < 0.05$).

Table 3. Comparison of apparent digestibility estimates (%) by direct (total fecal collection) and indirect (Cr_2O_3 marker) techniques

		Diet			
Diet fraction	Method	1	2	4	SED
Dry matter	Collection	81	89	84	1.4
	Marker	80	83	79	
Crude fiber	Collection	84	83	51	4.4
	Marker	82	76	36	
Crude fat	Collection	89	89	90	0.3
	Marker	87	85	86	
Crude protein	Collection	88	89	90	0.3
	Marker	87	86	87	

SED = standard error of differences of means between methods

Digestibility trials

Apparent nutrient digestibilities of the different diets obtained with the marker method are presented in Table 4. The apparent digestibility of dry matter, crude protein, crude fat and crude fiber varied among diets. The highest dry matter AD was found in diet 2 (86 %), the lowest in diet 3 with 64 % (white fish). The rest of the diets ranged from 75 to 83 %. The mean value of dry matter AD was 77 %. AD of crude fiber was highest within diet 1 and 2 (75 %), whereas the AD of the diet 3 and 4 was around 36 %. The mean value of crude fiber AD was 57 %. The AD of crude protein ranged from 79 to 88 % with a mean of 84 %. The digestibility of crude fat was highest in white fish (diet 3) and lowest in herring (diet 5). The other diets ranged from 85 to 88 %. The mean AD of fat was 85%.

Table 4. Apparent digestibility estimates by Cr_2O_3 marker technique[1]

Diet	Diet fraction			
	Dry matter	Crude fiber	Crude protein	Crude fat
1	76.4^{a} ± 3.8	74.8^{a} ± 10.2	83.2^{ac} ± 4.0	84.7^{a} ± 3.0
2	86.1^{b} ± 3.6	75.1^{a} ± 6.8	86.3^{ab} ± 4.5	86.0^{a} ± 4.8
3	64.5^{c} ± 7.5	36.7^{b} ± 12.8	84.5^{a} ± 3.4	95.7^{b} ± 1.8
4	80.3^{d} ± 3.0	36.2^{b} ± 12.3	87.8^{b} ± 2.2	87.6^{a} ± 5.5
5	74.7^{a} ± 3.4	63.0^{c} ± 13.8	79.4^{c} ± 4.2	70.2^{c} ± 4.5
Mean	77.0	57.2	84.2	84.8

± standard error

[1] Within a column, means with different superscripts differ ($p < 0.05$)

Discussion

In Eurasian otters digestibility trials are difficult to perform because it is rarely possible to conduct total feces collection trials. The animals are normally kept in large semi-natural enclosures for the presentation to the public. It is impossible to collect feces quantifiably from those enclosures. Additionally, the otters are normally not tame. So it would be stressful for untrained otters to be housed in small cages for assuring proper collection. Markers provide a method for indirect quantification of digestibility without collecting the total feces. To our knowledge, there are no marker studies in the Lutrinae to date. Appropriate markers must be selected for each species because of physiological variations in mammals due to their dietary adaptation (BERNARD et al. 1995). Cr_2O_3 is one of the mostly used markers for testing digestibility, but has not been extensively studied for non-domestic species (GUDMUNDSSON et al. 1998). Cr_2O_3 is the most commonly used marker for digestibility trials in captive zoo carnivores, e.g. with maned wolf (BARBOZA et al. 1994) or various Felidae (BARBIERS et al. 1982, MORRIS et al. 1974).

Ideally, using the collection or marker method should result in an identical estimation of the AD. Differences between the methods are not rare. In our study the AD of crude nutrients calculated for *Lutra lutra* with the marker method was lower than using the direct method. This is similar to results of other authors testing Cr_2O_3 in various other domestic species. Tests have been conducted with sows (EVERTS and SMITH 1987), cows (SOARES et al. 2003, PRIGGE et al. 1981, BRANDYBERRY et al. 1991), wethers (HATFIELD et al. 1991), dogs (HILL et al. 1996), or fish (DE SILVA et al. 1990) resulting in an underestimation of

the AD. In zoo animals a test for the validity of Cr_2O_3 in carnivores was made with lions, cougars, tigers and leopards (BARBIERS et al. 1982). This study also had lower AD values with the indirect method. By viewing the differences in values of each diet fraction, the differences in methods within dry matter, protein and fat are lower than with fiber. This is also seen in other species (MROZ et al. 1996, BARBIERS et al. 1982).

The underestimation of AD can be due to an overestimation of feces amounts with the indirect method or fecal losses in the collection method. Several reasons for divergent results are discussed. Chromium can sediment in the gastrointestinal tract (LATYMER et al 1990), gastric emptying and flow patterns of digesta can vary (LAPLACE et al. 1983), and a large circadian variation in the content of nutrients and chromium in digesta can occur (GRAHAM and AMAN 1986). The discontinuous gastric emptying can be a problem in ruminants but is not expected monogastric species. The influence of circadian variations was minimized through the 120 hours collection. Additionally, the otter has very short passage rates (minimum passage rate of 3 hours) what reduces the variation risk.

Errors in AD estimations with Cr_2O_3 have been reported by collecting feces only as spot collections by taking small samples from every defecation (PRIGGE et al. 1981). This is often done in trials with wild species producing large amounts of feces as rhinos (KIEFER 2002) or giraffes (BAER et al. 1985). That problem is absent due to the small amounts of feces of otters.

Some authors state that the total collection method, which serves as the reference method, has normally also unavoidable errors like losses of feed and feces (BEKKER and JONGBLOED 1994, EVERTS and SMITH 1987, MROZ et al. 1996). In this study the error of feed losses was excluded through feed bowls with intercepting tanks. For avoiding fecal loss the marking behaviour of otters is helpful. Every otter had chosen one of the edges of the cage for defecating. That facilitates collections and minimized the problem of collecting the feces from the ground. It can not be completely excluded that small amounts of feces could not be removed from the ground.

A problem in AD comparison between species is that different types of diets have been used in the different tests. In numerous digestibility trials concerning the carnivores mink (LOESCHKE 1959), fox (AHLSTROM and SKREDE 1998), dog (BURROWS et al. 1982) and domestic cat (KIENZLE 1994) commercial dried or canned diets were used. These processed diets are often more digestible than test diets made from fresh items.

By comparing the mean ADs with other carnivore species, the values of otters seem to be lower. Means for apparent fat digestion coefficients are reported for the non-domestic Felidae lion, tiger, leopard and puma fed with horse or beef meat from 97 to 99 %, protein ADs ranged from 91 to 96 % (MILLS 1980, HACKENBURGER and ATKINSON 1983). The otter ADs for beef meat based diet (diet 2) were 86 %. In polar bears fed with seals the fat digestion coefficients were 97 % and protein digestion 84 % (BEST 1985). For walruses fed herring, the digestibility of fat was reported to be 94 %, AD of protein was 93 % (FISHER et al. 1992). For Steller sea lions fed herring, the apparent dry matter digestibility was 90 % (ROSE and TRITES 2000). The otter values for herring were much lower (fat 70 %, protein 79 %, dry matter 75 %). There are no data within the Lutrinae and tests within Mustelidae fed unprocessed diets are also rare. For Black-footed ferrets fed mice ADs of 97 % for fat and 76 % for protein have been reported (HELLINGA et al. 1997). Fishers fed whole prey (hare, deer, vole, shrew, mice) had values of AD for fat of 92 % and for protein of 87 % (DAVISON et al. 1978). Within these comparisons it has to be stated that the feed items of Felidae and polar bear were given not as “whole prey” like in the otter.

The lower value of fat digestibility in otters is striking. The only diet approaching values normal for carnivores is diet 3, fish as single prey. That is a feed item on which otters would also prey on in the wild. The natural prey spectrum of the obligate carnivore is multifarious, but the main biomass consumption is freshwater fish in most habitats, beside some seasonal and habitat differences (GEIDEZIS 1999). The higher fat ADs of natural prey fish could be a hint that the low ADs within the tested zoo diets are not dependent on the species *Lutra lutra*, but are a sign towards a suboptimal choice of feed items used in dietary management in zoos. To prove that presumption more tests with natural feed items would be necessary. But by looking at the protein AD of white fish the digestibility coefficient of 84% is not higher in comparison to the other diets.

There is another hint that otters could be lower in utilizing their feed compared with other carnivores. Their gut passage rates of 3 hours minimum retention time are very short (GEIDEZIS 1999). The digestive efficiency and function of the otter seems to be adapted to continuous prey availability. This is also reflected in its ability to reproduce the whole year, also in regions with cold winters, and data of good body condition indices found in necropsy of wild otters during winter and summer time (SOMMER et al. 2005). The fact that otters have no fat deposits is also a hint that they have no problem with feed scarcity in winter (SOMMER et al. 2005). The circadian activity of otters shows an alternation of active and inactive

phases every one to three hours (KRÜGER 2006). The diet is ingested in nearly every activity phase. So the otter is probably not dependent on a high digestion efficiency like the large Felidae or polar bears which have irregular hunting success.

For the dietary management in zoos it can be assumed that the otter has a lower digestibility coefficient compared to other obligate carnivores. This has to be taken into account by calculating dietary plans for an optimal maintenance of *Lutra lutra* in husbandry.

4

COMPARISON OF DIGESTIBILITY AND PASSAGE RATE OF DIETS IN EURASIAN OTTERS (*LUTRA LUTRA*) AND MINK (*MUSTELA VISON*)

Katrin Ruff[1], Hans-Heinrich Krüger[1], Stephan Steinlechner[2], and Jürgen Zentek[3]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany

[2]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

[3]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany

submitted

Abstract

Adequate nutrition is essential to optimize husbandry of the captive population of the Eurasian otter (*Lutra lutra*), a severely endangered species. We evaluated the mink as a model for the otter by comparing apparent digestibility (AD) coefficients of crude nutrients and passage times. The apparent digestibility coefficients of 8 captive Eurasian otters and 8 captive mink were determined using the same diet. The experiment was conducted with the marker chromium oxide. Both species were adapted to the diet for 5 days, followed by a feces collection period of another 5 days. Proximate analyses were performed in the diet and feces to determine apparent digestibility of energy content, dry matter, crude fat, crude protein and crude fiber. Transit, retention and mean transit time (MTT) were determined in 8 mink and otters fed a chicken diet with plastic beads as marker.

The mean AD of dry matter was 69 %, crude protein 66 % and crude fat 84% in otters. The mean AD of dry matter was 65 %, of crude protein 71 % and crude fat 92% in mink. The digestibility of energy was lower in the otter (70%) than in the mink (75%). Transit time in mink was 195 min, retention time was 246 min and MTT was 305 min. The transit in otters was 115 min, retention 139 min and MTT 223 min.

Based on these results, the mink seems to differ to some extent from the otter. Lower digestive efficiency and faster passage times of the otter have to be taken into account when dietary recommendations for the mink are adopted for the otter.

Introduction

Concerted efforts have to be made to optimize the husbandry of the captive population of the Eurasian otter (*Lutra lutra*), a severely endangered species. Adequate nutrition is an essential prerequisite for successful husbandry. To enhance reproduction attempts in captivity, the otter is part of the European Endangered Species Programme (EEP) of the European Association of Zoos and Aquaria. Feeding recommendations for *Lutra lutra* are summarized in the EEP husbandry guidelines and are based on experience of different keepers and not on scientific data, except those for energy supply (MELISSEN 2000). Dietary management recommendations for other Lutrinae exist for Asian otters (*Aonyx cinerea*) and North American River otters (*Lontra canadensis*). Both are based on data derived from so-called model spe-

cies, mainly the domestic cat, farm mink and fox (CRISSEY and REED-SMITH 2001, MASLANKA and CRISSEY 1998). None of these models, however, have been tested for their comparability to the specific target species.

Using domestic animals as models to inherit the recommendations for non-domestic species is common (DIERENFELD 1996). It is often not possible to conduct trials with endangered exotic species concerning nutrient requirements because of the keeping situation and animal welfare constraints. But with wildlife and exotics, species specific differences are becoming apparent and limitations of domestic animal models have to be identified. Not only the species variation can be a problem but also the goals which should be achieved with the recommendations can differ between zoo populations and those of pet or livestock species. For pets, mainly cat and dog, much research was done to improve nutrition for supporting the well-being of the animals. In the field of farm animals the economic efficiency of production, like pelt quality in mink or fox, are emphasised (LEUS and MACDONALD 1997). This implements the limitations when models are used for zoo animals where longevity, well-being and reproductive success are in the focus. Before adopting recommendations from domestic animals to wild species, it is important to investigate possible differences in basic digestive functions (DIERENFELD 1997).

To ensure an optimal supply with nutrients for the otter, zoos have to design substitute diets based on a basic knowledge of the specific requirements of the species (HATT 2000). Because of a substantial lack of data for the otter and the difficulties to use otters in experiments, mink could serve as a suitable model. Mink is closely related to the otter: both belong to the Mustelidae. For the mink various nutrient recommendations are existing in the scientific literature and from feeding practice in pelt industry. The studies cover diverse basic data like digestibility coefficients, retention times and energy demands as well as nutrient recommendations (SINCLAIR et al. 1962, LOESCHKE 1959, GLEM-HANSEN 1980). For a comparison of the mink and the otter, data must be obtained with identical study design allowing a comparison of apparent digestibility coefficients of the species.

The aim of this study was to investigate the digestive efficiency of the otter and the mink with the same trial design and fed with the same diets. Additionally, gut passage time trials for both species were conducted using the same diet and marker. The comparative data are discussed in order to evaluate the mink as a model species for dietary recommendations for the Eurasian otter.

Materials and methods

Digestibility trials

The digestibility trials were performed with 8 adult otters (4 males, 4 females) and 8 adult mink (4 males, 4 females). All were in good health and not under medical treatment. The animals were housed individually in semi-natural enclosures, except for two of the female mink, which could only be kept together in an enclosure. The diet used for determining digestibility was 100 % chicken. The day-old-chicks were processed within one day after killing. They were minced as a whole with a meat grinder (perforated plate with holes of 0.5 cm diameter) and thoroughly mixed to assure that all nutrients were dispersed uniformly. For every animal the feed required for maintenance was calculated, for otters after MELISSEN (2000), for mink after NRC (1982), weighed to 1g accuracy and deep frozen in plastic bags at – 40°C. The diets were defrosted overnight before feeding. Water was supplied ad libitum. Both species were used to be fed with chicks, but as whole prey. Two weeks before the trials started they were accustomed to accept the minced diet. The Weende nutrient analysis (VDLUFA 2003) of the diet was performed and gave 21 % dry matter, 5 % crude ash, 2 % crude fiber, 52 % crude protein and 13 % crude fat (in dry matter). The measured energy content was 21 kJ/g.

The marker chromium oxide (Cr_2O_3) was used for the trials with a concentration of 0.2 % in the feed (fresh weight). Just before feeding, the diet was mixed and the markers were thoroughly stirred in. After the adaptation period the animals received the diet with the marker for 5 days, then the sampling period started for another 5 days. The feces samples were collected three times per day, pooled and frozen at – 40°C. The feces of the two female mink which were kept in one enclosure could not be assigned to the individual. Hence, the feces of this enclosure were pooled and regarded as one sample. Feces were freeze-dried and subjected to a Weende analysis. Energy content was measured with bomb calorimetry. Chromium oxide was determined by atomic absorption spectrophotometry (WILLIAMS et al. 1962).

Apparent digestibility was calculated by the following formula (MAYNARD et al. 1969):

AD % = 100 – 100 x ((% chromium in diet / % chromium in feces) x (% nutrient in feces / % nutrient in diet))

Gut passage

For investigating the passage rates 8 otters (6 male, 2 female) and 8 mink (4 males, 4 females) were used. The diet was the same as in the digestibility trial. The animals were adapted for one week to the diet. All animals were kept individually in enclosures covered with tilling to facilitate collection of feces. The markers were indigestible colored plastic beads with a diameter of 2.5mm. The trials started in the morning, the animals had access to feed for all the time. For the otters 50 beads were mixed in a feed mass of 150g, for the mink 25 beads were dispersed in 75g. The feed was offered to the animals and time measurement started after a minimum of 20 beads in mink and 40 beads in otter were ingested within 5 minutes. The number of ingested beads was recorded and the beads not ingested were removed. The animals were monitored to observe defecations. The time of each defecation and the number of excreted beads was noted. The trial was stopped after 100 % of the beads were found or at least 80 % of the markers could be detected, 6 hours passed after the last dropping and two defecations could be detected containing no beads.

Terminology associated with passage characteristics follows the convention of VAN SOEST (1982) and VAN SOEST et al. (1983). “Transit” was defined as the elapsed time between ingestion and first appearance of the marker in the feces; “retention” was defined as the interval between the recovery of 5 to 95% of the marker; “mean transit time” (MMT) was quantified as the integrated average of the marker excretion curve.

Statistics

Data were subjected to student’s t-test. The significance level was set at $P < 0.05$. The normal distribution was assessed with Kolmogorov Smirnov test (SACHS 1992).

Results

The results for the passage trials are presented in Table 1. The transit in mink was 195 min, in otters 115 min. The retention in the mink was 246 min, in otters 139 min and MTT of mink was 305 min, of otters 223 min. Transit, retention and MTT differed significantly ($p < 0.05$) between both species. By comparing the data of transit, the otter passage was 41 %, the retention 44 % and the MTT 27 % faster than in the mink. The recovery of the marker beads was 99% in mink; in otters it was 90 %.

Table 1. Transit, retention and MTT (min) of Eurasian otters and mink fed chicken diet

Animal No.	transit		retention		MTT	
	otter	mink	otter	mink	otter	mink
1	161	281	143	204	287	289
2	155	155	162	325	270	302
3	116	160	259	335	238	318
4	110	150	136	315	185	360
5	95	231	117	179	180	281
6	95	157	50	234	144	316
7	93	258	110	178	264	304
8	91	167	132	201	215	273
mean	115	195	139	246	223	305
±	28	53	59	68	50	27

± standard error

Apparent nutrient digestibilities of otters and mink are presented in Table 2. The apparent digestibility of energy, crude protein and crude fat differed among both species ($p < 0.05$). The dry matter AD for otters was 69 %, for mink 65 % (n.s.). The ADs for crude protein were 66 % for otters and 71 % for mink, crude fat in otters 84 %, in mink 92 %. The apparent digestibility of energy was lower in otter (70 %) compared to mink (75 %).

Table 2. Apparent digestibility coefficients (%) for Eurasian otter and mink fed chicken diet

	otter	mink
energy	70.0 ± 5.2	75.1* ±4.6
dry matter	68.5 ± 5.3	65.4 ±7.4
crude protein	65.7 ± 5.7	71.1* ±6.2
crude fat	83.5 ± 3.8	92.4* ±1.3

* $p < 0.05$; means ± standard error

Discussion

Passage rates are correlated to species specific physiological characteristics and feeding habits. Within the carnivores the felids are considered as obligate carnivores with a gastrointestinal tract adapted for that, having short passage rates contrary to canids accustomed to ingest also plant materials with longer rates (PEACHEY et al. 2000, FUCCI et al. 1995). Within the

mustelids the otter and the mink are strict carnivores. Therefore an adaptation towards short passage times was expected. This could be demonstrated with this study showing that the transit, retention and MTT in both species are rapid, for the otter even more than for the mink.

For Eurasian otters transit data from two studies exist reporting a mean transit time for fish of 186 and 195 min (JURISCH and GEIDEZIS 1997, LIBOIS et al. 1991) which are longer than our findings of 115 min for chicken diet. For the mink a passage trial using commercial mink feed revealed a retention of 142 min (SIBBALD et al. 1962), faster than our finding of 246 min.

By comparing the data of otters and mink with the cat, both have much shorter passages. MTT of cat were reported from 31 to 40 hours (FUCCI et al. 1995, PEACHEY et al. 2000).

The advantage of plastic markers is that they are easy to use, non-toxic and do not require laboratory analysis. They are mainly used in zoo animals for species with simple stomach systems. Plastic markers were previously used in Lutrinae (CARTER et al. 1999, JURISCH and GEIDEZIS 1997). In mink, mainly carmine-dyed rations were used (SIBBALD et al. 1962). Optimal markers should be non-absorbable, easy to recover, not interfere with the normal function of the gastrointestinal tract and should be in equilibrium with the pool of the fraction that it labels. The latter is often discussed as a problem with plastic markers (BERNARD et al. 1995). The diet has been minced with a meat grinder producing a feed mass still containing parts of the chicks larger than 3mm like feet, eyes or peckers. The diameter of the marker has been chosen to a size that represented the diameter of the smaller hard parts to have a medium size of the marker ensuring that it is closely associated with the diet fraction. The recovery rates of the marker in this study were optimal in mink, but also the rates in otters with over 90 % were good. Other studies with coloured plastic markers reported recovery rates of only 52 % (DIERENFELD et al. 1992).

Studies to investigate apparent digestibility coefficients are difficult to perform in Eurasian otters and mink kept in zoos because it is rarely possible to conduct total feces collection trials. The animals in this study were kept for the digestibility trials in their normal, semi-natural large enclosures covered with natural ground (soil and grass). It is impossible to collect feces quantitatively from normal enclosures. Therefore the trials for apparent digestibility coefficients were conducted with the marker chromium oxide. The marker provides a method for indirect quantification of digestibility without the need for collecting the total feces.

Cr_2O_3 is one of the most used markers for digestibility studies with carnivores (GUDMUNDSSON et al. 1998, BARBOZA et al. 1994, BARBIERS et al. 1982, MORRIS et al. 1974). For the mink the marker is commonly used, but the method was never validated in this species (SINCLAIR et al. 1962, ALLEN et al. 1964). For the otter no digestibility studies were done at all. In studies comparing the marker Cr_2O_3 with total collection method in various species, an underestimation of the AD occurs. This has to be kept in mind when comparing our data to total collection studies (HILL et al. 1996, BARBIERS et al. 1982).
The ADs for crude fiber, crude protein and crude fat are lower for otter than for mink.
For mink, mainly literature is available investigating ADs of energy, crude protein and crude fat. The studies were conducted with a variety of diets and therefore the results are differing within a large bandwidth. The results of this study are generally in accordance with corresponding literature data, but none has tested a chicken diet. For otters, no comparable studies seem to exist. The AD for crude protein in mink was reported in a range from 71 to 83 % (ALLEN et al. 1964, VHILE et al. 2005, LOESCHKE 1959); ADs for crude fat were 91 to 96 % (VHILE et al. 2005, LOESCHKE 1959); AD for energy was 72 to 80% (ALLEN et al. 1964, CHWALIBOG et al. 1979).
From our results it is apparent that the mink is more efficient in utilizing nutrients than the otter. The lower ADs of otters can be due to their faster passages. A fast passage rate through the small intestine can lead to a reduced digestibility (PEACHEY et al. 2000).
ADs from the cat and the otter are differing more than data between mink and otter. The AD of energy in cats was reported in a range of 86 to 94 % (BARBIERS et al. 1982, NOTT et al. 1994), for crude protein of 78 to 90 % (GREAVES and SCOTT 1960, MORRIS 1977) and for crude fat of 85 to 99 % (MORRIS 1977, KANE et al. 1981). All ADs are higher for the cat than they are for mink and otter. For determining the differences between otter, mink and cat, trials would have to be conducted with cats using the same diet. The available information does not allow to draw any further conclusions of species specific differences.

Mink and otters are obligate carnivores with simple stomachs. They are adapted to the same habitat as solitarily living, semi aquatic mustelids. Both prey on the same species spectrum in the wild consisting of fish, crayfish, reptiles, amphibians, insects, birds, small mammals and molluscs. The emphasis of otter is more on fish, of mink more on birds and small mammals (WISE et al. 1981). The better feed utilization of the mink as compared to the otter could be due to an evolutionary adaptation of the mink's gastro-intestinal system caused by the differ-

ing intake of biomass in the prey spectrum and prey availability. In general, the focus prey of mink (birds, small mammals) is harder to digest than that of otters (fish) (DRUMMOND 1918, JACQUOT and CREACH 1950). Both are eating the prey as a whole. The digestive efficiency and function of the otter seems to be adapted to continuous prey availability which is easy to digest. This is reflected in its ability to reproduce the whole year, also in regions with cold winters, and the good body condition indices found in necropsies of wild otters during both winter and summer time (SOMMER et al. 2005). The reproduction time of the mink is restricted to spring time (DUNSTONE 1993). That implicates that the mink would have problems due to food scarcity and climate in other seasons. Therefore the mink could be more dependent on a higher feed utilization due to feed scarcity, climate and a less digestible prey.

Recommendations for the mink are derived from production orientated studies. Any dietary parameters chosen to indicate the influence of an experimental treatment for the pelt industry are valid only if they are highly correlated to the more important characteristics of the ready-to-sell pelt (GLEM-HANSEN 1980). Another interest in mink studies is in the optimal reproduction and growth of the animals. Therefore many studies were only conducted with mink in the growth period up to pelting age at about 8 month and with lactating females (GLEM-HANSEN 1979 and 1980).

Conclusion

The mink seems to be comparable to some regards to the otter. A lower digestive efficiency and faster passage rates of the otter have to be taken into account by formulating diets for an optimal maintenance of *Lutra lutra* in husbandry.

5

COMPARISON OF THE NUTRIENT CONTENT OF EX-SITU AND IN-SITU DIETS OF EURASIAN OTTERS (*LUTRA LUTRA*)

Katrin Ruff[1], Hans-Heinrich Krüger[1], Jürgen Zentek[2], and Stephan Steinlechner[3]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany

[2]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany

[3]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

submitted

Abstract

For optimizing the husbandry of Eurasian otters (*Lutra lutra*) an adequate nutrition is essential, but no recommendations for adequate dietary nutrient levels are existing. Therefore the nutrient content of the ingested diet of wild otters was assessed to obtain hints on nutrient intakes of the species. The nutrient content of the in-situ diet was compared with zoo diets in order to give suggestions to optimize the ex-situ dietary management. The nutrient contents of ex-situ diets were obtained through a survey among keeping institutions. The in-situ nutrient values were calculated by literature review from 12 studies. Crude nutrients, calcium, magnesium, phosphorus, potassium, sodium, zinc and vitamin A, E and B_1 were calculated. The main parts of ex-situ diets consisted on average of 47% fish (13% trout, 9% herring, 5% roach) and 46% meat (21% chicks, 11% minced beef meat) differing considerably from the in-situ diet with 67% fish (12% carp, 11% trout, 11% perch, 6% pike), 4% meat and large amounts of crayfish and amphibians. The nutrient levels of the zoo diet exceeded the in-situ dietary fat content and vitamin A and B_1. The in-situ diet was higher in protein, zinc and vitamin E. Caution with the supplementation of vitamin A is proposed as well as with the deficiency of vitamin E.

Introduction

The Eurasian otter (*Lutra lutra*) was originally distributed throughout Eurasia. Due to a dramatic decline in range and number, otters are listed as severely endangered in many European countries (REUTHER 2004). To enhance captive reproduction attempts, the otter is often kept in European Zoos as part of the European Endangered Species Programme (EEP) of the European Association of Zoos and Aquaria. Additionally, otters are maintained in many non-European countries from Russia to Kuwait.

Frequent health problems in the husbandry of this species are low breeding success and nutrition related diseases. A high number of the zoo population is affected by urolithiasis (KEYMER 1981). Improper feeding can severely affect health and well-being of captive wild animals (HATT 2000) and improved nutrition has often positive effects on longevity, disease prevention, growth and reproduction (DIERENFELD 1997).

The otter is an obligate carnivore, feeding on a large variety of prey species in the wild, avoiding carcasses at all (GEIDEZIS 1999). In general, zoo diets are rarely adapted to the food in the natural habitat. The feedstuffs used ex-situ are at risk for nutrient imbalances, especially if the variety of offered feed items is small. It is not necessary to use the prey spectrum of the wild habitat in zoo diets, but the nutrient level should meet the requirements of the animal. Zoos should use diets that ensure an optimal supply with nutrients based on the knowledge of the specific requirements of the species (HATT 2000). Nutrient requirements of *Lutra lutra* are unknown to date. Existing recommendations for the species are adapted from experience of successful otter keepers. Beside the energy needs of the species (MELISSEN 2000), no scientific nutritional studies were carried out to test optimal nutrient supply for captive otters. Difficulties to conduct metabolic studies requiring feces, urine and blood samples under laboratory conditions are a major reason for that.

A possibility to assess nutrient requirements of a species without the need of metabolic studies is to consider natural feeding ecology. Presuming the unproven assumption of diet optimality in free-ranging animals as the optimal foraging theory implicates, nutrient requirements can be as first approximate derived by analysing the natural in-situ diet (DIERENFELD 1994, KREBS et al. 1978). Studies covering different habitats, seasonal changes and exact species specific analyses are a precondition for high-quality assessment of nutrient intakes with this method (DIERENFELD 1994). These basic studies have not yet been performed with *Lutra lutra*.

The aim of the study was to compare in-situ diets of *Lutra lutra* with zoo diets. Therefore the nutrient content of the ingested diet of wild otters was assessed to obtain hints on the requirements of the species. The nutrient content of the in-situ diet was compared with zoo diets in order to give suggestions helping to optimize the ex-situ dietary management.

Material and methods

Ex-situ diets

Ex-situ diets were surveyed by a questionnaire designed to collect detailed information on offered feed and its intake on a qualitative and quantitative level. As a basis the questionnaire of the EZNC (European Zoo Nutrition Centre) for a survey on nutrition of turtles was used (HELMINK et al. 2002) and modified for the purpose of the otter study. The questionnaire

was also modified to consider aspects to raise response rates of the survey, comprehensibility of the questions as well as to avoid ambiguity of answers (RAGHUNATHAN et al. 1995, ALWIN 1977). The questionnaire consisted of 8 questions in 5 sections including 3 general areas starting with an introduction letter, demographic data on otters (age, sex, weight), data on the keeping institution and an option for general comments of the participant. The most important section 3 and 4 covered the dietary management of the institutions. Section 3 referred to the feeding regime and the frequency of feeding or fasting days. Section 4 contained a list of feed ingredients open to extension. In this list the amounts of dietary ingredients should be recorded in gram or in mass %, all based on fresh matter. The list related feed amount per week because many zoos have feeding plans with different compositions for several weekdays but repeat the plans every week. The use of supplements was inquired with exact information on product names, manufacturers and amounts per week in gram. Another question asked if offered feed ingredients were regularly rejected from individuals in order to assess the difference between feed offered and ingestion.

Lists of otter keeping institutions were obtained from the European studbook, the EEP participant list and from a collection of the German Otter Centre. All institutions were contacted to receive information about the responsible person for dietary management. 68 institutions in 19 countries were contacted, 36 questionnaires were sent back (51.5%).

The nutrient content of the diets was calculated with the software ZOOTRITION Dietary management Version 2.0 (Wildlife Conservation Society 2001, New York, USA). Missing nutrient contents were added from literature (MEYER et al. 1983, MEYER et al. 2001, MASLANKA et al. 1998, DIERENFELD et al. 1996). Crude protein, crude fat, calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), zinc (Zn), and vitamins A, E and B_1 were calculated for every zoo diet. All nutrients are given as g per 100g dry matter. Zinc, vitamin E and B_1 were expressed in mg per kg dry matter, vitamin A as IU/kg (International Unit per kg). Energy (GE) is given as Kilojoule (kJ) per 100g dry matter. Data were summarized as means and the median range is given from 30 to 60 % of all data.

In-situ diets

In-situ data were compiled by a literature review. Dietary studies for otters have been made by direct observation (KRUUK et al 1978) and by stomach content analysis (ERLINGE et al. 1981, HOFMANN et al. 1992), but most have been based on spraint (feces) analysis (JACOBSEN et al. 1996, EWER 1973). To ensure comparability, only papers using the latter

method were included in this study. A problem is the presentation of the results in the different studies as well as the use of different methods to estimate the proportions of each prey category in the spraints. The “relative frequency of occurrence” method is the most used, but gives only data on how often prey species remains occurred in spraints (JACOBSEN et al. 1996). For quantitative estimates on the intake of prey species, the “range-bulk estimate” method was used. 12 of the studies existing for *Lutra lutra* used this method which is considered to be the best for calculating nutrient contents. Every included publication had spraint sample sizes of more than 100. The cumulative sample size of the studies was 27860 spraints. The in-situ data were similarly processed and presented as the ex-situ ones. For insects and reptiles few nutrient analyses are existing. Therefore all occurring insects and reptiles were summed in one section each. For the insects the nutrient data of flour beetles (*Tenebrio melitor*) were used, for reptiles those for Natrix (sp.). For four fish species no data could be found (*Chondrostoma nasus*, *Leuciscus cephalus*, *Vimba vimba* and *Rhodeus* sp.). All are Cyprinidae, therefore the nutrient value given in general for this family was used as approximation.

Statistics

Nutrient data were subjected to t-tests to test if the ex-situ and in-situ diet differs significantly in nutrient content (SACHS 1992). The significance level was set at $P < 0.05$.

Results

The typical ex-situ diet consisted of 47% fish, 46% meat, 1.2% plant material, 1.6% commercial cat diet and 4.3% others (Table 1). The most widely used fish species were trout (13%), herring (9.2%), roach (5.5%), bream (4.4%), mackerel (4.0%), carp (3.2%) and smelt (2.5%). The remaining 5.8% consisted of 14 other fish species, all fed less than 2%. Meat, including all mammal and bird species or products of them, was mainly chicken (21%) consisting of 15% day-old chicks and 6% adult chicken. Minced beef meat was fed at 11%. Small amounts of rabbits, mice and beef heart were reported, the remaining 6% were split in 11 feed items (e.g. guinea pig, beef liver, horse meat). Used plant material was mainly carrot (0.7%) and apple (0.2%). Two commercial canned cat diets were used. The section “others”

consisted of egg (1.3%), cheese and crayfish. The institutions used on average 6.7 different ingredients for the otter feed.

Table 1. Ingredients of ex-situ[1] and in-situ[2] diets with the percentage of ingested biomass

diet ingredient	biomass ex-situ %	biomass in-situ %
fish	**47.2**	**67.4**
trout	12.6	0
herring	9.2	0
roach	5.5	1.9
bream	4.4	0.6
mackerel	4.0	0
carp	3.2	12.4
smelt	2.5	0.2
other	5.8	52.3
meat	**46.2**	**4.3**
chicken (Gallus)	20.6	0
minced beef	11	0
rabbit	3.6	0
mice	2.7	0.9
beef heart	2.3	0
others	6.0	3.4
plant material	**1.2**	**0.1**
cat/dog food	**1.1**	**0**
other	**4.3**	**10.8**
amphibians, reptiles, insects	**0**	**17.5**

[1] ex-situ diet values derived from following institutions: Otterzentrum Hankensbüttel, Wildpark Schwarze Berge, Wildpark Schorfheide, Zoo Osnabrück, Zoo Rostock, Tierpark Hoyerswerda, Nationalpark Bayerischer Wald, Tierpark Kunsterspring, Tiergarten Weilburg, Tierpark Thale, Alpenzoo Innsbruck, Zoo Salzburg, Tierpark Dahlhölzli Bern, Fischotterverein Männedorf, Zoorama Europeen en foret de chize, Parc de Cigognes et des Loutres Hunawihr, Parc Zoologique de Paris, Parc Zoologique La Chaux-de-Fonds, Van Dierenpark Wissel, Skansen Foundation, Stiftelsen Skanus Djurpark, Ranua Wildlife Park, Zoo Helsinki, Zoo Poznan, Zoo Warszawa, Zoo Budapest, Attica Zoological Parc, Zoo Blackpool, Thrigby Hall Wildlife Gardens, Welsh Mountain Zoo, Highland Wildlife Parc, Cestnut Center Derby, Zoo Novosibirsk, Zoo Moskau, Lietuvos Zoologijos Sodas, The Scientific Center Kuwait

[2] in-situ diet values derived from LOPEZ-NIEVES et al. 1984, GEIDEZIS 1999, HARNA 1993, JEDREZEJEWSKA et al. 2001, MCFADDEN et al. 1983, KYNE et al. 1989, LIBOIS 1997, RUIZ-OLMO et al. 1997, RUIZ-OLMO et al. 2001, SULKAVA 1996, TAASTROM et al. 1999

The estimated nutrient contents (Table 2) of the ex-situ and in-situ diets differed significantly in energy content, crude protein, crude fat, Zn and the vitamins A, E and B_1. Energy density and fat content were higher in the zoo diet, crude protein in the wild. Within the minerals, only Zn was higher in the wild. Vitamins A and B_1 were higher in ex-situ diets, vitamin E was higher in-situ.

In-situ diets consisted of 67.4% fish, compared to the ex-situ diet with a different species spectrum. From the 21 fish species fed ex-situ, 11 could be found in the in-situ prey spectrum of the otter. The most preyed species were carp (12%), trout (11%), perch (11%), and pike (7.5%).

Only 4.3% of the biomass in the wild was meat. Within the meat section 8 of the 17 species of the ex-situ diet are also in the prey spectrum of wild otters (66% of biomass of meat section), but with a biomass part of 4.3% at a low level in total. From the plant material and the section “other” only crayfish belongs to the ex-situ diet. Crayfish (11%) was found quite often in the feed of free-ranging otter. Amphibians (15%), reptiles and insects which are common in the wild otters` diet are not used ex-situ.

Mineral and vitamin supplements were added to the diet in 25 of the 36 institutions (70%) from various manufacturers with differing mixtures. Often used were so called “fish eater tablets” and cod-liver oil.

Table 2. Calculated nutrient composition of in-situ and ex-situ diet in dry matter

	mean		range (30-60% of values)	
	in-situ	ex-situ	in-situ	ex-situ
Energy (GE)[1]	2055 ±217.6	2220 ±254.2	2040 - 2172	2195 - 2308
Crude fat (g/100g)	19.1 ±5.7	32.2 ±6.7	18.0- 21.8	29.3 - 34.6
Crude protein (g/100g)	72.7 ±7.2	57.5 ±7.4	72.2 - 74.5	53.6 - 61.7
Ca (g/100g)	1.0 ±0.6	1.1 ±0.6	0.9 - 1.2	0.6 - 1.6
Mg (g/100g)	0.3 ±0.3	0.2 ±0.1	0.3 - 0.4	0.2 - 0.3
P (g/100g)	1.2 ±0.5	1.2 ±0.4	1.3 - 1.4	1.1 - 1.4
K (g/100g)	1.3 ±0.4	1.1 ±0.2	1.3 - 1.4	0.9 - 1.2
Na (g/100g)	0.4 ±0.1	0.4 ±0.2	0.4 - 0.5	0.3 - 0.4
Zn (mg/kg)	93.7 ±41.4	75.0 ±18.5	69.1 - 115.1	67.5 - 82.0
Vit A (IU/kg)	21.1 ±9.3	38.0 ±29.6	18.3 - 24.2	25.1 - 36.6
Vit E (mg/kg)	106 ±73.0	71.5 ±74.5	88.4 - 114.5	36.4 - 63.7
Vit B_1 (mg/kg)	2.6 ±1.2	4.4 ±2.6	1.9 - 3.6	3.2 - 4.7

± standard error; [1]in kJ/100g

Discussion

Deriving ranges of nutrient requirements from diet compositions of free-ranging animals is a crude approximation and can only give hints on requirements of the species. High standard errors occurred in some nutrients indicating that the intake differed within a large bandwidth.

Diet optimality in the wild is a supposition of the optimal foraging theory (MCARTHUR et al. 1966, SCHOENER 1971). According to that theory animals, as a result of evolutionary pressure, tend to harvest their food efficiently and additionally make their choice to maximize their fitness. This includes a prey choice towards an optimal supply of energy, macro and micro nutrients (KREBS et al. 1978). Prey choice was often described for *Lutra lutra*. GEIDEZIS (1999) showed a species specific selection in a commercially-used pond area, influenced by nutritional values of the prey. Although carp was easy to hunt and available in huge amounts in comparison to other prey in the study area, the otters selected against this easy prey at the expense of other species. This behaviour could be a choice against a high intake of the enzyme thiaminase which destroys thiamine (vitamin B_1). Thiaminase is found in high concentrations in carp (TSCHIRCH 1978). Tests with captive otters showed the appearance of the Chastek paralysis under bream diet, which beside carp has a high concentration of thiaminase. In free-ranging animals a vitamin B_1 deficiency was never reported for a mammal (TSCHIRCH 1978). Other studies also found specific species selection in otters (TAASTROM et al. 1999, CALLEJO 1988), mainly when prey is abundant. This indicates that the otter is selective in hunting and optimal foraging theory could be to some degree applied to the species.
The 12 studies included for a retrospective data acquisition of in-situ diets cover all main otter habitats (river systems, lake districts, pond areas) and were conducted in various countries differing in prey species distribution and climate. Studies include all seasons of the year and are based on a large overall sample size. The literature data seem to be reasonably representative and avoid artefacts caused by differences in habitat, sampling, season or individual preferences being premise for the significance of nutrient content calculations from free-ranging animals (DIERENFELD 1994).

A certain level of inaccuracy in calculating the nutrient compositions of the diets was unavoidable. For many species a difference in nutrient values is known for different growth

levels and the use of different feed (CLUM et al. 1996). The nutrient calculations in literature are conducted with feed items of a certain size and reared with certain diets, but this could not be taken into account in this study due to the lack of available data. Another inaccuracy occurred by summing up four fish species as well as the insects and reptiles each in one group because no data were available. But the total biomass of affected species is low, thus avoiding a high influence on the nutrient calculation. The problem of nutrient loss due to the need to freeze feed ingredients for longer storage in zoos is not included in the discussion due to missing data concerning storage conditions and actual nutrient loss.

The biomass contents of ingested fish, meat, amphibians, reptiles, crayfish and other prey is strongly differing between ex-situ and in-situ. Especially amphibians, reptiles and crayfish as well as many fish species are often not available for zoos. The most fed prey ex-situ is day-old chicks, which is a common feed ingredient in zoos for many carnivores. In early diet recommendations chicks were added to the otter diet because the use of beef meat resulted in unfavourable stool qualities (REUTHER 1991). Rabbits and mice are common in carnivorous zoo diets. Birds, rabbits and mice are also preyed by wild otters but in very small biomass amounts (MASON et al. 1986). One of the most used fish species in zoos are herring and mackerel, which can not be preyed on by wild otters, even not in coastal areas. But these fish species are main feed sources for carnivorous marine mammals in zoos and therefore otters are also often fed with them. Only 7 feed ingredients (trout, herring, roach, bream, chicken and minced meat) make up 67% of the biomass of feed in zoos. The rare use of a commercial diet for *Lutra lutra* in Europe is different to the huge amounts which are fed in American zoos to otter species. Mainly commercial cat diets are used there (CRISSEY et al. 2001, MASLANKA et al. 1998).

Due to the difference in feed ingredients in zoos the nutrient composition ex-situ and in-situ is clearly different. The fat contents of zoo diets (32%) are higher than those of wild diets (19%). By looking at recommendations of other carnivores, the percentage of fat recommended for maintaining cat is 9% and for North American River Otter 23% (NRC 2006, CRISSEY 2001). Compared to the results for *Lutra lutra* of this study, the fat content of the ex-situ diet is far higher than the recommendations for cats and river otters. The higher fat contents of ex-situ diets are caused by the usage of beef meat and “oily” fish. Mackerels, herring, sprat, eel and trout are oily fish species, which have train oil not only in the liver but

also in the fillet. Of the oily fish trout and eel are preyed on in larger amounts by free-ranging otters (HARNA 1993, LIBOIS 1997, KYNE et al. 1989).

The intake of fat is important for the supply with essential fatty acids. The ability to convert essential fatty acids like linoleic, linolenic and arachidonic acids into other fatty acids is species dependent. Obligate carnivores such as cats require also pre-formed arachidonic acid. For the otter the requirements of essential fatty acids are not known, but it is presumed that they have similar requirements due to their obligate carnivorous status with simple gastrointestinal system like cats (CRISSEY 2001). A specific aspect related to fat intake is the intake of n3- and n6- fatty acids. Especially the ratio of n3/n6- intake is necessary for health (NÜRNBERG 2004). Fish is high in n3- fatty acids, chicken and beef in n6- fatty acids (BLOCK et al. 1985, NÜRNBERG 2004). No data have been published on the specific role of n3-, n6- intakes in otters.

Beside the need of fatty acids, fats are utilized by animals as energy. The dietary energy content of zoo diets is higher ex-situ, but obesity, which is often a major problem in zoo animals, is reported seldom in *Lutra lutra* (ALLEN et al. 1996). Many keepers even feed ad libitum and don't have problems with obesity.

The protein content is higher in-situ (73%) than ex-situ (58%). For cats and North American River otter 28% protein is recommended, for mink 24% (NRC 1982 & 2006, CRISSEY 2001). The protein content of the in-situ diet of the Eurasian otter exceeds the recommendations by far, but also the zoo diet is higher than protein levels recommended for other carnivores. The high protein intake indicates that otters are breaking down protein to a large extent to use it as an energy source.

The ex-situ and in-situ macro mineral contents were equal indicating that the requirements would be met on average in the zoo diet.

Within the trace minerals only Zn was calculated due to the incomplete data on other minerals for many prey items. Zn is a cofactor in many metabolic pathways and is involved in such vital functions as protein synthesis, immune competency, wound healing and DNA and RNA synthesis. During reproduction and growth the requirements are increasing (ALLEN et al. 1996). The Zn concentration was higher in the wild, but with a high standard error indicating that the content in the 12 studies differed in a large range. Recommendations for cats are 50 mg/kg, for North American River otter 72 mg/kg (NRC 2006, CRISSEY 2001). Latter is ac-

cording to the ex-situ values of this study (75 mg/kg) and lower than the concentrations in the wild (93 mg/kg).

Vitamins A, E and B_1 (Thiamine) were chosen for the calculation because they are known to be often a problem in captive fish-eating mammals. Thiamine deficiency can occur when thiaminase containing ingredients are fed. Fish species high in thiaminase are carp, roach, herring, mackerel and smelt (GERACI et al. 1980, AULERICH et al. 1995). ROBBINS (1993) also reports that thiaminase occurs in newly hatched chicks. Many zoos supplement vitamin B_1, some with the help of yeast. Caution is appropriate with the use of yeast due to the high purine values which can raise the risk of uric acid calculi in the kidneys, a common disease in captive otters (WEBER 2001). Vitamin B_1 concentrations were higher ex-situ, but due to the large biomass with thiaminase containing fish species and the high amount of day-old chicks this seems to be advantageous. The recommended supplementation regime for fish eating mammals is 25-30mg of thiamine per kg of fish fed on a fresh weight basis (BERNARD et al. 1997). The zoo diet concentration is in the bandwidth of recommendations for cats and the North American River otter (3-5 mg/kg, NRC 2006, CRISSEY 2001).
Vitamin A is one of the most oversupplemented nutrients in zoo diets (DIERENFELD 1994). A clear oversupplementation was also found in this study for *Lutra lutra* as the vitamin A concentration exceeds the amounts of the natural diet by 45%. Vertebrate prey given as a whole contains in general vitamin A levels that meet or greatly exceed dietary recommendations for wildlife and domestic species (1500 to 3000 IU/kg dry matter, ROBBINS 1993), but many zoos supplement the vitamin. Vitamin A is fat soluble and stored in the body and can cause toxicity. A study with seals showed a direct antagonism between vitamin A and E. A high vitamin A supplementations caused a decrease in vitamin E status (MAZZARO et al. 1995). By comparing the data with recommendations for cats (10 IU/kg) the values in the wild are higher (21 IU/kg), those of zoo diets almost 4 fold higher (38 IU/kg). Oversupplementation with vitamin A has been suggested as a relatively common cause of death in captive wildlife (ROBBINS 1993), however the calculated levels cannot be considered as toxic.
Vitamin E deficiencies have often been identified among zoo species, especially in fish-eating mammals (GERACI et al. 1980) fed with thawed frozen fish. Vitamin E is destroyed to large extent when fish is stored, even under optimal conditions, as polyunsaturated fatty acids undergo peroxidation (BERNARD et al. 2002). Much of the vitamin in fresh fish may be destructed even after a few weeks of frozen storage. Vitamin E is an important anti-

oxidant and is involved in a number of metabolic functions which include cell membrane integrity, enzyme and heme synthesis as well as steroidogenesis. Deficiency has far-reaching manifestations including reproductive failure, steatitis, muscular degenerations, liver necrosis and anemia (GERACI 1980). The concentration in the ex-situ diet (72 mg/kg) is lower than those of wild diets (106 mg/kg) and those recommended for cat (80 mg/kg, NRC 2006).

In conclusion, the data for the ex-situ diet present the mean values of 36 zoo diets. Therefore this study can only identify general problems in dietary management of *Lutra lutra*, not sparing that every institution has to calculate and compare its own diet individually with the recommendations for optimizing the nutrition of the otters.

6

DIETARY INFLUENCE ON URINARY MINERALS, METABOLITES AND AMINO ACID CONCENTRATIONS IN EURASIAN OTTERS (*LUTRA LUTRA*)

Katrin Ruff[1], Hans-Heinrich Krüger[1], Stephan Steinlechner[2], Jürgen Zentek[3], and Adrian C. Sewell[4]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany
[2]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany
[3]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany
[4]Department of Pediatrics, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

in preparation

Abstract

Urine trials were performed in captive Eurasian otters (*Lutra lutra*) to assess dietary influences on urinary mineral, metabolite and amino acid concentrations. Therefore, feeding trials were conducted with 5 otters and 6 dietary regimens. The animals were adapted to the diets for 5 days, followed by a urine collection period of 5 days. The urine was quantitatively collected in metabolism cages. Each urine was assayed for creatinine, sodium, potassium, calcium, magnesium, sulphate, phosphate, oxalate, citrate and free amino acids. The average urine volume was determined per 24 hours.

The mean concentration (expressed in mmol/mmol creatinine) of sodium was 3.7, of potassium 9.9, of calcium 0.13, of magnesium 0.13, of sulphate 39.6, of phosphate 19.6, of oxalate 0.5 and of citrate 0.3. Significant differences between diets on urinary mineral and metabolite concentrations were found for sodium, potassium, calcium, magnesium, sulphate and phosphate. No significant dietary influence was found for oxalate and citrate. The amino acid concentration in the urine differed between diets, no difference was detected for methionine, valine, glycine, phenylalanine and the dipeptide carnosine.

In conclusion, our results indicated that the diet influences the urinary concentration of minerals, metabolites and amino acids in otters. Therefore, dietary conditions should be considered when measuring these analytes in urine.

Introduction

Evaluation of urine is most important for the diagnostic of urinary tract infection and affection of the kidney. But also metabolic failure and liver diseases can often be discovered in an early stage through urinalysis (BARSANTI and FINCO 1979). However, dietary influences on urinary concentrations can have big impact on results (LULICH et al. 1991).

In the husbandry of the severely endangered Eurasian otter (*Lutra lutra*) health problems are occurring frequently (KEYMER et al. 1981). For example, urolithiasis is a common disease in the Eurasian otter, emerging in up to 23.4 % of otters in the wild population. In the captive population it can be as much as 69.2 % (WEBER 2001, KEYMER et al. 1981). The pathogenesis of stone formation is unknown for the otter. For investigating causes and risk factors concerning this disease, urine values of minerals, metabolites, amino acids and further or-

ganic substances are needed to detect abnormalities (LULICH et al. 1991). But urine studies are in general rare in zoo animals. Quantitative urine collection trials are difficult to perform because it is impossible to collect total urine under laboratory conditions in normal zoo enclosures with natural ground.

Only one study measured urinary compositions in *Lutra lutra*, based on spontaneous single urine samples, for uric acid and creatinine without including dietary influences (WEBER 2001). Beside *Lutra lutra*, captive Asian small-clawed otters (*Aonyx cinerea)* are often forming uroliths within the Lutrinae (PETRINI et al. 1999). For this species few urine data exist, including dietary influences or fasting on urinary concentrations (CALLE and ROBINSON 1985, PETRINI et al. 1996, PETRINI et al. 1999), indicating differences between diets and treatments.

Quantitative urine samples have never before been collected and analysed for *Lutra lutra*. The purpose of this study was to test dietary influences on urinary mineral, metabolite and amino acid concentrations (sulphate, phosphate, citrate, oxalate, sodium, potassium, magnesium, calcium and free amino acids) in Eurasian otters as a basis for further investigations, especially for urolithiasis research studies.

Materials and methods

Animals

The investigation was carried out in five otters, one male (2 years old) and four females (3, 5, 7, and 16 years old). The otters were housed individually in semi-natural enclosures, all were considered to be in good health; none was medically treated.

Diets

Six diets were used for obtaining basic data on urine composition and the dietary influence. The ingredients of diets 2, 3, 4 and 5 are listed in Table 1. Diet 1 consisted of one-day-old-chicken and diet 6 of herring.

The content of measured energy and macro nutrients of the diets are shown in Table 2. The diets were chosen because they represent those most commonly used in zoological facilities for feeding *Lutra lutra*.

Table 1. Composition of diets (%)

	Diet			
Ingredient	2	3	4	5
Day-old-	25	15		12.5
Bream	30	10	50	
Roach			50	
Herring				
Trout				31.25
Ground beef		65		6.25
Rumen	40			
Cattle heart	2.5	2		6.25
Cattle liver	2.5			
Chicken				12.5
Rabbit				6.25
Mouse				6.25
Rat				6.25
Guinea pig				6.25
Oat flakes		3		
Wheat bran		2		
Carrot		2		6.25
Yeast (fresh)		1		

Four feed ingredients were deep frozen (herring, rat, guinea pigs), the rest was purchased fresh. Before a trial started, all ingredients were minced with a meat grinder (perforated plate with holes of 0.5 cm diameter) to avoid selective feed intake. All feed animals were minced whole and only the fur of rabbits and guinea pigs and chicken feathers were removed. After mincing, the feed mash was thoroughly mixed to assure that all nutrients were dispersed uniformly. For each otter, the diet adjusted for maintenance was deep frozen in plastic bags at – 40°C. For the mixed diets, every single feed item was processed separately and mixed after thawing. The feed was defrosted overnight. The diet for one day was offered in one portion in the morning. Tap water was supplied ad libitum.

Table 2. Analysis of nutrient composition (% dry matter basis) and energy content (kJ/g) of the diets

	Diet					
	1	2	3	4	5	6
Energy (GE)	21.2	23.7	25.5	19.9	23.2	21.8
Dry matter*	21	26.7	34.3	26.5	27.5	20.2
Ash	5.2	7.1	4.1	15.2	8.6	11.8
Crude fiber	1.6	0.8	3.2	0.2	0.9	0.4
Crude protein	51.7	57.0	51.1	63.9	58.2	38.8
Crude fat	13.3	30.5	36.5	14.7	24.7	9.5
Sodium	7.9	4.2	2.4	2.4	4.5	3.9
Potassium	7.0	8.0	8.3	9.3	9.9	4.8

*fresh matter

Experimental procedure

To accustom the otters slowly to the new diet they were given minced chicks mixed with 12.5% of the new diet on the first day, 37.5% on the second, 50% on the third, 62.5% on the fourth, 75% on the fifth, and 87.5% on the sixth day. From the seventh day, 100% of the new diet was fed for another 5 days still within the normal enclosures. The otters were then moved to the metabolism cages and fed for one day without urine collection. Thereafter the collection period started for the 120 hour sampling.

Every metabolism cage (3.5 x 2.1 m) was situated in a separate room. The cage floor consisted of a wire mesh (5 x 5 mm), plastic sheets were formed into funnels to collect the urine into containers (1 litre). To provide water ad libitum without the danger that lost drinking water interfered with urine collection, water bowls with protective foils were installed. As enrichment, stones, branches, boxes and toys were put inside the cages.

Sample handling

Urine samples were collected in plastic vessels with thymol (0.2 g thymol in 3 ml deionised water and 2 ml ethanol) as a preservative. To reduce contamination, the urine passed through two dense meshes (grid size 1mm) placed above the containers. The urine samples were removed every 3 hours during the day and after a 9 hour period overnight. The samples were frozen at – 40°C. At the end of the trial, the urine of one period was thoroughly mixed,

pooled, and divided into aliquots of 100 ml. The aliquots were frozen at – 40°C for later analyses.

Analytical procedure

Diets were freeze-dried and subjected to a Weende analysis (VDLUFA 2003). Energy content was determined using a bomb calorimeter (IKA Kalorimeter C7000, IKA-Werke GmbH, Staufen, Germany). Sodium, potassium, magnesium and calcium were determined by HPLC (Dionex, type DX-120, Sunnyvale, USA). Sulphate, phosphate, oxalate and citrate were also analysed by HPLC (Dionex, type DX-500, Sunnyvale, USA).

An amino acid analyser (LC3000 Eppendorf/Biotronik, Hamburg, Germany) was used to quantify 28 urinary amino acids. After precipitation of the proteins, the free amino acids were separated via ion exchange chromatography, derivatised with ninhydrine and detected photometrically at 440 and 570nm.

Creatinine in urine was assayed by the Jaffé kinetic method using a Cobas Mira analyser (Roche, Indianapolis, USA).

The values for metabolites are given as mmol per mmol creatinine and mmol/l, those of amino acids as µmol/mmol creatinine. Data were summarized for metabolites as well as for amino acids as means and the median range is given from 5 to 95 % of all data (90 % percentiles).

Statistics

Differences between diets were tested by a one-factorial analysis of variance ANOVA, followed by t-tests (SACHS 1992). The significance level was set at $P < 0.05$.

Results

All animals tolerated the dietary manipulations well. The mean urine volume was 405 ml per 24 hours with a high standard error of 195.

Within the metabolites (Table 3), the highest urinary concentrations were measured on average for sulphate (39.6) and phosphate (19.6), the lowest for calcium (0.1) and magnesium (0.1). Urinary amounts of sodium (10.8), calcium (0.3), sulphate (62.9), oxalate (0.8) and citrate (0.5) were highest for diet 1 (chicken), of potassium (14.8) and phosphate (29.5) for diet 6 (herring) and of magnesium (0.2) for diet 4 (white fish). Lowest concentrations of sodium (0.9) and potassium (4.7) were recorded for diet 3, of sulphate (20.9) and phosphate (12.4) for diet 2. Calcium, magnesium, oxalate and citrate were low from diet 2 to 6. The urinary metabolite concentration differed between diets for sodium, potassium, calcium, magnesium, sulphate and phosphate. The urine concentration differences between the diets were highest for sodium, calcium and magnesium, the lowest were found for phosphate. Oxalate and citrate differed not significantly between diets.

Urinary amino acid concentrations are shown in Table 4. Proline, OH-proline and asparagine were in urine of all diets beside diet 1 under the limit of detection, arginine in all diets. 1-Met-histidine could only be detected in the urine collected while feeding diet 6. The highest urinary amino acid concentrations were measured while feeding diet 1, beside glutamine, 3-Met-histidine, ornithine and lysine which were highest while feeding diet 5. The concentrations differed between diets for all amino acids (for significance see Table 4) beside methionine, valine, glycine, phenylalanine and the dipeptide carnosine. For the amino acids standard errors were high.

Table 3. Analysis of urine metabolites in mmol/mmol creatinine*, except mean[1] of all diets, which is given in mmol/l

	diet												all diets			
	1		2		3		4		5		6					
metabolite	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	range	mean[1]
sodium	10.8^{a}	±2.7	1.6^{cb}	±0.5	0.9^{b}	±0.4	1.8^{bd}	±0.8	1.9^{cd}	±0.3	2.6^{cd}	±0.9	3.7	±3.9	0.8 - 11.9	20.2
potassium	10.3^{a}	±1.5	6.4^{bc}	±0.3	4.7^{c}	±1.1	10.4^{ab}	±2.9	10.3^{a}	±0.6	14.8^{a}	±3.9	9.9	±4.1	5.3 - 13.8	237
calcium	0.27^{a}	±0.07	0.09^{b}	±0.02	0.06^{b}	±0.02	0.08^{b}	±0.01	0.06^{b}	±0.01	0.08^{b}	±0.03	0.13	±0.08	0.1 - 0.2	0.7
magnesium	0.14^{ab}	±0.13	0.11^{ac}	±0.01	0.03^{a}	±0.05	0.15^{bcd}	±0.04	0.1^{acd}	±0.06	0.13^{bd}	±0.08	0.13	±0.08	0.1 - 0.2	0.8
sulphate	62.8^{a}	±17.1	20.9^{b}	±9.3	21.3^{b}	±10.4	38.2^{ab}	±13.3	38.6^{ab}	±9.7	44^{a}	±12.1	39.6	±19.4	13.5 - 64.3	230
phosphate	20^{ac}	±3.5	12.4^{bd}	±3.3	13^{bc}	±6.6	18.7^{abc}	±5.3	19.9^{acd}	±6	29.5^{a}	±11.8	19.6	±9.1	7.9 - 28.7	119
oxalate	0.83^{a}	±0.5	0.3^{a}	±0.2	0.42^{a}	±0.2	0.36^{a}	±0.1	0.56^{a}	±0.2	0.59^{a}	±0.5	0.53	±0.38	0.2 - 1.0	3.2
citrate	0.48^{a}	±0.3	0.17^{a}	±0.1	0.24^{a}	±0.1	0.21^{a}	±0.1	0.32^{a}	±0.1	0.34^{a}	±0.3	0.31	±0.22	0.1 - 0.6	1.8

* Within a column, means with different superscripts differ ($p < 0.05$)

Table 4: Analysis of amino acids in the urine in µmol/mmol creatinine [*]

	diet														
	1		2		3		4		5		6		all diets		
Amino acid	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	mean	±s	range
Taurine	490^{a}	±130	85.2^{b}	±71.1	155^{cb}	±140	436^{acd}	±214	305^{acd}	±77.7	447 ^{ad}	±173	332	±215	61.4 – 476
Asparatic acid	43.7^{ab}	±50.9	5.0^{a}	±1.3	3.9^{a}	±2.7	39.0^{b}	±21.9	5.9^{ab}	±1.2	13.7^{b}	±5.2	19.05	±29.7	2.7 – 40.5
Threonine	57.3^{ac}	±36.4	24.4^{a}	±12.0	27.7^{a}	±9.1	41.8^{ac}	±15.5	45.7^{bc}	±4.3	38.6^{ac}	±19.4	39.69	±23.4	16.1 – 48.8
Serine	155^{a}	±56.6	31.7^{b}	±12.3	33.5^{b}	±12.7	34.2^{b}	±11.9	41.9^{b}	±5.5	18.0^{b}	±6.2	55.42	±57.0	15.9 – 138
Asparagine	12.2	3.4	nd		nd		nd		nd		nd		nd		
Glutamic acid	91.4^{ab}	±112	13.0^{a}	±4.2	16.8^{ab}	±8.8	28.9^{ab}	±21.5	19.0^{ab}	±3.6	29.8^{b}	±12.1	36.18	±59.9	12.0 – 54.2
Glutamine	26.5^{ab}	±29.9	14.7^{ac}	±3.7	12.7^{a}	±2.7	21.6^{ab}	±10.6	19.2^{bc}	±2.1	30.1^{b}	±9.2	22.35	±15.7	13.7 – 34.7
Proline	57.1	55.6	nd		nd		nd		nd		nd		nd		
Glycine	116^{a}	±72.9	41.5^{a}	±26.9	39.5^{a}	±19.6	75.9^{a}	±35.7	62.7^{a}	±18.6	66.8^{a}	±30.3	69.16	±48.9	21.6 – 115
Alanine	117^{abc}	±121	56.8^{ab}	±27.8	42.6^{a}	±18.0	48.0^{a}	±29.7	97.4^{b}	±11.3	50.4^{ac}	±21.5	68.65	±64.5	21.3 – 98.2
Citruline	26.6^{ab}	±26.6	1.8^{ac}	±0.6	1.7^{a}	±1.7	9.9^{bc}	±5.5	8.3^{b}	±2.4	7.2^{ab}	±5.1	9.91	±15.4	0.2 – 16.1
Valine	49.4^{a}	±63.7	4.0^{a}	±1.9	5.8^{a}	±3.4	15.0^{a}	±13.5	5.5^{a}	±0.2	4.0^{a}	±3.5	15.31	±34.5	1.4 – 30.9
Cystine	54.3^{a}	±24.1	18.6^{b}	±4.1	21.8^{b}	±16.7	25.2^{ab}	±18.4	42.0^{ab}	±17.5	34.3^{ab}	±19.9	33.24	±22.6	10.3 – 70.1
Cystathionine	87.7^{a}	±43.3	18.0^{b}	±9.6	20.4^{b}	±12.0	13.4^{b}	±9.6	27.7^{ab}	±8.0	9.7^{b}	±4.2	32.97	±36.6	3.0 – 68.9
Methionine	21.6^{a}	±25.4	3.1^{a}	±0.8	3.3^{a}	±1.0	11.3^{a}	±13.0	1.3^{a}	±0.3	4.7^{a}	±6.2	8.24	±15.0	1.9 – 14.6
Isoleucine	37.1^{ab}	±49.7	1.4^{ad}	±0.2	2.2^{ac}	±1.1	11.4^{ab}	±10.7	2.8^{bc}	±0.3	7.4^{bd}	±4.7	11.63	±26.7	1.6 – 19.4
Leucine	51.4^{ab}	±72.7	1.8^{a}	±0.8	2.9^{a}	±2.3	39.5^{ab}	±45.8	11.7^{b}	±1.2	32.3^{b}	±17.1	25.21	±42.7	1.5 – 59.5
Tyrosine	27.1^{ac}	±30.1	3.2^{a}	±0.9	3.7^{a}	±2.0	15.8^{ac}	±13.7	6.1^{bc}	±0.6	14.9^{bc}	±7.2	12.76	±17.3	2.6 – 23.7
Phenylalanine	40.6^{a}	±43.9	6.2^{a}	±1.3	6.1^{a}	±2.3	25.1^{a}	±25.7	5.4^{a}	±1.5	15.5^{a}	±9.3	17.84	±26.5	31.6 – 74.2
Histidine	24.7^{ac}	±21.4	6.7^{a}	±3.0	6.0^{ad}	±2.5	21.0^{bd}	±11.6	14.1^{bc}	±1.9	20.5^{b}	±8.5	16.11	±13.5	4.6 – 34.5
1-Met-Histidine	nd		nd		nd		nd		nd		67.6	10.7	nd		
3-Met-Histidine	24.7^{a}	±10.3	56.5^{a}	±16.5	63.2^{a}	±15.6	76.0^{ac}	±16.6	65.0^{ac}	±20.4	96.7^{bc}	±20.7	71.09	±22.3	41.5 – 103
Tryptophan	14.6^{ab}	19.3	0.3^{a}	0.3	1.5^{ab}	1.3	3.3^{b}	1.4	2.8^{b}	0.2	nd		nd		
Carnosine	5.8^{a}	±2.7	3.7^{a}	±0.3	5.1^{a}	±3.1	2.3^{a}	±1.2	3.7^{a}	±2.8	nd		4.34	±2.7	0 – 8.2
Ornithine	7.0^{b}	±6.7	8.1^{b}	±4.6	7.2^{b}	±1.5	3.1^{b}	±3.9	40.4^{a}	±7.4	2.5^{b}	±1.4	9.68	±12.6	1.7 – 28.5
Lysine	50.5^{ab}	±56.3	32.5^{ab}	±17.1	19.9^{a}	±8.0	57.2^{b}	±24.8	57.8^{b}	±19.0	54.4^{b}	±26.4	45.47	±34.4	10.7 – 92.1

[*] within a column, means with different superscripts differ ($p < 0.05$); nd: not determinable (<1mmol/l)

Discussion

Our results indicated that diet affects the concentration of excreted sodium, potassium, calcium, magnesium, sulphate and phosphate. This was also reported, amongst others, for dogs, cats and Asian small-clawed otters. Studies with these species also showed a large dietary influence on urinary sodium, potassium, calcium and magnesium concentrations according to our results (LULICH et al. 1991, PETRINI et al. 1996). This is due to the regulation of these metabolites that are primarily eliminated by the kidney (LULICH et al. 1991). Sodium, potassium and calcium concentrations in the blood are regulated hormonally very tense, so the urinary excretion is strongly dependent on blood levels. When a deficit is occurring, the renal excretion is reduced, and vice versa for a surplus to maintain optimal blood values. Therefore, deficits can be detected earlier with urine instead of blood analysis. Problems can occur while examining a dietary influence of hormonally regulated minerals when a deficiency is emerging not directly caused by the diet. For example, potassium efflux is rising during muscular effort and gets partly lost via kidney (MEYER and ZENTEK 2001). This can raise the urinary concentration although no dietary influence is existing. In general, also the influence of interactions between minerals with each other and with other nutrients can have an influence on renal excretion (ROBBINS 1993).

Citrate, an anorganic acid freely filtered by glomeruli, is almost completely reabsorbed by proximal tubules. In dogs, less than 1% of filtrated citrate is excreted in urine (BARUCH et al. 1975). Dietary influence on urinary citrate concentrations is not caused directly by citrate intake, but variations in acid-base balance can affect the quantity of excretion: alkalosis promotes citrate excretion. Acidosis minimizes it (SIMPSON 1964). The low differences between diets for citrate in this study, which were not significant, are in accordance with that.

Oxalic acid, an end product of the metabolism of either ascorbic acid or glyoxylate, is in humans to 80 - 90 % derived from endogenous metabolism, the rest of dietary sources (WILLIAMS and WANDZILAK 1989). For the otter we found no significant dietary influence, so it is assumed that endogenous production of oxalate accounted for most of the excretion in Eurasian otters. A low dietary influence on citrate was found in Asian small-clawed otters, no significant difference occurred for oxalate (PETRINI et al. 1996). This study reported also of dietary differences between sulphate and phosphate in similar intervals like we have found for *Lutra lutra*.

No data on a possible dietary influence on urinary amino acid excretion are available for any mustelid species.
Due to the dietary influence on measured urinary metabolite and amino acid concentration shown in this study, feeding conditions should be always considered when measuring urinary excretions, no matter if tests are done for routine health check or for investigations concerning specific health problems like renal calculi. Because of the lack of data concerning the content of minerals, metabolites and amino acids in the diets, no comparison of dietary intake and urinary excretion can be made in this study.

By comparing the means of all diets of urinary mineral, metabolite and amino acid concentrations we found for *Lutra lutra* with other species, it has to be considered that the studies are rarely conducted with equal diet compositions. In the dog, normally low calcium amounts are excreted via the kidney (MEYER and ZENTEK 2001). The low calcium concentrations (0.04 mg/mg creatinine, 0.68 mmol/l) found in this study indicate the same for *Lutra lutra,* but trials with exact intake would be necessary for reliable data. For the River Otter (*Lontra canadensis)* reported calcium mean is higher (2.8 mmol/l), but the study gives no hint towards diet (HOOVER and TYLER 1986). The low oxalate and citrate concentration we found in *Lutra lutra* is in accord with CALLE´s (1993) values for Asian small-clawed otters (0.03 mg/mg creatinine for citrate, 0.08 mg/mg creatinine for oxalate). PETRINI´s (1996) data (0.09 mg/mg creatinine for citrate, 0.11 mg/mg creatinine for oxalate) for Asian otters are higher. The mean of sulphate measured for *Lutra lutra* (3.6 mg/mg creatinine) is within the bandwidth of PETRINI (1996), magnesium concentration found in *Lutra lutra* (0.03 mg/mg creatinine) is lower (2.7 - 7.8 mg/mg creatinine for sulphate, 0.14 - 0.18 mg/mg creatinine for magnesium). Phosphate concentrations of this study (5.4 mg/mg creatinine, 119 mmol/l) are within the bandwidth reported for Asian otters (PETRINI 1996: 3.4 - 5.6 mg/mg creatinine) and higher than those of *Lontra canadensis* (HOOVER and TYLER 1986: 40.4 mmol/l). Sodium concentration (20.2 mmol/l) of Eurasian otters is in accordance with findings of River otters (HOOVER and TYLER 1986: 16.5 mmol/l), but potassium levels (238 mmol/l) are exceeding those by far (12.6 mmol/l).
For amino acids only values of Asian otters exist concerning valine, isoleucine, leucine, phenylalanine, methylhistidine and arginine with a range from 0.03 – 0.1 µg/mg creatinine. All amino acid concentrations found in *Lutra lutra* are exceeding these values beside arginine, which was under the detection level of the analyser.

7

DIETARY RISK FACTORS FOR URATE UROLITHIASIS IN EURASIAN OTTERS (*LUTRA LUTRA*)

Katrin Ruff[1], Hans-Heinrich Krüger[1], Adrian C. Sewell[2], Astrid Günther-Weigl[3], Stephan Steinlechner[4], and Jürgen Zentek[5]

[1]Otter Centre, Sudendorfallee 1, 29386 Hankensbüttel, Germany

[2]Department of Pediatrics, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

[3] Leibnitz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str.17, 10315 Berlin, Germany

[4]Institute of Zoology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

[5]Institute of Animal Nutrition, Freie Universität Berlin, Brümmerstr. 34, 14195 Berlin, Germany

submitted

Abstract

Urolithiasis due to ammonium urate calculi is a significant problem in captive Eurasian otters (*Lutra lutra*). To assess the risk factors for otters to form ammonium urate calculi, feeding trials were conducted in a group of 5 captive otters under seven dietary regimens. The animals were adapted to the appropriate diet for 5 days, followed by a urine collection for 5 days. The urine was quantitatively collected in metabolism cages. The composition of each diet was assayed for purine content, dry matter, crude ash, crude fiber, crude protein and crude fat. Each urine collection was assayed for pH, uric acid, creatinine, ammonium and allantoin concentrations. The average urine volume was 405 ml per 24 hours. For the blood tests, plasma from 15 otters was obtained and the uric acid concentrations were determined.
High concentrations of uric acid (3.3 mmol/l; 0.5 mmol/ mmol creatinine) and ammonium (103 mmol/l; 15 mmol/mmol creatinine) were found. The impact of purine intake on the renal uric acid excretion was significant. The urinary allantoin excretion, expressed as mmol/mmol creatinine, was 2.7 and higher than that reported for dogs. The mean plasma uric acid level of our otters (0.15 mmol/l) was higher than that in dogs, but not reaching critical levels above which uric acid is prone to precipitate.
In conclusion, risk factors for the formation of urate calculi in captive otters can be influenced by diet. The correlation between purine intake and renal uric acid excretion provides an opportunity to minimize this risk factor for urate urolithiasis in the captive population.

Introduction

Urolithiasis is a common disease in the Eurasian otter (*Lutra lutra*), occurring in up to 23.4% in the wild population (WEBER 2001). In the captive population it can be as much as 69.2% (KEYMER et al. 1981, WEBER 2001). In mustelids, urolithiasis is a problem only in minks (*Mustela vison*) and ferrets (*Mustela putorius* f. furo), which form primarily struvite uroliths (EDFORDS et al. 1989, WEBER 2001). Within the Lutrinae, the problem of urolithiasis has been reported in the Asian small-clawed otter (*Aonyx cinereus*). Data from the wild are rare, but in captivity up to 66.1% develop stones, mainly of calcium oxalate (CALLE 1988). Single case studies of the North American river otter (*Lontra canadensis*) have also described uric acid calculi (GROOVE et al. 2003).

The uroliths of the Eurasian otter are almost exclusively located in the kidneys (BAITCHMAN and KOLLIAS 2000, GEISEL 1979, MADSEN et al. 1999). The calculi are mainly composed of ammonium urate (80 % of calculi), with additional components (sodium urate, phosphate, struvite, oxalate) occurring in 20 % of stones (WEBER 2001).
Ammonium urate precipitation occurs when the urine is oversaturated with urate and ammonium ions (KLOHN et al. 1986). An important factor for the solubility of urate is the urine pH. Above pH 5.75, uric acid dissociates to its salt (urate) and is relatively insoluble. The solubility rises with the pH (THORNHILLS 1980). The uric acid pool is derived from both endogenous metabolism and exogenous intake, which varies with diet and its purine content (SHEKARRIZ and STOLLER 2002).

Normally the uric acid excretion in mammals is low because it is converted by the enzyme uricase to allantoin (THORNHILL 1980). This substance is much more soluble compared with uric acid and can pass through the kidney without the risk of crystallisation (SHEKARRIZ and STOLLER 2002). This is the reason why uric acid stones are rare in mammals. Exceptions, however, are humans, apes and Dalmatian dogs, where the final product of purine metabolism is uric acid (KEELER 1940). In humans, uricase is virtually absent but in apes, it is present at a low activity. In the Dalmatian dog, uricase is synthesized but the specific transport system for uric acid in the liver cell membranes has minimal capacity. Therefore, uricase which is present in normal activity is supplied only to a limited extent with uric acid for the transformation into the easily soluble allantoin (GIESECKE et al. 1985).
Not all Dalmatian dogs with high urinary uric acid concentrations are urolith formers, the reason is as yet unknown. The risk factors, i.e. high concentrations of uric acid and ammonium with a pH of around 6.0, are not the only reasons for the formation of calculi and the aetiology seems to be multifactorial (SORENSEN and LING 1993). Nevertheless, these factors seem to be a precondition for the formation of calculi (OSBORNE et al. 1986, SORENSEN and LING 1993, THORNHILL 1980).

In humans, high serum uric acid concentrations promote gout caused by interference of purine metabolism through multiple defects. Urate forms crystals when the serum concentration is above 0.5 mmol/l. The crystals accumulate in the peripheral joints and generate inflammation. The uric acid concentration in the serum of Dalmatian dogs never reaches the

critical mark (GIESECKE et al. 1985). As no data are available for the Eurasian otter concerning possible gouty disease, we determined their serum uric acid status.

The pathogenesis of stone formation is unknown for the otter and quantitative urine samples have never before been collected and analysed. The aim of the present study was to assess risk factors (uric acid, ammonium, pH) for the formation of urate calculi. For a possible prevention of the disease in captive otters, we tested how far the purine intake affects the urinary uric acid excretion. Furthermore, data on allantoin are presented to compare the process with that in humans and Dalmatian dogs.

Materials and methods

Animals

The dietary investigation was carried out in five otters, one male (2 years old) and four females (3, 5, 7, and 16 years old). One male otter was used for the fasting uric acid excretion study. The otters were housed individually in semi-natural enclosures, all were considered to be in good health, none was medically treated. Blood samples were obtained from 10 males and 5 females.

Diets

Seven diets were used for testing the correlation between the urinary uric acid excretion and purine intake as well as for obtaining basic data on urine composition. Diet 1 was the Hills prescription diet u/d (Hill's Pet Nutrition Inc., Topeka, USA), diet 3 was one-day-old-chicken and diet 7 was herring. The compositions of diets 2, 4, 5 and 6 are listed in Table 1.

Table 1. Composition of diets (%)

	Diet			
Ingredient	2	4	5	6
Day-old-	15	25	12.5	
Bream	10	30		50
Roach				50
Herring				
Trout			31.25	
Ground beef	65		6.25	
Rumen		40		
Cattle heart	2	2.5	6.25	
Cattle liver		2.5		
Chicken			12.5	
Rabbit			6.25	
Mouse			6.25	
Rat			6.25	
Guinea pig			6.25	
Oat flakes	3			
Wheat bran	2			
Carrot	2		6.25	
Yeast (fresh)	1			

The purine content of the diets varied from 0.03% up to 0.22% (Table 2). The diets were chosen because they represent those most commonly used in zoological facilities for feeding *Lutra lutra*.

One diet was a commercial canned formula; four feed ingredients were deep frozen (herring, rat, guinea pigs), the rest was purchased fresh directly from the abattoir and processed within one day. Before a trial started, all ingredients were minced with a meat grinder (perforated plate with holes of 0.5 cm diameter) to avoid selective feed intake. All feed animals were minced whole and only the fur of rabbits and guinea pigs and chicken feathers were removed. After mincing, the feed mash was thoroughly mixed to assure that all nutrients were dispersed uniformly. For each otter, the diet adjusted for maintenance was deep frozen in plastic bags at – 40°C. For the mixed diets, every single feed item was processed separately and mixed after thawing. The feed was defrosted overnight. The diet for one day was offered in one portion in the morning. Water was supplied ad libitum. Every otter was familiarised for two weeks with the minced feed before the trials started.

The animals for the blood sample trial were fed with diet 4 (Table 1). The blood samples were drawn in the morning after an overnight fast.

Table 2. Analysis of nutrient composition (% dry matter basis) of the diets

	Diet						
Diet fraction	1	2	3	4	5	6	7
Dry matter*	25.7	34.3	21	26.7	27.5	26.5	20.2
Purine	0.03	0.06	0.09	0.11	0.13	0.14	0.22
Ash	2.6	4.1	5.2	7.1	8.6	15.2	11.8
Crude fiber	0.6	3.2	1.6	0.8	0.9	0.2	0.4
Crude protein	12.7	51.1	51.7	57	58.2	63.9	38.8
Crude fat	19.1	36.5	13.3	30.5	24.7	14.7	9.5
Chloride	0.44	0.2	0.7	0.32	0.33	0.2	0.85

* fresh matter

Experimental procedure

To accustom the otters slowly to the new diet they were given minced chicks mixed with 12.5% of the new diet on the first day, 37.5% on the second, 50% on the third, 62.5% on the fourth, 75% on the fifth, and 87.5% on the sixth day. From the seventh day, 100% of the new diet was fed for another 5 days still within the normal enclosures. The otters were then moved to the metabolism cages and fed for one day without urine collection. Thereafter the collection period started for the 120 hour sampling. For the fasting uric acid levels, one male was fasted for four days in two trials. Subsequently urine was collected for three days under fasting conditions.

Every metabolism cage (3.5 x 2.1 m) was situated in a separate room with a glass door providing natural light. The cage floor consisted of a wire mesh (5 x 5 mm). Plastic sheets were formed into funnels to collect the urine into containers (1 litre). To provide water ad libitum without the danger that lost drinking water interfered with urine collection, water bowls with protective foils were installed. As enrichment, stones, branches, boxes and toys were put inside the cages. Chloride was used as a marker to compensate for potential urine loss assuming that absorbed chloride is excreted to 100% via the kidneys. The exact amount of chloride intake compared to that excreted in the urine provided a double check of urine loss. To calculate losses caused through the design of the cages and through evaporation, trials with known amounts of water were performed. Different volumes of water were poured into various

places in the cage. The amount was measured in intervals of 1, 2, 3, 6 and 9 hours. Averages of lost water were calculated and the average losses added to the measured urine volumes.

For the collection of the blood samples, the otters were sedated in a Plexiglas box using isoflurane. The blood was drawn from the Vena cephalica antebrachii.

Sample handling

Urine samples were collected in plastic vessels with thymol (0.2 g thymol in 3 ml deionised water and 2 ml ethanol) as a preservative. To reduce contamination the urine passed through two dense meshes (grid size 1mm) placed above the containers. The urine samples were removed every 3 hours during the day and after a 9 hour period overnight and the pH measured within 30 min (Microprocessor pH/mr-Meter 196, WTW GmbH, Weilheim, Germany). The samples were frozen at – 40°C. At the end of the trial, the urine of one period was thoroughly mixed, pooled, and divided into aliquots of 100 ml. The aliquots were frozen at – 40°C for later analyses.

The blood samples (5 ml) were drawn into EDTA-treated tubes, centrifuged within the next 24 hours (10 min at 3000U) and the resulting plasma stored at – 20 °C.

Analytical procedure

Urinary ammonium was determined by HPLC (Dionex, type DX-120, Sunnyvale, USA). Creatinine in urine was assayed by the Jaffé kinetic method using a Cobas Mira analyser (Roche, Indianapolis, USA). For the uric acid analysis, urine samples were heated for 30 minutes at 60°C to ensure complete solubility and diluted to 0.5 mmol/l creatinine prior to analysis by HPLC with diode-array detection (Shimadzu, type LC-10AD, Columbia, USA).

Allantoin in urine was determined as dinitrophenylhydrazone by HPLC (Shimadzu, type LC-10AD, Columbia, USA) after precolumn derivatisation (CHEN et al. 1993).

Results obtained are presented as mmol/l; uric acid and ammonium are also given as a ratio to creatinine (U/C, Am/C) (FINCO and BARSANTI 1982).

Uric acid in plasma was assayed enzymatically using a routine method. Uric acid, which absorbs light at 293 nm is converted with uricase to allantoin. Allantoin does not absorb light at 293 nm. The difference in extinction, which is caused by the decrease in uric acid, is directly

proportional to the concentration in the sample. The difference is measured bichromatically at 293 nm and 700nm (Dade Behring, type Dimension RXL, Illinois, USA).

Statistics

Linear regression equations and correlation coefficients (Pearson) were calculated according to Sachs (1992). The significance level was set at $P < 0.05$.

Results

All animals tolerated the dietary manipulations well.

The mean urinary pH was 6.1 (Table 3). The lowest urinary pH values occurred while feeding diets 1 (pH 5.8) and 4 (pH 6.0), the highest on diets 3 (pH 6.4) and 6 (pH 6.2). The mean urine volume was 405 ml per 24 hours with a standard error of 195.

The mean uric acid excretion (Table 3) was 3.3 mmol/l with a mean uric acid/creatinine ratio (U/C) of 0.5. Feeding diet 1 caused the lowest uric acid concentration (1.2 mmol/l; U/C 0.09) and diet 7 the highest (4.9 mmol/l; U/C 0.86).

Table 3. Analysis of uric acid, ammonium, allantoin and pH in the urine

	Diet							
	1	2	3	4	5	6	7	mean
Uric acid (mmol/l)	1.2	2.9	2.5	3.6	4.8	3.3	4.9	3.3 ±1.6
U/C	0.09	0.36	0.55	0.41	0.67	0.52	0.86	0.5 ±0.29
Ammonium (mmol/l)	141.7	90.1	122.2	104.8	95.2	77.3	86.0	102.8
AM/C	10.2	10.9	27.3	12.6	12.9	11.7	15.2	15.0 ±6.6
Allantoin (mmol/l)	1.1	1.3	1.2	1.3	1.3	1.4	1.2	1.2 ±0.2
A/C	0.02	0.17	0.3	0.16	0.18	0.23	0.24	0.2 ±0.1
U/A	1.2	2.5	1.9	2.7	3.7	2.4	4.3	2.7 ±1.4
pH	5.8	6.1	6.4	6.0	6.2	6.2	6.1	6.1 ±0.2

± standard error

U/C: Uric acid in mmol/ mmol creatinine; A/C: Allantoin in mmol/mmol creatinine

AM/C: Ammonium in mmol/mmol creatinine; U/A: U/C in mmol/ mmol A/C

Higher amounts of dietary purine increased the renal excretion of uric acid (Figure 1). The lowest uric acid excretion of 0.12 mg per 5 days occurred while feeding the low purine diet. The uric acid excretion increased to 3.3 mg per 5 days with the high purine diet (herring) equivalent to a multiplication factor of 27. Each mg of purine intake increased the renal elimination of uric acid by 0.39 mg. The correlation between purine intake and uric acid excretion was significant ($P > 0.001$). The lowest renal uric acid excretion (mean: 1.25 mmol/l) occurred while feeding the low purine diet, the highest (4.93 mmol/l) with the high purine diet (herring).

An approach to quantify endogenous purine metabolism was made by collecting urine from a fasted male otter and by calculating the regression equations of renal uric acid excretion compared to dietary purine intake. The relationship between purine intake and renal excretion of uric acid (Figure 1) suggested that the endogenous uric acid production averaged 0.12 mg (per day). The uric acid excretion of one male under fasting conditions was 0.18 mg and 0.28 mg and in a similar magnitude as suggested by the regression equation.

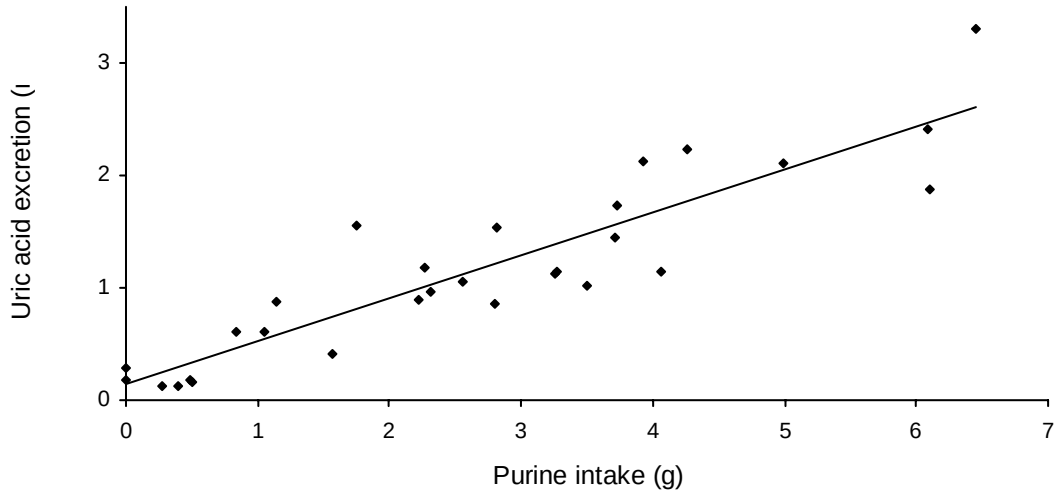

Figure 1. Relationship between purine intake (x) and renal excretion of uric acid (y)
$y = 0.38 x + 0.14$; $r = 0.91$; $P < 0.001$.

The mean urinary ammonium concentration (Table 3) was 103 mmol/l with a mean ammonium/creatinine ratio (AM/C) of 15. The lowest ammonium concentrations were recorded while feeding diets 6 (77 mmol/l) and 7 (86 mmol/l) and the highest with diets 1 (142 mmol/l) and 3 (122 mmol/l).
Allantoin was detected in all urine samples ranging between 1.1 and 1.4 mmol/l with a mean allantoin/creatinine ratio (A/C) of 0.9. The U/A (ratio of U/C in mmol/mmol A/C) ranged from 1.2 on diet 1 to 4.3 (diet 7). In mean the U/A was 2.7, stating that otters excreted nearly 3 fold more uric acid than allantoin. The lowest allantoin excretion was found with diet 1 (1.2) the highest with diet 7 (4.3).
The mean plasma uric acid concentration was 0.15 mmol/l (range 0.03 –0.26 mmol/l, n=15).

Discussion

The formation of urate crystals depends on the concentration and solubility of uric acid and its salts in the urine. Uric acid forms salts, mainly ammonium urate and sodium urate, above a urine pH level of 5.75. The solubility of urate is low at ca. pH 6.0 and increases with pH (BOWYER et al. 1979). At an urine pH of 7.0 there is a more than 20-fold increase in the solubility of urate in urine (THORNHILL 1980). With a mean pH of 6.1 in otter urine found in this study, uric acid is present as salt and the solubility is low. Urate salts form molecular aggregates in urine and assume a colloid state. These colloid particles are small and tend to remain suspended in solution. Therefore they can exist in a state of high supersaturation for prolonged periods and can be excreted in the urine (BOWYER et al. 1979). As long as urate is available, the aggregates continue to increase. If their size increases and cations are available in high amounts, the colloids can flocculate. The cations with the greatest flocculation power are trivalent ions such as ammonium. Ammonium ions are generated from ammonia, which is produced by the tubular cells. Ammonia acts as a buffer for hydrogen ions (SENOIR 1989). Ammonium ions can cause flocculation of ammonium urate colloids followed by precipitation. Hydrogen ions are also able to cause flocculation, but to a lesser degree (SORENSEN and LING 1993). High hydrogen ion concentrations are formed after ingesting a high protein meal, which is accompanied by an increased ammonia production (HALPERIN et al. 2001). The risk for the formation of ammonium urate calculi is a high urate and ammonium ion concentration (DEVRIES 1983, OSBORNE et al. 2000).

Published data on uric acid excretion in otters are scarce, therefore we have compared our data with those known for dogs, bearing in mind that dogs are relatively omnivorous and that otters are pure carnivores. Our otters had a mean U/C ratio of 0.5 exceeding the values reported for dogs (except Dalmatians) that have ratios of 0.1 and 0.26 (FRIEDMAN and BYERS 1948, HOFFMANN 1993). The mean urinary uric acid concentration in otters was 3.28 mmol/l and exceeds that of dogs (except Dalmatians) that have a range from 0.23 to 1.28 mmol/l (BRIGGS and HARLEY 1986, HOFFMANN 1993, OSBALDISTON and LOWREY 1971, PORTER 1963). In Dalmatians, the U/C ratio is higher than in normal dogs but lower than in otters (0.34 and 1.0) (HOFFMANN 1993, OSBALDISTON and LOWREY 1971, PORTER 1963).

Two studies on the Asian small clawed otters reported U/C ratios of 0.15 and 0.4 (CALLE and ROBINSON 1985, PETRINI and TRECHSEL 1996). Spontaneous urine samples collected after feeding had uric acid concentrations as high as 4.75 mmol/l (WEBER 2001). These levels can be attributed to the nutritional status.

The lowest uric acid concentration and U/C ratio were found while feeding the low purine diet (diet 1), the highest while feeding the high purine diet (diet 7) which reflects the direct influence of dietary purine content on the uric acid excretion.

The mean AM/C ratio of 15.01 found in this study is very high compared to that reported for dogs (3.7). Similar levels have only been found in dogs with chronic metabolic acidosis (up to 21) (HALPERIN et al. 2001). The mean ammonium concentration of 103 mmol/l is higher than that reported for dogs (30.5 – 51.9 mmol/l) and for the Dalmatian dog (28.9 – 57.9 mmol/l) (HOFFMANN 1993, PORTER 1963). Ammonium concentrations above 100 mmol/l were found while feeding diets 1, 3 and 4, and when comparing AM/C ratios, diet 3 gave the highest levels. Diet 3 consisted solely of chicken which seems to provide easily digestible protein. Diet 1 had low protein concentrations but is a commercial canine diet which, according to the manufacturer, provides ‘highly digestible protein quality’. Diets 3 and 4 were similar in protein content. Both fish diets (6 and 7) produced low ammonium concentrations although the white fish diet (diet 6) had the highest protein level.

For captive otters, the risk factors depend on the feeding practice. As we know from a zoo survey conducted in 2004, otters are often fed with diets high in protein which are highly digestible compared to their natural prey. In addition, many ingredients high in purine content are used, which will increase the uric acid excretion. For example, the most fed fish species in zoos is herring which has an exceedingly high purine content. Yeast is often used as a

vitamin supplement and is also high in purine. This could be a reason for the high rates of uric acid calculi in captive otters compared to the wild populations.

Our otters responded to increased amounts of dietary purine by increasing the rates of renal excretion of uric acid. The fasting trial showed a low endogenous production of uric acid. Therefore the uric acid concentration is strongly influenced by the dietary intake. This was also reported for the Dalmatian dog (GIESECKE et al. 1985).
Limiting the dietary purine helps to lower the risk of high uric acid concentration in the urine. Animal products can have a high purine content. Within the feedstuffs of animal origin the purine contents vary from low (e.g. bream, muscle meats) to high (herring, salmon, innards). Complete canine diets vary but specific products may have low purine contents. They can be used for dietetic reasons but not exclusively. Lowering the protein content of the diet would help reduce the hydrogen and ammonium ion load.
Thus it would be possible to reduce the purine and protein intake in captivity to lower the risk factors renal concentrations of uric acid, ammonium and hydrogen ions for the formation of calculi.

Otters are able to convert uric acid into allantoin. In the otter the U/A was 2.7 exceeding that reported for both, normal (0.05-1.02) and Dalmatian dogs (0.83-1.7) (HOFFMANN 1993). The U/A ratio was lowest with the low purine diet and highest with the high purine diet, again reflecting the response to a high purine load.
Further research would be necessary to check if a defective hepatic transport system or another defect causes the high uric acid excretion in otters (GIESECKE et al. 1985).

The serum levels of uric acid in non-Dalmatian dogs range from 0.01 to 0.04 mmol/l (APPLEMAN et al. 1966, GIESECKE et al. 1990, KUSTER et al. 1972, LING 1995, SIMKIN 2005), those of Dalmatian dogs from 0.03 to 0.2 mmol/l (GIESECKE et al. 1985, LEMIEUX and PLANTE 1968, LING 1995, SIMKIN 2005) and those of humans from 0.15 to 0.42 mmol/l (LING 1995). The mean plasma concentration of 0.15 mmol/l in the otter exceeds that of both non-Dalmatian and Dalmatian dogs, but even the highest measured value of 0.26 mmol/l does not come close to the critical level of 0.5 mmol/l where the uric acid crystallizes. It should be noted that the blood collection was conducted in the morning after fasting the animals over night. Additionally, the diet given to the otters during the time before the collec-

tion (diet 4) was in the middle of the range of purine content (Table 2). So it could be expected that the values after ingesting a diet high in purine content are higher than those we obtained in our trials.

Conclusion

Risk factors for the formation of urate calculi are pH, uric acid and ammonium under different diets. The impact of purine intake on the renal uric acid excretion offers the opportunity of dietary management to minimize the risk for urate stone formation in the captive otter population. Feed ingredients with low purine contents should be chosen for substitute zoo diets. To reduce the urinary ammonium concentrations, the protein intake should be adequate according to the requirement of *Lutra lutra*, paying particular attention to the quality of the protein and its digestibility.

8

DISCUSSION

Discussion

The Eurasian otter is an interesting carnivore for nutritional studies due to the lack of knowledge in most aspects of nutrition of this semi-aquatic mammal. The only sector of nutrition research in *Lutra lutra* with surpassing knowledge for a wild species is the prey spectrum with biomass intakes in the field. From data of respiratory trials a high energy demand for the species was calculated (PFEIFFER 1996, IVERSEN 1972). Hints like the predicted high metabolic rate, the semi-aquatic pattern of life and the focus on fish as prey gave rise to the assumption that the otter shows characteristic peculiarities in his nutrition and digestive physiology. The supposition was partly confirmed in the present study and in parts parallels could be found to the pattern in dog and cat, the best researched carnivorous domestic animals. None of the results presented here have ever been studied in the Eurasian otter before, except for the passage rates and energy intake.

Energy

In Chapter 2, a detailed analysis of energy intake on the basis of digestive energy is provided. The results show that the high energy demand predicted by PFEIFFER (1996) and IVERSEN (1972) was confirmed. Mainly the costs for thermoregulation and locomotion could be a reason for the high energy needs being related to the body shape and way of life. Most mustelids, including *Lutra lutra*, have an elongated body shape. Due to a greater surface area this long and thin shape is energetically not efficient e.g. for thermogenesis compensating heat losses (BROWN and LASIEWSKI 1972). For a semi-aquatic mustelid like the otter the heat loss is not only a problem on land but especially in water. The thermal conductivity of water is 25 fold higher than that of air (SCHMID-NIELSEN 1990). PFEIFFER (1996) measured an energy consumption for an inactive otter on land of 4.1 W/kg, for an inactive otter in the water of 6.4 W/kg and for a diving otter (speed 1.3 m/s) of 11.8 W/kg showing the higher energy consumption in water. The good insulation of otters through its dense fur keeping an air film between skin and guard hairs helps to reduce heat loss to a certain degree (WEISEL et al. 2005).

The otter spends not only a large amount of time for foraging in the water but also playing behavior and intraspecies quarrels were observed. Mating takes also place in water. In the case of imminent danger the otter is seeking refuge in the water body (REUTHER 1993). So a large part of activity is spent in aquatic habitats.

However, not only thermoregulation is a problem but also transport costs. Otters are adapted as semi-aquatic mammals to swimming, diving and walking (on land), hence none of the modi are optimized and transport costs are for every type of locomotion higher than those of strictly terrestrial or exclusively aquatic mammals. In comparison the otter seems to be better adapted in his locomotion to the aquatic habitat because transport costs for diving are lower than in many other semi-aquatic mammals (PFEIFFER 1996).

Large semi-natural ponds were always available for our otters in this study beside large enclosure sizes. I did not observe the otters for e.g. knowing the activity periods or time spend in water. KRUUK (1995) found a maintained body temperature even in cold water. So energy need probably varies with the time spent in water. But also different activity levels should cause different energy demands. Therefore, it would be interesting to measure these relations. In other institutions with differing keeping facilities, mainly in those providing not such large water bodies and enclosure sizes like we did, energy demands could be lower. A problem of our semi-natural enclosures is that prey could enter from outsight through the fences or from above. Three times during the 2-year-period an otter (male 1) captured a buzzard and ingested parts. It is also possible that mice or amphibians were eaten. The enclosures are covered with natural soil offering the possibility to forage for earth worms. These additional feed was impossible to measure in the trials and could have lowered our measured intakes.

The energy loss through urine or metabolic energy transformation could not be determined. This would be interesting for future research to be able to calculate the metabolizable and net energy.

Digestibility

The digestive energy efficiency (Chapter 2) as well as the apparent digestibility of dry matter, protein and fat (Chapter 3) was low in comparison to other carnivores. The only diet approaching values normal for carnivores was the freshwater fish diet. That was the only feed item tested for which otters also would prey on in the wild to a large extend. The higher ADs of this natural prey could be a hint that the low ADs within the tested zoo diets are not specific for *Lutra lutra*, but are a sign towards a suboptimal choice of feed items used in dietary management in zoos. It is possible that the ADs are in general higher for natural prey items and the otters are adapted to that specific prey species. To prove that presumption more tests with natural feed items would be necessary.

The assumption that otters could have short passage rates in general was verified in Chapter 4. So the lower ADs of otters can be due to their faster passage rates. A fast passage rate through the small intestine can lead to a reduced digestibility (PEACHEY et al. 2000).

In Chapter 3 it was also presumed that the otter is probably not dependent on a higher digestive efficiency because the species is adapted to continuous prey availability. Several hints suggest that the otter has no feed scarcity in winter. Among these are its ability to reproduce the whole year, data of good body condition indices found in necropsy of wild otters during winter and summer time, as well as the lack of fat deposits which many mustelids have to a big extend to survive the cold season (SOMMER et al., 2005). Prey is foraged and captured in nearly every activity phase (KRUUK 1995). So the otter is probably not dependent on a high digestion efficiency like the large Felidae or polar bears which have irregular hunting success. By linking the results of the energy study with higher energy demands in the winter period as compared to the summer period with the assumption that otters are not adapted to feed scarcity in winter, otters must capture even more prey in the cold period to cover their energy requirements for not loosing weight or even to raise cubs. It would be interesting to know if the foraging success of *Lutra lutra* is higher in the cold season and for what reason. Perhaps there is a cause in winter dormancy of prey species that make them easy to catch for the otter.

Testing of the marker chromium oxide resulted in an underestimation of AD in comparison with the total collection method, in a similar range as it was found for other carnivores (HILL et al. 1996). For getting the exact AD values of a diet with the marker method without an underestimation, it would be necessary to conduct trials with both methods for every diet. Then the factor of underestimation for every nutrient and diet could be calculated in the marker method, but that has not been determined for every diet in our trials.

Model species mink

The marker choice was also a problem in the study comparing the two species otter and mink (Chapter 4). For determining passage rates plastic beads were chosen as markers because they can be detected visually without needing laboratory analyses. A problem with markers for passage trials is that markers should be in equilibrium with the pool of the fraction that it labels. This is often discussed as a problem with plastic markers (BERNARD et al. 1995). We expected the problem of possible differing passage speeds of the marker and feed as not

severe in our trials because the used chicken diet contained parts of the same size like the beads had. In mink, mainly carmine-dyed rations were used in prior studies, but also Cr_2O_3 was taken (SIBBALD et al. 1962). To detect differences in passage rates using different markers, it would be necessary to conduct trials measuring passage rates of the same diet with each of the markers. Our intention was to compare passage rates of mink and otter, not to determine the exact passage rate values for the species. Because we used the same marker for both species, it is not expected that the comparison of passage rates was influenced by marker errors.

Both species had short passage rates and low ADs in comparison to other carnivores. Otters had even shorter passages and lower ADs than mink. Some studies recommended taking the cat as a domestic model for the Lutrinae (CRISSEY and REED-SMITH 2001, MASLANKA and CRISSEY 1998). But ADs from the cat and the otter are differing more than those between mink and otter (BARBIERS et al. 1982, GREAVES and SCOTT 1960, KANE et al. 1981, MORRIS 1977, NOTT et al. 1994). All ADs are higher for the cat than they are for mink and otter. But none of the cat studies used chicken as diet. For future research it would be necessary to conduct trials with chicken or diets used for the otter in Chapter 3 and the same marker to have more data available for further conclusions of species specific differences. In general it would be interesting to have more model species due to the difficulties to perform studies on nutrient requirements with *Lutra lutra*. Dogs and cats are probably the best researched domestic carnivores, much better than the mink. Therefore, trials concerning these species to evaluate them as a model species as well as more data for mink and otter would be desirable.

Ex-situ and in-situ diets

In Chapter 5 we could demonstrate differences between the natural diets of *Lutra lutra* and those fed to captive otters in keeping institutions. This provides another indirect method to obtain hints on the optimal nutrient intake through learning from nature.

The nutrient levels of the zoo diet exceeded the in-situ dietary fat content and vitamin A and B_1. The in-situ diet was higher in protein, zinc and vitamin E. Vitamin A is one of the most oversupplemented nutrients in zoo diets (DIERENFELD 1994), therefore caution is proposed when vitamin supplements are fed containing vitamin A. Problems with a vitamin E deficiency occur often in fish-eating mammals because the vitamin is destroyed to large extent

when fish is stored. Therefore, a supplementation of vitamin E is to be considered in husbandry.

Rare data on nutrient content of natural prey species was a limiting factor for calculating nutrient compositions of free-ranging otters. For various fish species, insects and reptiles no data could be found at all concerning nutrient contents. To raise accuracy of calculations, it would be favourable to analyse prey items for which only incomplete data are available. Another problem for the calculation is that the size of prey and status of reproduction often has a large influence on nutrient composition (CLUM et al. 1996). Studies investigating the prey size are rare but would also be important for calculating nutrient compositions (GEIDEZIS 1999).

The intake of fat provides the body with essential fatty acids. The ability to convert essential fatty acids like linoleic, linolenic and arachidonic acids into other fatty acids is species dependent. Obligate carnivores such as cats require also pre-formed arachidonic acid. For the otter the requirements of essential fatty acids are not known, but it is presumed that they have similar requirements due to their obligate carnivorous status with simple gastro-intestinal system like cats (CRISSEY 2001). A specific aspect related to fat is the intake of n3- and n6-fatty acids. Especially the ratio of n3/n6- intake is necessary for health (NÜRNBERG 2004). Fish is high in n3- fatty acids, chicken and beef in n6- fatty acids (BLOCK et al. 1985, NÜRNBERG 2004). Because data concerning fatty acid content are rare for most natural prey as well as for feedstuffs used ex-situ, we abstained from calculating these nutrients. For future research analyses of fatty acids are necessary to know whether the otter has resembling needs as cats.

The same applies to amino acids. The knowledge on otter requirements is the more important by looking at the requirements of cats. Cats as obligate carnivores with simplistic gastro-intestinal tract, comparable to the otter, need an array of pre-formed amino acids from the diet with additional needs such as taurine. For calculating the amino acid pattern of natural forage more data must be obtained on the compositions of prey species which otters ingest in the wild to learn about the species needs.

Dietary influences on urine composition

For several metabolic studies urine is needed, e.g. to investigate the pathogenesis of urolithiasis (Chapter 7). Using different diets can have a big impact on the urinary excretion of cer-

tain minerals, metabolites and amino acids, as we could demonstrate in Chapter 6. Parallels to dogs and cats were detected like the significant differences between diets on urinary mineral and metabolite concentrations for sodium, potassium, calcium, magnesium, sulphate and phosphate. No significant dietary influence was found for oxalate and citrate for the otter which was also reported for dogs and cats (LULICH et al. 1991, BARUCH et al. 1975, WILLIAMS and WANDZILAK 1989).
All analytes measured in urine are not yet analyzed in the diet, so Chapter 6 presents preliminary data. The exact intake of feed is known for the trials, making it possible to assess the intake and excretion.

Urolithiasis

Urolithiasis due to ammonium urate calculi is a significant problem in captive Eurasian otters (WEBER 2001). In the study of Chapter 7 risk factors were assessed for otters to form ammonium urate calculi and the dietary influence on uric acid excretion could be demonstrated. High urinary concentrations of uric acid and ammonium with a mean urine pH of 6.1 were found. All three factors are proposed to promote the formation of calculi in dogs and human (DEVRIES 1983, GIESECKE et al. 1985).
But these factors could not be the only reason of a formation of calculi because not all Dalmatian dogs with high urinary uric acid and ammonium concentrations are urolith formers. Three theories are discussed as a reason for that. The first step in all theories is the formation of a urolith which involves the formation of a nidus of crystals. Nucleation depends on supersaturation of the urine with urolith-forming crystalloids. Mechanism of nucleation is not known. One theory is that of precipitation-crystallization, in which the primary cause for the formation of a nucleus is supersaturation of urine with crystalloids, which then precipitate to form the nucleus. Another theory is matrix-nucleation in which an organic matrix forms the nidus, facilitating the precipitation of mineral crystals, a mechanism similar to the mineralization of the bone matrix. The last theory is the crystallization-inhibition theory in which there is a reduction or lack of inhibitors of crystallisation. The lack of these organic and inorganic inhibitors allows precipitation from supersaturated urine (SORENSON and LING 1993). It was never proven if one of the theories is true for the formation of ammonium urate calculi. Therefore, we can only regard the high concentration of uric acid, ammonium and the urinary pH as risk factors for the otter. The formation of calculi and the aetiology seems to be multifactorial (SORENSEN and LING 1993).

Beside the three general theories of stone formation additional evidence on the formation of ammonium urate calculi were detected in humans. Monoammoniumurate was found to be prone to preticipate in urine with low phosphate concentrations (ROTH and FINLAYSON 1983). KLOHN (1986) reported on urease producing bacteria which cause through the break-down of urea an increased ammonium production. These hints should be investigated for the otter in future research.

The probable multifactorial cause of stone formation has to be kept in mind when testing possibilities of preventing the formation of renal calculi. We found an impact of purine intake on the renal uric acid excretion which offers the opportunity of dietary management to minimize the risk for urate stone formation in the captive otter population. Feed ingredients with low purine contents should be chosen for substitute zoo diets. But long-term effects of feeding severe purine-reduced diets should be determined before giving a recommendation to otter keepers.

Another possibility to prevent stone formation is to increase the urinary pH, e.g. through a supplementation of sodium carbonate (WEBER 2001). Surprisingly, we found the lowest urinary pH of 5.8 while feeding the canned dog diet which has, after manufacturers´ data, potassium citrate as an additive to increase the urinary pH of dogs. An urine pH of 7.0 to 7.5 is the aim of the diet in dogs. Tests would be necessary to determine why potassium citrate is not suitable to increase the pH in *Lutra lutra*, for what reason ever, or to find other additives which could be used to increase pH.

It is also possible to lower urinary urate concentration through administering allopurinol what is the prominent therapy in Dalmatian dogs (GIESECKE 1985, SORENSEN and LING 1993). Surgical removal of calculi, the conventional approach to manage existing calculi in dogs beside the allopurinol treatment, was never reported in *Lutra lutra*. A problem is the diagnosis of urolithiasis in otters. The limited possibility to examine the health status of otters leads to the detection of stones just after death during necropsy. For detecting uric acid calculi, ultrasonic examinations are necessary (WEBER 2001). But these are barely feasible in Eurasian otters due to their dense coat.

Maintaining urine at low specific gravity is recommended for treating all types of uroliths in humans and is important in the dissolution of urate uroliths due to decrease urate saturation (SORENSEN and LING 1993, ORBORNE et al. 1986). Keeping institutions usually provide a water area for the otters which ensures an ad libitum supply with water (REUTHER 1991).

To increase water consumption a supplementation with salts e.g. sodium chloride could be possible but was never tested for *Lutra lutra* (WEBER 2001).

We measured uric acid concentrations in blood detecting values that did not reach critical levels above which uric acid is prone to precipitate. The diet which was used for the otters prior to blood collections was not high in purine content (0.11% of dry matter). This raises the question as to whether the urate concentration in the blood is influenced by the diet. Feeding a diet high in purine could probably cause critical levels of urate in the blood.

As it could be demonstrated in Chapter 7, the otter is able to convert uric acid into allantoin. Hence, the otter has the enzyme uricase which is necessary for the conversion. The urinary allantoin excretion was 2.7 and higher than that reported for dogs, meaning that otters excreted nearly 3 fold more uric acid than allantoin and at least double that of Dalmatian dogs. In the latter a defect of the hepatic transport system is the cause for the low conversion rate of uric acid to allantoin even though uricase is available in normal amounts and activity. In contrast to other dogs (and humans), most of the urate filtered by the glomeruli is not reabsorbed, tubular secretion continues, and uric acid excretion is therefore high. Concurrently, urate ions in plasma have limited access to the abundant uricase in hepatic cells, and allantoin production is thereby impaired (SIMKIN 2005).
Further research would be necessary to check if a defective hepatic transport system or another defect causes the high uric acid excretion in otters.

Besides obtaining additional data for the Eurasian otter it would be in general interesting for future research if the results of *Lutra lutra* could be adapted for other species of the Lutrinae. This will not be the case for the results of urolithiasis because the type of calculi is species specific in otters (KEYMER et al. 1981, CALLE 1988, PETRINI et al. 1999). But the results of energy requirement, digestibility data and testing a model species are interesting because for none of the Lutrinae such studies are existing. In general, nutritional studies are rare in Lutrinae, for the wild but also especially in the sector of captive dietary management (MASLANKA et al. 1998, CRISSEY et al. 2001, SYKES-GATZ 2001). All members of the Lutrinae, consisting of 13 species, are semi-aquatic except for the sea otter (*Enhydra lutris*) which is truly aquatic (FESTETICS 1980, CHANIN 1985, FOSTER-TURLEY et al. 1990,

KRUUK 1995). Beside the latter, all Lutrinae prey on aquatic forage as well as terrestrial prey species, to a more or less focus on specific prey, e.g. *Aonyx cinerea* is specialised on crabs (CHANIN 1985, TOWEILL and TABOR 1982). Some species seem to be comparable to a large extend with *Lutra lutra* and captive dietary management is needed. For example, the North American river otter (*Lontra canadensis*) is a close relative to *Lutra lutra* within the Lutrinae which is often kept not only in American zoos, but also in Europe due to the possibility to maintain them in groups. They are comparable towards habitats, prey species, morphological data and anatomy with *Lutra lutra* (SHELDOM and TOLL 1964, KNUDSEN and HALE 1968, BAITCHMAN and KOLLIA 2000). No scientifically founded dietary management exists for the species (CRISSEY et al. 2001). Therefore, it would be important to conduct trials to compare both species so that recommendations for *Lutra lutra* could be adopted for *Lontra canadensis*.

In general the survey described in Chapter 4 pointed out that Eurasian otters in captivity are often treated either as a terrestrial carnivore or to the other extreme, as an aquatic carnivore. Some keepers take the same diets for the otter as they use for the piscivorous sea lion, some orientate themselves on the diet used for lions. The true needs of this semi-aquatic species are supposed to be somewhere between lion and sea lion. The results of the comparison of ex-situ and in-situ diets indicated mistakes in dietary management.

Conclusion

In conclusion, otter keepers have to consider species specific peculiarities of *Lutra lutra* like the high energy demand and lower digestibility compared to many other species. Further studies into the specific requirements of otters are needed to optimise animal health and welfare and to minimize nutrition-related disease problems like urolithiasis.

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SUMMARY

Katrin Ruff

Nutritional and energetic studies on captive Eurasian otters (*Lutra lutra*)

Concerted efforts have to be made to optimize the husbandry of the captive population of Eurasian otters (*Lutra lutra*), a severely endangered species. There are still problems in the husbandry of this species with low breeding success and nutrition-related diseases (KEYMER 1981). Improper feeding can severely affect health and well-being, hence adequate nutrition is an obligatory prerequisite for successful husbandry (HATT 2000). Up to now, feeding recommendations for *Lutra lutra* are based on experience of different keepers and not on scientific data, except those for energy supply (MELISSEN 2000). Therefore, the aim of this study was to conduct nutritional trials with the species to investigate basal data on nutrition in order to provide information to improve the captive dietary management.

An adequate energy supply is essential for animal husbandry in captivity. We measured a high digestible energy intake (721 kJ/ kg body mass $(BM)^{0.75}$/d) in trials of 2- and 5- year periods with 14 otters in comparison with other carnivores. Seasonal and sex differences occurred with higher intakes in winter than in summer and females with a higher intake than males. For the formulation of suitable diets for captive otters, the knowledge of their digestive efficiency of different diets is important. Therefore, we conducted digestibility trials of 9 otters determining dry matter, energy, crude protein and crude fat apparent digestibility (AD) resulting in low AD coefficients (75-85% for dry matter AD) in comparison to other carnivores. The study was conducted with eight diets, typical for otter husbandry, to facilitate diet calculations for keeping institutions. Prior to the digestibility trials we tested the marker chromium oxide which facilitates AD studies because it does not require that the feces are collected quantitatively. A trend towards underestimation of AD with the marker method was found in comparison to total feces collection.

The low AD coefficients of the otter were also confirmed in a trial evaluating the mink as a model species for which various nutrient recommendations exist from pelt industry. Adopting these recommendations for the otter would be an indirect method to obtain nutrient requirements because studies are difficult to perform with the Eurasian otter due to animal welfare constraints and keeping situation. Trials with otters and mink using the same diet and marker

method showed low ADs as well as fast passage rates for both species, otters had even shorter passages and lower ADs than mink. This must be considered by taking the mink as model species for the otter.
A comparison of nutrient contents of in-situ and ex-situ diets used for captive otters showed differences mainly in vitamin A, B_1 and E, zinc, protein and fat between the natural diets of *Lutra lutra* and those fed to captive otters in keeping institutions. This provides another indirect method to obtain hints on the optimal nutrient intake through learning from nature.

The nutrition-related disease urolithiasis based on ammonium urate calculi is a significant problem in captive Eurasian otters which has to be included in nutritional studies to consider dietary influences promoting the disease. To assess the risk factors for otters to form ammonium urate calculi, feeding trials were conducted under varying dietary regimens collecting the urine quantitatively. The high concentration of uric acid and ammonium in the urine, accompanied by a urinary pH of 6.1, are factors which are proposed to promote the formation of ammonium urate calculi in dogs (GIESECKE et al. 1985). The impact of purine intake on the renal uric acid excretion demonstrated a dietary influence. The correlation between purine intake and renal uric acid excretion provides an opportunity to minimize the risk factor of high urate concentration for urate urolithiasis in the captive population through avoiding diets high in purine content.
Additionally, uric acid concentrations were determined in blood samples of otters to assess the risk of gout for the species. The mean plasma uric acid level of our otters was higher than that in dogs, but not reaching critical levels above which uric acid is prone to precipitate.

In conclusion, otter keepers have to consider species specific peculiarities of *Lutra lutra* like the high energy demand and lower digestibility compared to many other species. Further studies into the specific requirements of otters are needed to optimise animal health and welfare and to minimize nutrition-related disease problems like urolithiasis.