Czech University of Life Sciences Prague

Faculty of Forestry and Wood Sciences

Department of Game Management and Wildlife Biology

Towards a better understanding of ungulate diets: a methodological approach

Doctoral Thesis

Author: Michaela Holá, MSc.

Supervisor: prof. Ing. Jaroslav Červený, CSc.

2016

Declaration

“I declare that I have written this doctoral thesis entitled “Towards a better understanding of ungulate diets: a methodological approach” independently, relying on the literature sources listed below and under the supervision of prof. Ing. Jaroslav Červený, CSc.

I hereby agree with the publication of my doctoral thesis in accordance with Law no. 111/1998 on Higher Education Institutions as amended, irrespective of the outcome of its oral defence.

I followed the citation format of the Forest Ecology and Management journal while writing this doctoral thesis.”

30 May 2016, Prague

…………………….

Michaela Holá, MSc.

Acknowledgements

I would have never been able to finish this thesis without the guidance, support and help of my family, friends, and colleagues. I would first like to thank my supervisor prof. Ing. Jaroslav Červený, CSc. for his guidance. This thesis was written at the Department of Game Management and Wildlife Biology of the Faculty of Forestry and Wood Sciences. I owe my thanks to all the colleagues for an inspirational working environment. It has been a great pleasure working with you all. I am particularly thankful to my officemates and friends Miloš Ježek and Tomáš Kušta.

I am grateful to the Institute of Animal Science Uhříněves, Prague where I could perform most of my experiments, particularly to prof. Ing. Luděk Bartoš, DrSc. and Ing. Radim Kotrba, Ph.D. for their contributions to my manuscripts and help with sample collection. I would also like to thank Petr Janovský and Vratislav Kšáda at the Institute of Animal Science Uhříněves, and Jindřich Brožovský and Michal Švec at the game park Sedlice for taking care of the study animals and assisting in hair sampling during my experiments. I also thank Ing. Petr Sedlák, Ph.D. from the Department of Genetics and Breeding, and doc. Ing. Pavel Klouček, Ph.D. from the Department of Crop Production at the FAFNR CULS Prague for allowing me to use the lyophilisator and spectrometer machines. I also thank Mark Sixsmith for language-related help.

This thesis was supported by the Grant Agency of the Czech University of Life Sciences Prague, CIGA (Grants No. 20134314 and 20164312), by the Internal Grant Agency of the Faculty of Forestry and Wood Sciences (IGA FLD), Czech University of Life Sciences Prague (Grants No. 20134368, A15/15), and by the Ministry of Agriculture of the Czech Republic, Grant No. QJ1220314.

Last but not least, I want to thank my incredibly supportive family. Thank you mom, dad, and Vládíku for always being there for me. My eternal thanks go to my dearest sister Barbora for her support, guidance and help. Without her none of this would have been possible.

Abstract

Populations of European ungulates have grown substantially over recent decades, resulting in considerable environmental and socio-economic impacts. Availability and quality of natural and supplemental food sources are among the main factors driving their population dynamics. Detailed knowledge of feeding strategies of management-targeted species is therefore of primary importance for their successful management. Over time, methods to study the feeding strategies of animals have also evolved considerably but each has its advantages as well as limitations. This doctoral thesis uses a combination of traditional methods (i.e. stomach content analysis) and novel methods (i.e. stable isotope analysis, near infrared reflectance spectroscopy) to achieve a better understanding of feeding strategies of two important ungulate species (i.e. wild boar and red deer) in the Czech Republic, where their populations are on the rise and supplementary feeding is rampant. Next, this dissertation aims to introduce and to establish a basis for these novel methodological approaches for the study of free-ranging individuals. In this respect, it also addresses methodological issues related to their application in this field.

The diet composition of wild boar was investigated by examining stomach contents in order to identify their dependence on food resources of human origin (i.e. agricultural crops and supplemental foods). Foods of human origin were the dominant diet type and constituted the bulk of wild boar's diet throughout the year. A necessary prerequisite for application of stable isotope analysis is the trophic discrimination factor (i.e. systematic difference between the isotopic composition of the consumer tissues and that of the diet), which was experimentally determined for stable carbon and nitrogen isotopes, the two most commonly used in ecology, in hair tissue of wild boar and red deer. Furthermore, possible sources of variation (such as sex, age, body weight, and lactation) on isotopic discrimination were investigated. The results of the experiments provide a starting point for the successful use of stable isotope analysis in field studies on wild boar and red deer. Finally, application of near infrared reflectance spectroscopy was proven to be useful in measuring faecal indices of food quality for red deer. The results of this thesis will contribute towards efficient management of wild boar and red deer in the Czech Republic.

Key words: stable isotope analysis, near infrared reflectance spectroscopy, stomach content analysis, food quality, wild boar, red deer

Abstrakt

Početní stavy spárkaté zvěře v posledních desetiletích narůstají téměř na celém území Evropy, což má za následek významné environmentální a ekonomické dopady. Kvalita a dostupnost přirozených i umělých zdrojů potravy patří mezi nejvýznamnější faktory ovlivňující populační dynamiku spárkaté zvěře, a proto je nezbytné, aby byl efektivní management zvěře založen i na poznatcích z oblasti potravní ekologie. S postupem času se metody studia složení potravy značně vyvinuly, nicméně každá má své výhody a nevýhody. Cílem této dizertační práce je s použitím kombinace tradičních (tj. analýza obsahu žaludků) a moderních (tj. analýza stabilních izotopů, spektroskopie v blízké infračervené oblasti) metod studia složení potravy získat nové poznatky o potravní ekologii dvou významných zástupců spárkaté zvěře (tj. prase divoké, jelen evropský) na území České republiky. Jejich populace zde neustále narůstají a zároveň jsou intenzivně přikrmovány. Dále si tato práce klade za cíl vytvořit podklady pro následnou aplikaci těchto metod v myslivecké praxi a zaměřit se na metodologické otázky spojené s jejich použitím.

Na základě rozboru žaludků byl zhodnocen rozsáhlý soubor dat za účelem určit, do jaké míry jsou prasetem divokým přijímány složky potravy pocházející z lidské činnosti (tj. zemědělské plodiny a přikrmování). Potrava produkovaná člověkem tvořila dominantní a nejobjemnější složku potravy prasete divokého během celého roku. Nezbytným předpokladem k využití analýzy stabilních izotopů je znalost tzv. trofických diskriminačních faktorů (tj. systematický rozdíl v izotopovém složení mezi potravou a tkání živočicha, jež danou potravu přijal), které byly v rámci této práce experimentálně určeny pro stabilní izotopy uhlíku a dusíku v srsti prasete divokého a jelena evropského. Dále byly zhodnoceny možné zdroje variability (pohlaví, věk, váha, laktace) hodnot izotopové diskriminace. Získané výsledky představují nezbytné podklady pro použití analýzy stabilních izotopů k rekonstrukci potravy volně žijících jedinců prasete divokého a jelena evropského. V rámci této práce byla dále prokázána spolehlivost spektroskopie v blízké infračervené oblasti k určení ukazatelů kvality potravy v trusu jelena evropského. Výsledky této dizertační práce přispějí k efektivnímu mysliveckému managementu spárkaté zvěře na území České republiky.

Klíčová slova: analýza stabilních izotopů, spektroskopie v blízké infračervené oblasti, analýza obsahu žaludků, kvalita potravy, prase divoké, jelen evropský

Contents

Chapter I

Diet composition of wild boar and its dependence on food sources of human origin in the Czech Republic

Chapter II

Experimental determination of diet-hair isotopic discrimination for stable carbon and nitrogen isotopes in wild boar hair

Chapter III

Carbon and nitrogen isotope discrimination between diet and hair of red deer: the effect of sex, age, body weight, and lactation

Chapter IV

Use of near infrared reflectance spectroscopy to assess nitrogen and fibre fractions in red deer faeces and evaluation of winter food quality and its variability for red deer

Introduction and Aims

What do free-ranging ungulates eat? Finding clear answers to this fundamental ecological question is of particular importance to wildlife management. Food quality and availability along with other external and internal factors determine the habitat selection behaviour of animals, which in turn determines their survival and reproduction and hence their population dynamics. Food availability for animals, in today´s rapidly changing world, is constantly affected by human modifications of landscapes and management practices. It is therefore of prime importance to study how human modifications of landscapes and food availability affect the habitat selection behaviour and feeding strategies of wild animals, especially those of management concern. Over time, the methods to study feeding strategies of animals have also evolved, thereby providing unprecedented opportunities in understanding how changing landscapes and management practices affect species dietary adaptations. This doctoral thesis is focused on addressing this vital question, using a combination of traditional (i.e. stomach content analysis) and novel methods (i.e. stable isotope analysis, near infrared reflectance spectroscopy) for investigating the dietary ecology of two important ungulate species which are of prime management concern in the Czech Republic, i.e. wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). I also aim to establish baseline values for interpreting and applying these methods on free-ranging individuals.

Specifically, I aim to:

(i) determine the diet composition of wild boar and its dependence on food sources of human origin by applying stomach content analysis (*Chapter I*)

(ii) provide a foundation for application of stable isotope analysis on free-ranging animals (i.e. experimental determination of isotopic discrimination, identification of sources of variation in discrimination values; *Chapters II and III)*

(iii) develop calibration models for predicting food quality of red deer via faecal near infrared reflectance spectroscopy (*Chapter IV*)

(iv) evaluate winter food quality and its variability for red deer using faecal indices and near infrared reflectance spectroscopy (*Chapter IV)*

Alongside the Introduction, Literature Review, and Conclusions and Management Implications, this thesis is divided into four main chapters based on the respective question; Methods, Results and Discussion are simultaneously provided within each chapter. The individual chapters are based on four manuscripts.

Chapter I was submitted to the Wildlife Research Journal as: Ježek, M., Holá, M., Kušta, T., Červený, J.: Creeping into wild boar stomach to find traces of supplementary feeding.

Chapter II was published as Holá, M., Ježek, M., Kušta, T., Košatová, M., 2015: *Trophic* Discrimination Factors of Stable Carbon and Nitrogen Isotopes in Hair of Corn Fed Wild Boar. PLoS ONE 10(4).

Chapter III is based on a manuscript to be submitted to PLoS ONE journal: Holá, M., Ježek, M., Kotrba, R., Bartoš, L.: Diet-hair discrimination factors of stable carbon and nitrogen isotopes in red deer (*Cervus elaphus*): the effect of sex, age, body weight, and lactation.

Chapter IV is based on a manuscript to be submitted to Wildlife Biology journal: Holá, M., Ježek, M., Kušta, T., Červený, J.: Winter food quality and its variability for red deer in forest environment: overwintering enclosures vs. free-ranging areas.

The thesis ends with the conclusions summarizing the findings and their implications in ungulate management and offering suggestions for future research.

Literature Review

Populations of wild European ungulates have been substantially increasing over recent decades, both in density and distribution (Apollonio et al., 2010). Several factors are simultaneously driving this increase, such as favourable weather conditions (Mysterud et al., 2001), ongoing management strategies (Putman and Apollonio, 2014), long absence of large carnivores (Kaczensky et al., 2013; Kuijper, 2011) as well as changes in forestry and agricultural practices (Borowik et al., 2013; Mysterud et al., 2002). The Czech Republic has 11 ungulate species, out of which four are native (i.e. red deer *Cervus elaphus*, wild boar *Sus scrofa*, roe deer *Capreolus capreolus*, and moose *Alces alces*) and other species have been either introduced or accidentally escaped from captivity and became established (i.e. fallow deer *Dama dama*, sika deer *Cervus nippon*, white-tailed deer *Odocoileus virginianus*, chamois *Rupicapra rupicapra*, mouflon *Ovis musimon*, Barbary sheep *Ammotragus lervia*, wild goat *Capra aegagrus*; Bartoš et al., 2010). Populations of most of these species are following the same trend as other European populations, and their hunting bags have risen on average by 700 percent since 1980 (source: Ministry of Agriculture of the Czech Republic, hereinafter MACR, 2013).

While these increasing ungulate populations are more favourable for hunters, more and more concerns are emerging about their environmental and socio-economic impacts (Côté, 2004; Massei and Genov, 2004; Weisberg and Bugmann, 2003). Overabundant ungulate populations frequently exert enduring effects on ecosystem structure and function (Côté, 2004; Massei and Genov, 2004; White, 2012). By directly affecting the structure, regeneration, and reproduction of vegetation (e.g. Brookshire et al., 2002; Palmer et al., 2004), ungulates also alter ecosystem processes such as energy flow and nutrient cycling (Frank et al., 2000; Hobbs, 1996), and have cascading effects on many invertebrates and birds (e.g. Carpio et al., 2014a, 2014b). They can also cause considerable human-wildlife conflicts through damage to forests and crops (e.g. Bleier et al., 2012; Didion et al., 2009), vehicle collisions (e.g. Groot Bruinderink and Hazebroek, 1996a; Kušta et al., 2015), and disease transmission to livestock (Gortázar et al., 2007; Martin et al., 2011).

In light of this increase in populations of ungulate species and the growing ecological and economic costs related to it, there is an urgent need to investigate the relationships between the quantity and quality of the available food and resource use on multiple scales (Tremblay et al., 2004). The theoretical basis of this study is shown in Figure 1.

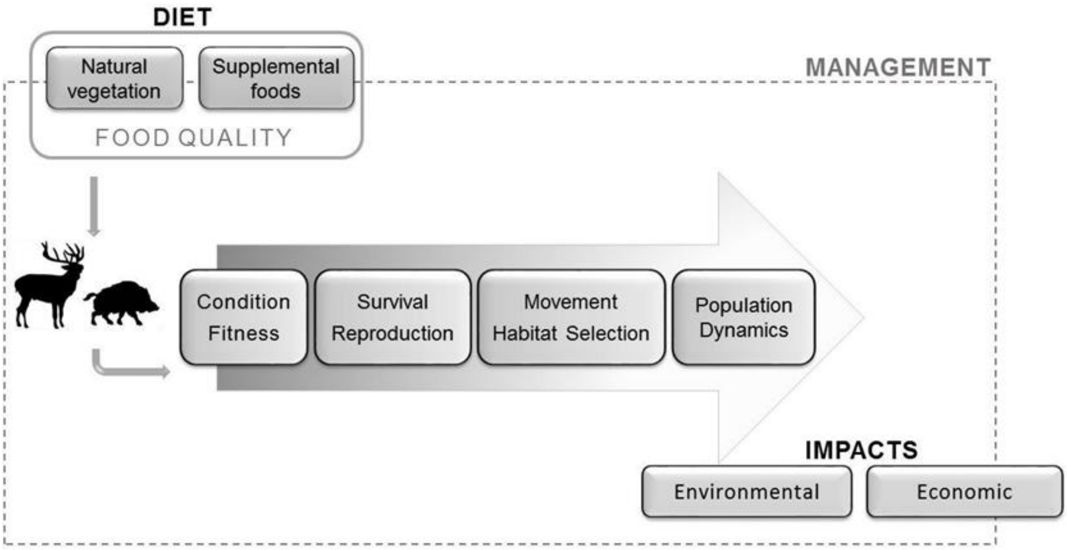


Figure 1. The theoretical basis of this study. Food is the basis of life for all living animals. Food quality and availability of both natural and artificial sources provided by humans are among the main factors affecting an individual´s habitat selection behaviour, which in turns determines its survival and reproduction and therefore its population dynamics. Many populations of European ungulates have grown over recent decades, causing environmental and socio-economic impacts. Accordingly, knowledge of foraging ecology of large herbivores is essential for efficient management of these animals.

Importance of diet to ungulates

Information on botanical composition and quality of ungulate diets is crucial for better understanding of their behaviour and ecology (Apollonio et al., 2010; Prins and Van Langevelde, 2008). Indeed, diet influences life-history traits, such as body mass, litter size, longevity, as well as demography and population dynamics (e.g. Parker et al., 2009; Pettorelli et al., 2003). Both food quality and availability drive the distribution of ungulates, their habitat selection, or seasonal movements and thus their impacts on environment at different spatiotemporal scales (Mobæk et al., 2009; Singh et al., 2010; Van Beest et al., 2010). Ungulates are long-lived species with a broad diet breadth and given favourable conditions they can rapidly grow in numbers (Baskin and Danell, 2003). Ungulate communities comprise of both herbivores (e.g. red deer) and omnivores (e.g. wild boar), which differ in their feeding strategies.

Red deer are classified as intermediate feeders; they feed opportunistically on a mixed diet of grass and concentrate food, such as browse, forbs and fruits (Gebert and Verheyden-Tixier, 2001; Hofmann, 1989; Krojerová-Prokešová et al., 2010). The quality of the food (nutrient content, digestible energy and digestibility-reducing compounds) is particularly important to red deer and other herbivorous ungulates (Robbins, 1993; Van Soest, 1994). They do not consume just any plant species they encounter, but prefer species containing higher amounts of nitrogen and digestible energy as well as lower amounts of digestibility-reducing substances, such as fibre and secondary metabolites (e.g. Forsyth et al., 2005; Iason, 2005; Robbins, 1993). Herbivorous ungulates need a continuous intake of dietary nitrogen and digestible energy because it affects their reproduction and survival (Mattson, 1980; Robbins, 1993; White, 1993). In addition, lower concentrations of foliar nitrogen are associated with higher levels of fibre and secondary metabolites, so consumption of forage containing low levels of nitrogen leads to low nutrient intake and reduced digestibility (Palo et al., 1985; Robbins, 1993; Van Soest, 1994). A diet high in levels of secondary metabolites (i.e. total phenolics and condensed tannins) leads to reduced voluntary food intake because condensed tannins are able to bind or precipitate with plant proteins and gastrointestinal enzymes, thus significantly reducing protein digestion (e.g. Palo and Robbins, 1991; Estell, 2010). These plant nutrients are all highly variable in space and time; herbivores thus have to make foraging decisions and deal with variations in food resources at different spatiotemporal scales which will consequently affect the extent and level of their environmental impacts (e.g. Nurmi et al., 1996; Palo et al., 1985; Parker et al., 2009).

Wild boar are omnivorous and will feed opportunistically on all types of organic matter (e.g. Schley and Roper, 2003). Although they primarily consume plant matter (80-90% of total food mass), they can also consume small vertebrates and invertebrates, as well as inorganic material on occasion (e.g. Ballari and Barrios-García, 2013; Fernanda Cuevas et al., 2013; Schley and Roper, 2003). However, some wild boar populations have been seen to feed predominantly on food sources of human origin (i.e. agricultural crops in fields and supplemental foods from hunters during lean periods to assist population persistence or to bait animals for hunting; e.g. Leránoz, 1983; Herrero et al., 2006; Amici et al., 2012). Little is still known, however, about the importance of such diet type in the bulk diet of wild boar and their possible effects on the species.

In general, the diet composition of both red deer and wild boar is mainly determined by the spatiotemporal availability and quality of food items in an area (Gebert and Verheyden-Tixier, 2001; Schley and Roper, 2003). Therefore, a detailed knowledge of seasonal diet composition as well as spatiotemporal variation in nutritional quality of forage species is of particular importance for efficient management of these ungulate species (Apollonio et al., 2010).

Supplementary feeding of wild ungulates

With the restricted distribution and population size of large carnivores in most European countries including the Czech Republic, ungulate communities are released from top-down control and appear to be more regulated by food and hunting (Apollonio et al., 2010). While the ungulate populations have increased due to the long term lack of large carnivores and better protection, the extent and intensity of supplementary feeding has also increased thereby providing food subsidies during the lean period of the year (Putman and Staines, 2004).

The main purpose of supplementary feeding is to increase the productivity of animals, their nutritional status, survival during harsh winter conditions, as well as the quality of trophies (Peek et al., 2002). However, its effectiveness in achieving these objectives is rather dubious (Putman and Staines, 2004). Moreover, winter feeding of ungulates is generally considered as an efficient method to reduce damage to forests and crops by controlling animal movements and distribution (Sahlsten et al., 2010), but it has often been found to be counter-productive in preventing negative ungulate impacts (Putman and Staines, 2004). In most cases feeding is associated with maintaining high densities of animals for hunting (Peek et al., 2002; Putman and Staines, 2004). As a consequence, high densities and increased social interactions around feeding sites may limit the access of food to the lower-ranked individuals (Donohue, 2010), or increased risks of disease transmission (Thompson et al., 2008).

In the Czech Republic alone, 84 665 ungulate feeding sites (i.e. 12.3 feeding sites/1 000 ha) were recorded in 2013 (MACR, 2013). Supplementary feeding of wildlife during lean periods is obligatory by law and, therefore, conducted intensively. For example, in the case of wild boar, it has been suggested that consumption of foods of human origin may increase their reproductive potential and thereby contribute to the increase of their population densities (Cellina, 2008; Gortázar et al., 2000; Herrero et al., 2008). Crop raiding or deliberate provisioning (i.e. supplementary feeding and baiting) mean that foods of human origin are available to wild boar all year-round in the Czech Republic. Agricultural land totals over 50 % of the country's area (over 4.2m ha), with wheat, barley, rye, oat, rapeseed, and maize being the main crops. Most of these crops are available in fields for free-ranging wild boar only from June until the harvest in late September (particularly the seeds, which wild boar find most attractive). Regarding the provision of supplemental feed, wild boar are mainly fed with grain (i.e. wheat, barley, rye), and maize. Potatoes, fruits, and pastry are also provided, but to a lesser extent. In addition, baiting is conducted by hunters throughout the year, with maize and wheat being among the most used baits to lure wild boar into hunting areas (Bartoš et al., 2010).

Although supplementary feeding is widespread, our understanding of its long-term physiological and life-history consequences on wild ungulates is very limited. The crucial point here is to acquire detailed knowledge on the extent of the reliance on supplemental food by individual animals. Moreover, considering high financial costs associated with supplementary feeding programmes (Calenge et al., 2004), such knowledge will also contribute towards cost-effectiveness of this management practice.

Dietary assessment methods

A variety of methods exist for studying foraging ecology of ungulates and they have undergone many transitions in the past. Each method has its advantages as well as limitations (Litvaitis, 2000). So far, studies of diet composition of free-ranging ungulates have been primarily based on direct observation in the field or examination of stomach and faeces contents. Direct observation is feasible on a small group of animals only and, despite the immense work involved, it usually does not provide accurate estimations of numbers and proportions of the consumed species, especially for diverse ungulate diets (Holechek et al., 1982a; Litvaitis, 2000). Stomach and faeces content analyses provide important information about the recent diet of animals and if a large number of samples are regularly collected over the year, they are capable of providing a thorough seasonal picture of dietary changes (e.g. Baubet et al., 2004; Krojerová-Prokešová et al., 2010). Individual food items are detected in stomachs and faeces and identified either directly by visual inspection, followed by traditional taxonomic identification from reference database, or indirectly via DNA-based identification methods, such as DNA barcoding or n-alkanes (e.g. Bugalho et al., 2002; Holechek et al., 1982a; Valentini et al., 2009). Traditional stomach and faeces content analyses can be time consuming and may require thorough training and experience and an extensive reference database of food items. Moreover, it has been suggested that these techniques may not always give a true measure of diet composition, as the amount of unidentifiable food items can be high and identified food items may be biased towards less digestible material in the diet (Holechek et al., 1982a; Litvaitis, 2000). Nevertheless, many previous studies have found stomach and faeces content analyses to be reasonably reliable (e.g. Alipayo et al., 1992; Hanley and McKendrick, 1985).

Stable isotope analysis

Stable isotope analysis offers an alternative method for diet reconstruction; it has become increasingly used to study a variety of topics related to wildlife management, including food web interactions, animal movement, as well as diet composition and habitat use of various species (e.g. Ben-David and Flaherty, 2012; Walter et al., 2014a). Naturally occurring stable isotopes of carbon (^{13}C, ^{12}C) and nitrogen (^{15}N, ^{14}N) are present in all organic compounds and are the two most commonly used in trophic ecology. Stable isotope analysis uses the ratio of a heavier, less common isotope to a lighter, more common isotope (reported as δ^{13}C for carbon and δ^{15}N for nitrogen; Fry, 2006). The δ^{13}C and δ^{15}N in a consumer´s tissues are related ultimately to those in its diet, thus allowing the determination of relative contributions of isotopically distinct food sources to the consumer´s diet (DeNiro and Epstein, 1978, 1981; Hobson, 1999; Post, 2002). Dietary inferences based on plant foliar δ^{13}C values generally rely on divergent photosynthetic pathways of C3 (Calvin-Benson photosynthetic cycle; Bassham et al., 1953) and C4 (Hatch-Slack cycle; Hatch et al., 1967) plants. The majority of C4 plants have δ^{13}C values between –16 and –9 ‰ with an average of –13.1 ± 1.2 ‰, while most C3 plants range from –35 to –22 ‰ with an average of –27.1 ± 2.0 ‰ (Farquhar et al., 1989; O´Leary, 1988). In contrast, the δ^{15}N values of vegetation integrate terrestrial nitrogen cycling; they can vary greatly due to a number of abiotic (Craine et al., 2009) and physiological (Ambrose, 1991) factors.

Measuring δ^{13}C and δ^{15}N in animal tissues yields time-integrated information about assimilated and not just ingested diet; this avoids the limitations often associated with more traditional methods, such as stomach content analyses (Peterson and Fry, 1987; Tieszen et al., 1983). The integration period for diet-derived isotopic signature depends on tissue-specific isotopic turnover (Tieszen et al., 1983); this is defined as the time it takes for a given consumer tissue to reflect the isotopic composition of the diet, which results from both tissue growth and tissue metabolic activity (MacAvoy et al., 2006; Tieszen et al., 1983). Different tissues therefore provide records of an animals' dietary history over different time periods (Hilderbrand et al., 1996; Peterson and Fry, 1987; Tieszen et al., 1983). Within a single species, isotopic turnover can vary from a few days up to several months for different tissues (e.g. Hilderbrand et al., 1996). Recent dietary intake is reflected in tissues with higher metabolic activity (e.g. blood plasma, liver), whereas tissues with lower metabolism (e.g. red blood cells, bone collagen) reflect diet integrated over longer time-periods (Hilderbrand et al., 1996; Tieszen et al., 1983).

Metabolically inert tissues that continuously grow provide a time line of the diet consumed during their growth (Schwertl et al., 2003). An example are hair tissues, which reflect the diet consumed during the period of their growth and store this information in a chronological manner (Schwertl et al., 2003). All the growth sections of the hair reflect the isotopic information of the diet during the time period when it was produced. The most recent dietary information is represented by the proximal end of hair, whereas the distal end represents dietary information from earlier (Holá et al., 2015; Schwertl et al., 2003; Tyrrell et al., 2013). A sequential analysis of hair (i.e. hair sectioned into sequential segments to allow analysis of temporal isotopic variation along the hair length unit) therefore allows assessment of the recent feeding history of an animal; in contrast serially collected hairs (i.e. hairs collected at different time periods) provide information on long-term dietary intake (e.g. Cerling and Viehl, 2004; Cerling et al., 2006; Iacumin et al., 2005). However, converting the isotope data along the length of hair into a temporal record requires estimating the average hair growth rate of the species in question (Schwertl et al., 2003). Various animal tissues can be used for stable isotope analysis, but hairs are particularly valuable because they can be sampled and stored easily, non-invasively, and cost-efficiently.

A requirement for the accurate use of stable isotopes in ecology is the establishment of physiologically mediated diet-tissue discrimination factors for the specific species and tissue analysed; this is because the isotopic ratios of diet sources are not transmitted directly to a consumer’s tissue. Isotopic discrimination is a systematic difference between the isotopic composition of the consumer tissues and that of the diet (Fry, 2006; Phillips, 2012; Ben-David and Schell, 2012). The discrimination values need to be taken into account while reconstructing animal diet by stable isotope analysis (Ben-David and Schell, 2012; Fry, 2006; Phillips, 2012; Tieszen et al., 1983). Precise estimates of isotopic discrimination are a fundamental prerequisite for modern isotope-mixing models; they are used to derive quantitative estimates of dietary contributions of isotopically distinct food resources (Ben-David and Schell, 2012; Caut et al., 2008b; Bond and Diamond, 2011). The discrimination factor is usually expressed as $\Delta^{13}C$ for C and $\Delta^{15}N$ for N, where Δ represents the difference in isotopic composition between the diet and animal tissue (Fry, 2006).

These discrimination measurements can vary due to many factors; thus far, experimental studies have shown variation which may be due to the tissue and species analysed, age, body size, nutritional or reproductive state, diet composition, or growth rate (e.g. Caut et al., 2009; Hobson and Quirk, 2014; Kurle et al., 2014; Lecomte et al., 2011). Isotopic discrimination values may thus be unique for species, tissue and diet (Martínez del Rio et al., 2009; McCutchan et al., 2003). Although there have been numerous efforts to identify the sources of variation in discrimination and tissue turnover, many of the variables have yet to be investigated in detail, e.g. effect of gender, age, body weight, and lactation.

The δ^{13}C values in animal tissue reflect dietary carbon sources; there is usually only a small enrichment (0.5 - 1 ‰) in δ^{13}C at each trophic level (e.g. DeNiro and Epstein, 1978). For δ^{15}N, a constant discrimination factor of 3.5 ‰ is commonly used for diet analysis; this is because there is a general assumption that a consumer's δ^{15}N value increases predictably with every step in the food chain (Peterson and Fry, 1987). However, using generalized, assumed and not experimentally derived discrimination factors can lead to incorrect interpretation of stable isotope data (Caut et al., 2008b; Bond and Diamond, 2011). It is essential, therefore, to have an experimental determination of species- and tissue- specific discrimination factors under controlled conditions. This may greatly improve our understanding of the mechanisms underlying the discrimination variation and help to improve our capacity to use stable isotope data collected from free-ranging animals (Caut et al., 2008b; Martínez del Rio et al., 2009).

Despite rapid progress in stable isotope analysis in mammalian research, the isotopic discrimination values of stable carbon and nitrogen isotopes in various tissues have only been determined for a small number of ungulates (e.g. Darr and Hewitt, 2008; Nardoto et al., 2006; Sponheimer et al., 2003a). There only seem to be three experimental studies which have established diet-hair discrimination factors of stable carbon and nitrogen in large herbivores: white-tailed deer *Odocoileus virginianus* (Darr and Hewitt, 2008), cattle *Bos taurus*, goat *Capra hircus*, alpaca *Vicugna pacos*, llama *Lama glama* (Sponheimer et al., 2003a,b). Regarding suids, only two studies are available on isotopic discrimination in multiple tissues of domestic pig (Nardoto et al., 2006; Warinner and Tuross, 2009), with none for wild boar (*Sus scrofa*).

Mathematical mixing models are the tools used to convert isotopic data into estimates of proportional contributions of food sources to the isotopic composition of the consumer´s tissues (Fry, 2006). A variety of mixing models have been proposed to reconstruct consumer diet (e.g. Phillips, 2012). The IsoSource mixing model (Phillips and Gregg, 2003) and Bayesian mixing models such as MixSIR (Moore and Semmens, 2008; Semmens et al., 2009) and SIAR (Parnell et al., 2010) have been among the most commonly used by researchers in wildlife studies.

For rapidly expanding species such as red deer and wild boar, which are increasingly being fed with supplementary food and also depend upon agricultural crops, stable analysis may help to determine the ratio of these artificial food sources and natural vegetation. The assumption here is that crops in fields (C3 type plants such as barley *Hordeum vulgare*, oat *Avena sativa*, and wheat *Triticum* spp.), which are also consequently utilized as supplementary food provided by hunters, are chemically fertilized in the Czech Republic and thus are isotopically distinct from natural vegetation (e.g. Bateman and Kelly, 2007). Also, isotopically different maize (*Zea mays*) with C4 photosynthetic pathway (Farquhar et al., 1989) is one of the most commonly used supplementary foods and among the crops raided by ungulates (e.g. Herrero et al., 2006).

Faecal indices of food quality and use of near infrared reflectance spectroscopy

Direct measurements of food quality for herbivorous ungulates can be both time-consuming and expensive (Leslie et al., 2008). An alternative approach is to measure nutritional characteristics of faeces that bear a relationship to the quality of ingested diet (Holechek et al., 1982a; Leslie and Starkey, 1985). Faecal material offers a convenient, non-invasive method as it is readily available and easy to obtain. Faeces collection is also not restricted to hunting seasons, so year-round information on food quality can be found without the need to kill animals. Several faecal constituents demonstrate the relationship with food quality (e.g. dietary nitrogen, fibre, and digestible energy) of herbivorous ungulates (Belovsky, 1981, Robbins, 1993). Faecal nitrogen (FN), faecal acid detergent fibre (FADF), and faecal neutral detergent fibre are among the most widely applied faecal indices of food quality (FNDF; e.g. Dixon and Coates, 2009; Hodgman et al., 1996; Leslie et al., 2008). Despite its broad application, the use of FN as a food quality indicator for ungulates remains controversial (see review by Leslie et al., 2008); some researchers promote its use (e.g. Hodgman et al., 1996), while others warn of limitations (e.g. Hobbs, 1987). FN combines metabolic nitrogen (microbial as well as endogenous) and a small amount of undigested nitrogen excreted in faeces (Schneider, 1935). The digestibility of the diet affects the FN levels because bacterial fermentation activity as well as turnover increases with higher digestibility (Robbins, 1993). This leads to a positive linear relationship between FN and digestibility (Holechek et al., 1982b). Because nitrogen content shows a positive linear relationship with digestibility in plants, FN also correlates with dietary nitrogen; this is one of the most important parameters of food quality for herbivorous ungulates (Robbins, 1993; Hodgman et al., 1996; Holechek et al., 1982b; Leslie a Starkey, 1985). Woody plant species, however, contain secondary metabolites (i.e. condensed tannins); these are known to bind with plant proteins and gastrointestinal enzymes during chewing and digestive processes. These complexes are not digestible at rumen pH and are excreted in the faeces, thereby raising FN concentrations (Palo and Robbins, 1991; Robbins, 1993). Thus, the presence of tannins in ungulate diets can complicate the interpretation and comparison of FN levels among diets which contain different plant species, particularly different amounts of secondary metabolites. FN levels of two food items might be similar, despite one of them being high-quality with high digestibility, while the other is low-quality and has high secondary metabolite levels (Min et al., 2003). Feeding experiments on red deer and white-tailed deer have shown that an experimental diet which is high in tannins can cause elevation of FN, thus overestimating dietary nitrogen (Mould and Robbins, 1981; Osborn and Ginnett, 2001). Nevertheless, it has been suggested that under natural conditions, where free-ranging herbivores choose their own plant species for consumption, there is a relatively consistent correlation between FN and dietary nitrogen (Caughley and Sinclair, 1994). In addition, free-ranging animals are less likely to select food items with a high tannin content (Verheyden-Tixier and Duncan, 2000). Although its application might sometimes be problematic, FN provides a viable index and allows for comparisons within-season for free-ranging ungulates occupying similar habitats and consuming similar diets (Leslie a Starkey, 1985).

In terms of the fibre fractions in faeces, NDF consists predominantly of hemicellulose, cellulose and lignin (Van Soest et al., 1991); it has also been demonstrated that increasing levels of NDF in diet can reduce voluntary food intake (Van Soest, 1994). ADF is a subset of NDF, which includes lignin, cellulose and cutin – i.e. the least digestible compounds for most herbivores (Van Soest et al., 1991). As the content of ADF in a diet increases, digestibility and available energy decrease (Van Soest, 1994). Red deer are known to have a higher preference for plants containing a lower level of fibre (Forsyth et al., 2005). However, sufficient fibre levels are required in a diet to maintain normal rumen function (Van Soest, 1994).

Plant nutrients and thus quality of food consumed by herbivorous ungulates are highly variable in space and time (e.g. Crawley, 1997; Holá, 2012; Palo and Robbins, 1991). Such variation in food quality at different spatiotemporal scales must be considered when addressing many of the questions of interest for wildlife managers. However, this requires an enormous number of samples and the wet-chemistry methods used to measure FN, FADF, and FNDF in faeces frequently become very time-consuming and expensive. Near infrared reflectance spectroscopy (NIRS) is a useful method for overcoming these drawbacks – it allows rapid, low-cost, chemical-free, and non-destructive analyses of a large number of samples (Foley et al., 1998). Wildlife nutrition research has widely used NIRS over the past four decades with numerous studies using NIRS to measure food quality through faecal indices in herbivorous ungulates (e.g. Dixon and Coates, 2009; Kamler et al., 2004; Showers et al., 2006).

NIRS operates on the general principle that the electromagnetic spectrum is reflected due to the number and type of molecular bonds C-H, N-H, and O-H (i.e. the primary constituents of the organic compounds of plant and animal tissues) in a sample together with the extent of molecular vibrations emitted after interaction of these bonds with the electromagnetic radiation absorbed in the NIR wavelength region (750-2 500 nm). The nature and number of bonds present in a sample are determined by its chemical composition and thus also the wavelengths and amount of light that is absorbed. The spectrum of light that is reflected from the sample therefore represents details of the sample's chemical composition (Cen and He, 2007; Foley et al., 1998; Shenk and Westerhaus, 1993). As well as chemical information, the spectrum also contains noises which reflect the physical properties of the sample, e.g. particle size and the conditions under which samples were scanned, such as temperature and humidity (Cen and He, 2007; Stuth et al., 2003). Appropriate pre-treatment methods can eliminate these noises. Many pre-treatment methods are available nowadays, including multiplicative scatter corrections (Martens and Naes, 1991) and detrending or standard normal variate transformations (Barnes et al., 1989) – these reduce the effect of particle size (scatter). Calculating derivatives of the spectra can remove the effects of overlapping absorption bands and increase the spectral resolution (Cen and He, 2007). The calibration process relates chemical information contained in the spectral properties of a substance to the chemical information revealed by traditional wet-chemistry methods. Calibration is designed to develop a predictive regression equation so the constituents of interest can be quantified using NIRS alone, eliminating the need for expensive wet-chemistry methods (Stuth et al., 2003). The basic aim of any calibration procedure is to ensure that the initial sample subset selected for calibration represents the total spectral variation found in all samples (Foley et al., 1998). The calibration involves any of multivariate regression procedures, e.g. multiple linear regression, principal component regression or partial least squares regression (Cen and He, 2007; Foley et al., 1998; Shenk and Westerhaus, 1991). The most commonly applied method for validating the calibration model is cross validation (or the “leave one out method”). During this process, one sample (i.e. the validation sample) is removed from the data set, while a prediction of the constituents of interest is made on the remaining samples based on the developed calibration (Foley et al., 1998). After the spectrometer has been calibrated, up to 100 samples per day can be simultaneously analysed for multiple constituents (Rothman et al., 2009).

NIRS analysis generally involves the following five steps: (i) spectra acquisition, (ii) spectra pre-treatment to eliminate noises, (iii) calibration model development using a subset of samples with known analysed concentration obtained by wet-chemistry methods, (iv) validation of the calibration, (v) prediction of unknown samples (Cen and He, 2007). NIRS provides a powerful alternative method to investigate many questions of interest for the management of herbivorous ungulates, such as evaluation of food quality and its variability in space and time. However, development of robust calibration is a necessity for its successful application.

Chapter I

Diet composition of wild boar and its dependence on food sources of human origin in the Czech Republic[*]

Materials and Methods

Study sites

The intact stomachs of 345 hunted wild boar were collected from four study sites in the Czech Republic from 2010 to 2012 (Figure 2). The stomach samples were collected mostly during driven hunts (i.e. collective hunts performed with beaters and hunting dogs), and thus it can be assumed that the dataset was not biased towards those wild boar individuals preferably occurring at feeding sites. The stomach samples were collected during *spring* (April-June; *SPR* hereinafter), *summer* (July-September; *SUM* hereinafter), and *winter* (October-March; *WIN* hereinafter). Age of hunted individuals was determined based on the pattern of tooth eruption and each individual was placed into one of the following age classes: (i) juveniles (<12 months), and (ii) subadults (12 – 24 months; Briederman, 1970). Adults (>24 months) were not considered since the Czech law prohibits hunting of this age category during driven hunts.

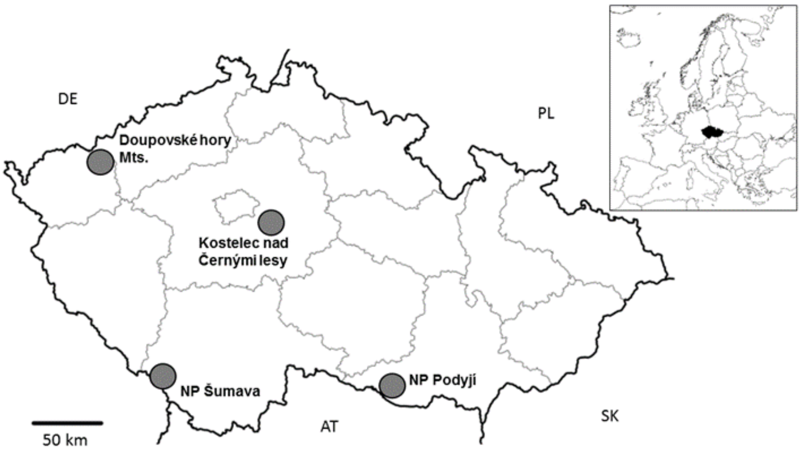


Figure 2. Location of the study sites within Europe and the Czech Republic.

---

[*] This chapter was submitted to the Wildlife Research Journal as: Ježek, M., Holá, M., Kušta, T., Červený, J.: Creeping into wild boar stomach to find traces of supplementary feeding.

Podyjí National Park (48°51′ N, 15°54′ E) has a total acreage of 63 km^2. Forests cover 84% and agricultural land 16% of the national park. The predominant vegetation type is broad-leaved forest. The main tree species are sessile oak (*Quercus petraea*), European hornbeam (*Carpinus betulus*), and European beech (*Fagus sylvatica*). Acidophilous and thermophilous oak forests are predominant throughout the lower altitudes of the eastern part of the national park. Mixed oak-hornbeam forests dominate the vegetation above 300 m a.s.l. in the west. Sub-montane beech forests occupy the highest locations in the western part of the national park (Škorpík et al., 1993).

Šumava National Park (48°46′ N, 13°51′ E) covers an area of 681 km^2. Forests are predominant at 80% of the area, followed by non-forest lands (20%), other areas (10%), agricultural lands (9%), and water surfaces (1%). The natural climax spruce forests with Norway spruce (*Picea abies*) and mountain ash (*Sorbus aucuparia*) dominate the vegetation above 1 200 m a.s.l. Mixed montane forests with Norway spruce, silver fir (*Abies alba*), European beech (*Fagus sylvatica*), and sycamore maple (*Acer pseudoplatanus*) are found between 600 and 1 000 m a.s.l. (Šumava NP, 2015).

Hradiště military training area (50°16′ N, 13°7′ E) covers almost 331 km^2 in the Doupovské hory Mountains. Forests cover 41% of the area, agricultural lands 8%, other land cover types 51%, and water surfaces 0.2%. Deciduous forests (71%) consist mainly of Norway spruce, European larch (*Larix decidua*), and pine (*Pinus sylvestris, Pinus nigra*). Broad-leaved forests (29%) consist mainly of beech (*Fagus sylvatica*), maple (*Acer pseudoplatanus*), and ash (*Fraxinus excelsior*). The predominant forest type is herb-rich beech forest. The other forest types are ravine forests, alluvial forests, oak-hornbeam forests, and thermophilous oak forests (Vojta and Kopecký, 2006).

The study site surrounding the town of Kostelec nad Černými lesy (49° 59′N, 14°51′E) has a total acreage of 80 km^2. Forests cover 43% of the site, agricultural lands 47%, other land cover types 9%, and water surfaces 1%. The main tree species are Norway spruce and beech. The predominant forest type is oak-hornbeam forests (Chytrý, 2013).

Regarding the intensity of food supplementation in individual study sites, the intensity is higher in Podyjí National Park and in Kostelec nad Černými lesy compared to Doupovské hory Mountains and Šumava National Park. The density of baiting and supplementary feeding stations in Podyjí National Park and Kostelec nad Černými lesy is higher (5 stations/1 000 ha) than in Doupovské hory Mountains and Šumava National Park (1-2 stations/1 000 ha).

Sample preparation and food item identification

All the collected stomachs were frozen at -20°C after collection and kept at the same temperature until further examination. Each stomach wall was removed and the volume of the food content in the stomach was measured (to a precision of 10 ml). The procedure described by Fournier-Chambrillon et al. (1995) was followed for handling the stomach contents. In order to facilitate identification of different food particles by size, the samples were washed in five consecutive sieves of decreasing mesh size (5, 2, 1, and 0.8 mm and 40 µm). Individual plant and animal items were identified to the lowest possible taxon using a reference collection, published keys, and guides. They were then classified into 15 main categories: *foods of human origin* (i.e. cereals, oilseeds, root crops), *herb seeds*, *tree seeds*, *fruits*, *roots and rhizomes*, *leaves of trees*, *herbs*, *grasses*, *mushrooms*, *mammals*, *fish*, *birds*, *amphibians*, *invertebrates*, *others* (i.e. unidentifiable, non-organic material). Two parameters were determined for each food item: (i) *frequency of occurrence*, which was calculated as: $\%F_i = \left(\frac{N_i}{N}\right) \times 100$, where %Fi = percent frequency of food item i, N_i = number of stomachs containing a given food item i, N = total number of stomachs; and (ii) *percentage of total biomass consumed* (%B) calculated as: $\%B_i = \left(\frac{Wt_i}{W}\right) \times 100$, where %Bi = percentage of total biomass consumed, Wt_i = total weight (g) of a given food category i, Wt = total weight (g) of all food categories identified in all of the examined stomachs. The frequency of occurrence provides a better picture of wild boar ecology and food habits, as well as highlighting the importance of food items occurring in very small numbers or volume. On the other hand, the percentage of total biomass consumed, emphasizes larger food items or those which are consumed in larger quantities.

Statistical analyses

The consumption of foods of human origin by wild boar in individual study sites and its possible differences between seasons, sexes, and age classes (juveniles and subadults) were evaluated using generalized linear mixed-effects models (GLMMs) using the values of the percentage of total biomass consumed. Due to the nature of the data, a binomial family was used for the correct formulation of a linear mixed model (Crawley, 2007). To appropriately model the proportion as a response variable with binomial family, a two vector response variable was created - the proportion of foods of human origin (as FoodHumanOrigin) and 1-FoodHumanOrigin. We then combined the two vectors into a single vector response variable (FoodHumanOrigin, 1-FoodHumanOrigin) and then regressed it against season, sex, and age. The study site was chosen as a random effect to account for spatial stochasticity (Pinheiro and Bates, 2000). Model selection was performed using AIC model selection criteria; this was calculated by *dredge* function to determine best-fit models (Burnham and Anderson, 2002). Then the best candidate models with ΔAICc ≤ 2 were selected within the MuMIn library (Bartoń, 2013), followed by model-averaged parameter estimates calculated for the variables included in those models (Burnham and Anderson, 2002). Significant effects are reported for $P < 0.05$. All statistical analyses were performed with the R software, version 3.1.2 (R Development Core Team).

Results and Discussion

Overall composition of the diet

Wild boar had a broad diet breadth comprising both plant and animal matter. The diet was dominated by plant matter, totalling 90.2% of stomach-content biomass; animal matter only accounted for 7.5% (Figures 3 and 4). This difference was not, however, that significant in terms of frequency of occurrence (i.e. 99% for plant matter and 56% for animal matter). This finding is in accordance with previously published studies, all of which reported that the diet of wild boar is primarily plant-based, both in terms of frequency of occurrence and percent of biomass consumed (e.g. Giménez-Anaya et al., 2008; Hafeez et al., 2011; Schley and Roper, 2003).

The most consumed food category in all seasons were *cereals* (i.e. wheat, barley, oat, rye, maize; %F: WIN=76; SPR=54.8; SUM=59.3; %B: WIN=42; SPR=27.9, SUM=35.2), followed by *roots and rhizomes* (%F: WIN=47.3; SPR=43.7, SUM=42.6; %B: WIN=20.5; SPR=13.3, SUM=15.6). The other frequently consumed food type throughout the year were *grasses* (%F: WIN=36.4; SPR=46.1, SUM=51.9; %B: WIN=10.2; SPR=23.8, SUM=12.1). Oilseeds (i.e. rape, field mustard, sunflower) and root crops (i.e. potatoes, beet) were rarely consumed and only in small volumes (%F$_{\text{OILSEEDS}}$: WIN=8.2; SPR=7.0, SUM=1.9; %B$_{\text{OILSEEDS}}$: WIN=0.8; SPR=0.1, SUM=0.0; %F$_{\text{ROOT CROPS}}$: WIN=2.7; SPR=0.0, SUM=1.9; %B$_{\text{ROOT CROPS}}$: WIN=1.5; SPR=0.0, SUM=1.3). Small volumes (%B=1.4%) of *herb seeds* were frequently consumed in summer (%F=27.8). *Tree seeds* (i.e. beechnuts, acorns etc.) were consumed frequently in all seasons (%F: WIN=25.5; SPR=33.8, SUM=22.2) but in larger volumes during spring (%B: SPR=13.4). *Herbs* were relatively frequent in spring (%F: SPR=23.4) and *leaves of trees* in summer (%F: SUM=15.2). *Fruits* were consumed more frequently and in larger volumes in summer (%F=44.4; %B=13.5). *Mushrooms* were relatively frequent in the winter (%F=13.6) and spring (%F=12.7) diet, but occurred in a small volume (%B: WIN=1.0; SPR=0.1). Regarding the animal matter in the diet of wild boar, *invertebrates* were the most frequent food category throughout the whole year (%F: WIN=38.6; SPR=42.0, SUM=50.0). *Invertebrates* included molluscs (Mollusca), spiders (Araneae), beetles (Coleoptera), earthworms (Haplotaxida), ensifera (Orthoptera), ants (Hymenoptera), and larvae and caterpillars. *Mammals* were other frequently consumed animal matter (%F: WIN=14.1; SPR=12.7, SUM=16.7). Mammals included ungulates

(Artiodactyla), lagomorphs (Lagomorpha), rodents (Rodentia), and insectivore (Eulipotyphla; Figures 3 and 4). Interestingly, wild boar remains were identified in four collected stomachs. *Birds*, *amphibians*, and *fish* were consumed rarely and in negligible volumes (Figures 3 and 4). Detailed table showing frequency of occurrence (%) of all diet items identified in wild boar stomachs is included in the supplementary material Table S1.

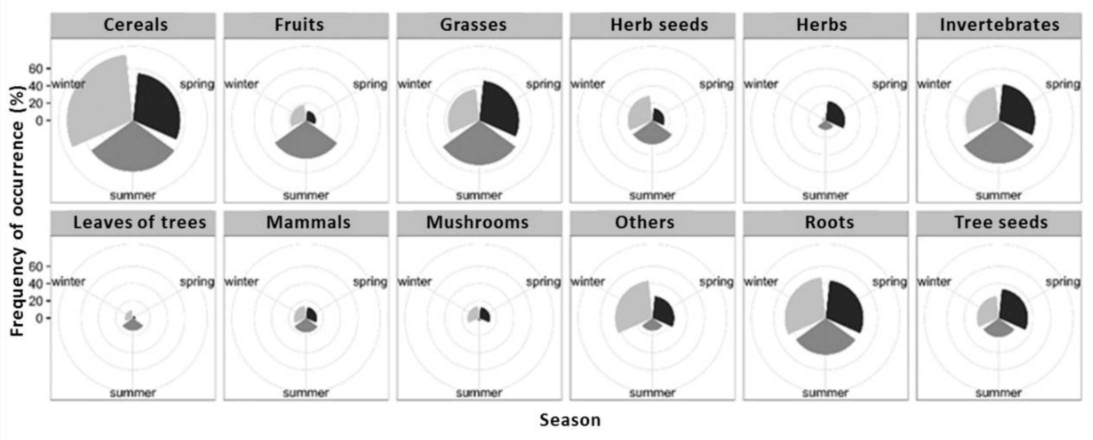


Figure 3. Frequency of occurrence (%) of the twelve major food categories which were frequently consumed by wild boar in four study sites in the Czech Republic during 2010-2012. Some food categories were ignored as they were eaten rarely (i.e. Amphibians, Fish, Root crops, Birds, Oilseeds).

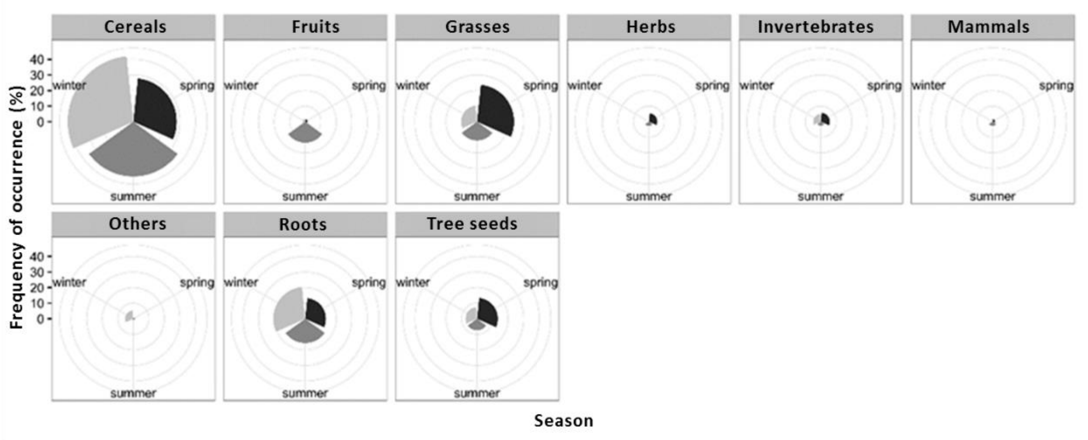


Figure 4. Total biomass (%) of the nine major food categories, which were consumed by wild boar in largest volumes in four study sites in the Czech Republic during 2010-2012. Some food categories were ignored as they were consumed in small volumes (i.e. Amphibians, Fish, Birds, Herb seeds, Leaves of trees, Mushrooms, Oilseeds, Root crops).

Frequent occurrence of food sources of human origin in wild boar diet has been already reported in other countries (Ballari and Barrios-García, 2013; Herrero et al., 2006; Schley and Roper, 2003). Hahn and Eisfeld (1998) in a study from Germany reported that agricultural crops (mainly corn) played a key role in the diet of wild boar throughout the whole year. Fournier-Chambrillon et al. (1995) showed that cereals, particularly corn, were eaten constantly throughout the year except in winter and accounted for one-third of the annual diet of wild boar in France. Likewise, Cellina (2008) reported that supplemental foods provided by hunters constituted up to 50% of stomach contents and were eaten year-round in Luxembourg.

It has been reported that, when available, consumption of agricultural crops and supplemental foods may strongly impact wild boar’s movement, behavioural patterns and also distort their regular diet (e.g. Groot Bruinderink et al., 1994; Hahn and Eisfeld, 1998; Mysterud, 2010). The analyses showed that roots and invertebrates were consumed frequently and in relatively high volumes throughout the year, which might be a consequence of large proportions of foods of human origin in wild boar’s stomachs. Presence of such diet type in wild boar’s diet may increase the consumption of protein-rich food items such as roots and invertebrates (Groot Bruinderink et al., 1994, 1996b). The reason for this is probably related to the low nutritional quality of agricultural crops since many cereals are rich in carbohydrates but low in protein. Therefore, wild boar consuming high proportions of carbohydrate-based cereals may compensate their protein deficiency by consuming higher amounts of roots and invertebrates (Groot Bruinderink et al., 1994, 1996b; Arentson and Zimmerman, 1995).

Interestingly, seeds of trees (such as acorns and beechnuts) were consumed frequently and in larger volumes during spring. Most of the stomachs containing this food type came from the Podyjí National Park. Among the study sites, the Podyjí National Park is the only site with high abundance of acorns and beechnuts and thus the only site where mast years can be assumed. Moreover, as shown in the study of Zeman et al. (2016), acorn seedling survival in the Czech Republic (i.e. area with high availability of agricultural crops and supplemental foods) is very high and therefore they remain as an abundant diet source for wild boar even in spring. Furthermore, the gained results showed a presence of uncommon diet type for wild boar, i.e. fish.

However, fish identified here came exclusively from baiting sites, where they were deliberately provided by hunters. However, the use of animal residues for baiting is prohibited by law in the Czech Republic. Also, the observed cannibalism in the results deserves increased attention from researchers and wildlife agencies as a consumption of infected carcases could pose a serious risk for rapid spread of the African swine fever (EFSA AHAW Panel, 2015).

Consumption of human based food sources of human origin

Food sources of human origin, especially cereals, occurred most frequently in the diet of wild boar and had the largest volumes during the year (Figures 3 and 4). Out of all GLMMs formulated to explain the consumption of agricultural crops and supplemental foods, the best-fit models (with ΔAICc ≤ 2) included age, season, and sex (Table 1).

Table 1. Comparison of candidate GLMM models with ΔAICc ≤ 2 explaining consumption of food sources of human origin (i.e. agricultural crops and supplemental foods provided by hunters) by wild boar in relation to age (i.e. juveniles and subadults), season, and sex.

Model	*df*	Fixed effects	AICc	ΔAIC	AIC weight
1	7	age + season + sex	351.0	0.00	0.284
2	5	age + season	351.3	0.22	0.254
3	5	study site	351.9	0.87	0.184

Generalized linear models (GLMMs) were fitted with a binomial distribution. **AICc** is the second-order Akaike's Information Criterion, Δ**AICc** is the difference of AICc compared with the best model, ***df*** are model degrees of freedom.

The origin of foods of human origin in wild boar's diet is however disputed. Here, the cereals found in the analysed stomachs may come from both cultivated fields as well as from deliberate provisioning from hunters. The cereals identified in the stomachs were mainly wheat, corn, barley and oat which are widely distributed agricultural crops in the Czech Republic. Wild boar preferably consumed seeds since other parts of these crops are more fibrous, lignified and therefore less digestible (Hacker and Minson, 1981). Stomachs collected in winter contained largest volumes of seeds of cereals (0.632±0.594), with the smallest volumes detected in spring (-0.659±0.467; Table 2). In the Czech Republic, seeds of the above mentioned crops are available in fields for free-ranging wild boar from June until the harvest in August (for wheat, barley, and oat), and corn in September. They are not available in fields after the harvest for the rest of the year (i.e. from October to May) since, due to current advanced harvest technologies, the post-harvest losses are negligible (<1%; Břečka et al., 2001). Harvested seeds are stored in closed stocks which cannot be accessed by wild boar. Therefore, it is possible to assume that the cereals found in the stomachs of wild boar in winter and spring (from October to May) might come exclusively from baiting and supplementary feeding carried out by hunters. It is not possible to determine the exact origin of the cereals found in the wild boar stomachs in summer since at this time of the year they can come from both cultivated fields as well as from deliberate provisioning from hunters. In comparison to other studies, which in general detect large amounts of foods of human origin in wild boar diet occurring predominantly in summer – e.g. 32% (Fournier-Chambrillon et al., 1995); 36.7% (Giménez-Anaya et al., 2008), we found over 70% share of this food type (i.e. higher share) occurring in particular during winter and to a lesser extent in spring and summer (>50%). Therefore, the gained results indicate that deliberate provisioning from hunters is the major food source for wild boar in winter and spring.

Females consumed less supplemental foods than males, whereas juveniles depended on such feed less than subadults (Table 2). The observed difference between males and females may be again attributed to the relatively low nutritional quality of cereals given their known deficiencies in essential amino acids (Arentson and Zimmerman, 1995). Low quality protein derived from cereals is probably not sufficient to meet nutritional demands of reproducing and lactating females and that is why females consumed this food type in smaller volumes (Wilcox and Van Vuren, 2009). Another possible explanation might be that males are more successful at obtaining foods at feeding sites and thus might get proportionally more foods of human origin than females (Clutton-Brock and Albon, 1985). Similarly, differences in consumption of cereals between juveniles and subadults may be attributed to different nutritional requirements of these two age classes (Dardaillon, 1986).

Table 2. Model-averaged parameters for the effect of age, season, and sex included in the candidate GLMM models (ΔAICc ≤ 2) for consumption of food sources of human origin (i.e. agricultural crops and supplemental foods provided by hunters) by wild boar.

Variable	Parameter estimate ± SE	95% CI LB	95% CI UP
Intercept	-0.659 ± 0.467	-1.638	0.232
Season (summer)	0.186 ± 0.398	-0.627	1.199
Season (winter)	**0.632 ± 0.594**	0.059	1.882
Age (juveniles)	**-0.677 ± 0.304**	-1.292	-0.117
Sex (males)	-0.323 ± 0.400	-1.310	0.167

Spring, subadults, and females represent the base. Wald's test (z-score) was used to calculate the significance of each variable and therefore the respective *p*-values. Statistically significant coefficients are in bold. **SE**: standard error; **CI**: confidence interval; **LB**: lower bound; **UP**: upper bound.

Among individual study sites, the consumption of cereals was highest in the Podyjí National Park, followed by Kostelec nad Černými lesy, Doupovské hory Mountains, and Šumava National Park (Figure 5). The observed differences in consumption of cereals in individual study sites appeared to be caused by different intensities of food supplementation. The highest amount was found in the stomachs collected in the Podyjí National Park and in Kostelec nad Černými lesy. The intensity of food supplementation in these two study sites is higher compared to Doupovské hory Mountains and the Šumava National Park, where wild boar fed less on supplemental foods. Moreover, obtaining supplemental foods is easier for hunters in the Podyjí National Park and Kostelec nad Černými lesy since these sites are both surrounded by intensively managed agricultural landscapes.

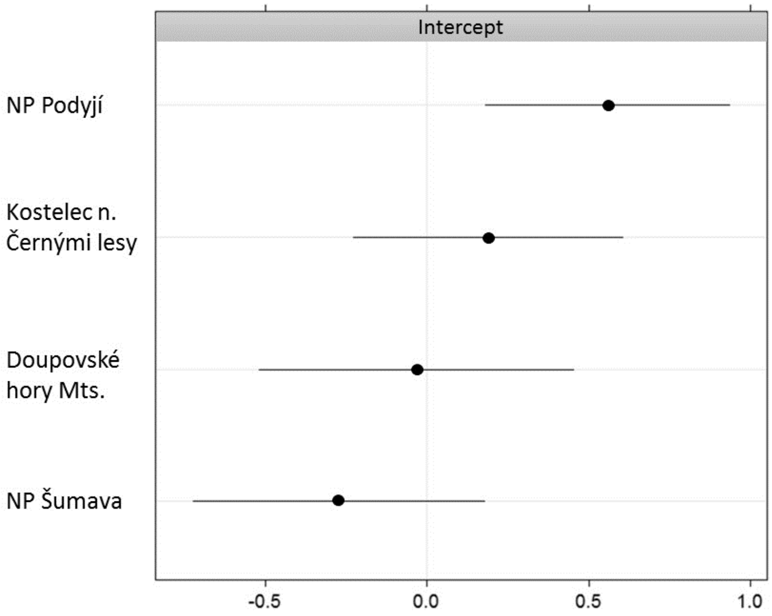


Figure 5. Estimates of random study site-effect (deviations from the fixed intercept). The consumption of food sources of human origin by wild boar was highest in Podyjí NP, whereas lowest in Šumava NP.

Nowadays baiting and supplementary feeding are among the most widespread practices of wild boar management throughout most of central Europe. Winter feeding of ungulates is even obligatory by the law in the Czech Republic as well as in other central European countries, such as Slovakia, Poland and Hungary. It is clear that this regulation comes from historical times when ungulates were not abundant, and in need of protection. This practice has been widely debated, particularly in relation to its efficiency, as in the case of wild boar when intensive winter feeding may prevent mortality due to starvation and thus promotes already high reproduction of this species, and consequently fails to prevent damage (e.g. Cellina, 2008; Keuling, 2009). As demonstrated by results of this and other similar studies, supplemental foods provided by hunters has become a major item of wild boar´s diet in most of European countries (e.g. Amici et al., 2012; Herrero et al., 2006; Leránoz, 1983).

In the Czech Republic, the most common way of food supplementation for wild boar is baiting conducted in order to increase hunting success. The less common way is to use supplementary feeding as a tool for controlling wild boar movement and distribution (Červený et al., 2013). Consequently, a high proportion of supplemental foods in wild boar's diet found in this study indicates a frequent visits to baiting stations by wild boar and therefore better hunting success of those hunters that supply feed. In addition, the more than 50% share of foods of human origin in the wild boar diet identified here points to the fact that wild boar may use baiting stations as their main dietary source. This is a bit surprising given the fact that this method of wild boar management is denoted as "supplementary feeding". The fact that, supplemental foods dominate the wild boar diet, is a consequence of the behaviour of hunters due to the absence of any legal regulations restricting supplementary feeding and baiting in the Czech Republic (Bartoš et al., 2010). In particular, hunters do not strive to maximize their hunting success for the individuals visiting the baiting stations, but instead seem to maximize their individual hunting success. In general, although hunters may visit the baiting stations infrequently but they often want to maximize their own hunting success. In order to lure wild boar individuals to baiting stations, hunters "overfeed" them by frequent provision of feed to make sure that wild boar individuals are present as much as possible so that if hunters come to hunt (which does not happen very frequently) they score a success. By doing this, hunters act in defiance of the purpose of baiting stations, which is a reduction of wild boar population size.

To conclude, the results gained in this study confirmed consistent dependence of wild boar on food sources of human origin throughout the year in all study sites. These findings indicate that supplementary feeding of wild boar is widespread throughout the Czech Republic and feed is constantly available in large quantities. Year-round consumption of supplemental foods by wild boar individuals may improve their condition, reproduction success and thereby contribute to the rapid increase of their population numbers. Therefore, there is an urgent need for management agencies to control feeding practices of wild boar populations so as to contain their undesirable consequences. Restrictive measures for supplementary feeding and baiting of wild boar should be implemented in the Czech Republic. Specifically, concrete regulation measures need to be designed concerning baiting. These regulations should specify maximal amount of food that can be used for baiting (i.e. up to several kilograms). Regarding the supplementary feeding, this management practice should be limited and at least concrete time periods for supplementary feeding should be specified. Further, techniques which will prevent non-target species from accessing the food provided by hunters have to be defined. Another step towards reducing wild boar numbers in the Czech Republic and consequently their impact on the environment should be to allow adult individuals to be hunted during driven hunts. However, further studies are needed in order to provide a deeper understanding of the effects of supplementary feeding and baiting on wild boar morphology, physiology and reproductive efficiency. Since the wild boar populations continue to increase and expand throughout the whole Europe, these insights are of particular relevance in the context of emerging African swine fever and re-emerging tuberculosis among wild boar in Europe.

Chapter II

Experimental determination of diet-hair isotopic discrimination for stable carbon and nitrogen isotopes in wild boar hair[*]

Materials and Methods

Ethics statement

This experiment was carried out in accordance with the recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague. The protocol was approved by the Animal Care and Use Committee of the Czech Ministry of the Environment (Permit number: 15106/ENV/14-825/630/14). All care was undertaken to minimize stress and suffering to the animals.

Study design

From June to October 2013 the feeding experiment was conducted at Sedlice game park (49°21.705´, E13°59.343´), Czech Republic. Four three-year old male wild boar were fed with maize grain (*Zea mays,* crude protein = 8.68 %, carbon content = 40.72 %) for the duration of the experiment (4 months). This time period was chosen because it coincides with the availability of maize growing in fields for free-ranging wild boar. To minimize isotopic heterogeneity, the maize grain was obtained from a single batch. During the study period the four wild boar were kept in an experimental outdoor enclosure. Feed and water were freely available. A four-month period was chosen as it is considered to be sufficient to allow hair to reach an isotopic equilibrium of δ^{13}C and δ^{15}N with diet (Caut et al., 2008a; Jones et al., 1981; Zazzo et al., 2007).

Sample collection

At the beginning of the experiment (day 0, when the wild boar were given maize grain), two patches of hair (c. 6 cm^2) from two different body parts from each

---

[*] This chapter was published as Holá, M., Ježek, M., Kušta, T., Košatová, M., 2015. Trophic Discrimination Factors of Stable Carbon and Nitrogen Isotopes in Hair of Corn Fed Wild Boar. PLoS ONE 10(4): e0125042. doi:10.1371/journal.pone.0125042.

individual (i.e. shoulder and rump; Figure 6) were shaved to the skin. Because they reflected only the diet prior to the experiment, these hair samples were not analysed for isotopic signatures. A bundle of hair (including hair root) was plucked from the previously shaved patches from each wild boar in the first sampling period (day 42). At the end of the experiment (day 140), hair was again plucked from the same patches sampled during the first sampling period, but in a different place within the same patch. The hair samples collected at the end of the experiment thus reflected the dietary information since day 0. As the wild boar hairs were shaved at the beginning of the experiment period, their isotopic signatures can be assumed to reflect only the research diet. Only guard hairs were selected from each animal. Each month the maize grain samples were collected.

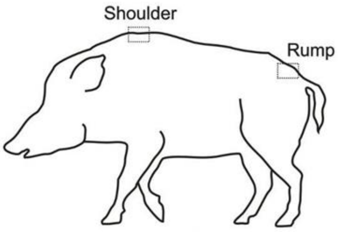


Figure 6. The sampling locations (shoulder and rump) of the hair samples collected on individual wild boar.

Hair growth rate

All of the hair samples collected from the wild boar individuals in the first sampling period (day 42) were measured and the mean length (mm) of the shoulder and rump hair was determined. The growth rate for the shoulder and rump hair of each animal was determined by dividing the mean hair length by 42 (i.e. the number of days since the beginning of the experiment).

The differences in hair growth rate between individual body parts were minimal, so a mean growth rate was taken for further calculations. The animals were on a constant diet during the experiment, so accounting for the slight observed difference would not affect the interpretation of the results gained. Therefore, average growth rate of wild boar hair was used for all calculations (i.e. the mean length of both shoulder and rump hair (46.48 mm) divided by 42).

Sample preparation

To allow comparison of the degree of incorporation of δ^{13}C and δ^{15}N values of the maize grain into hair at different periods of the experiment, 10 mm sections were taken from both the distal end and from the proximal end (hair root included) of the hair samples collected at the first sampling period (day 42); only these sections were used for isotope analysis. The average hair growth rate of wild boar calculated previously (i.e. 1.1 mm/day) meant that the 10 mm distal end sections hair represent the information from the first c. 9 days of the experiment (i.e. day 0-9) while the proximal ends sections reflect the most recent information prior to the first sampling period (i.e. day c. 34-42). The most recent dietary information prior to the end of the experiment was obtained by cutting 10 mm sections from the proximal end (hair root included) of the hair collected at the end of the experiment (day 140). Dietary information on the last c. 9 days of the experiment (i.e. 132-140; Figure 7) was therefore collected.

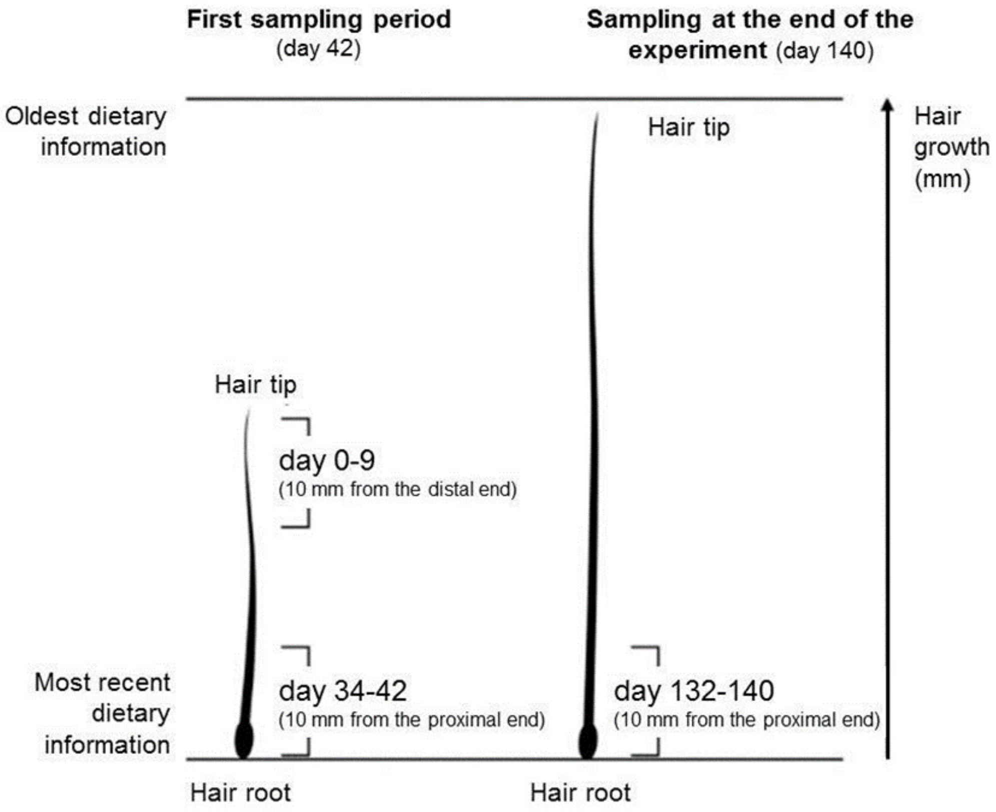


Figure 7. Sequences of wild boar hair used for isotope analysis of δ^{13}C and δ^{15}N. The wild boar hair were collected in the first sampling period (day 42) and at the end of the experiment (day 140) from the shoulder and rump of each wild boar.

To remove any contaminants and oil residues, the individual hair samples were washed in 0.25M sodium hydroxide solution followed by two separate washes in purified water. Then the washed hair samples were placed in sanitized screw top vials and dried overnight at 60°C. The maize grain samples were ground to a fine powder, homogenized, and also dried overnight at 60°C.

Stable isotope analyses

The δ^{13}C and δ^{15}N values of all samples were determined using EA-IRMS (Elemental Analysis-Isotope Ratio Mass Spectrometry) coupled with Europa Scientific 20-20 IRMS at Iso-Analytical Ltd. (Cheshire, UK). The results of isotope analyses are presented as δ^{13}C (‰) relative to the Vienna PeeDee Belemnite (V-PDB) standard and δ^{15}N (‰) relative to nitrogen in air and were calculated as follows:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \, ,$$

where δX is δ^{13}C or δ^{15}N, and R is the respective ^{13}C/^{12}C or ^{15}N/^{14}N ratio. The internal standards, IA-R042 (powdered bovine liver, $\delta^{13}C_{V\text{-}PDB}$ = –21.6 ‰, $\delta^{15}N_{AIR}$ = 7.6 ‰) for hair samples and IA-R001 (wheat flour, $\delta^{13}C_{V\text{-}PDB}$ = –26.4 ‰, $\delta^{15}N_{AIR}$ = 2.5 ‰) for maize samples, were used as reference material to ensure the analytical precision of the measurements. IA-R042, a mixture of IA-R005 and IA-R045 and a mixture of IA-R006 and IA-R046 were analysed for quality control of the hair samples. IA-R001, a mixture of IA-R005 and IA-R045 and a mixture of IA-R006 and IA-R046 were analysed for quality control of the maize samples. These working standards were then calibrated against IAEA standards N1 and CH6. The precision of measurements was better than 0.1 ‰ (one standard deviation) for both elements.

Isotopic discrimination

The diet-hair discrimination factors (i.e. differences in the isotopic ratio between the consumer and its diet) of carbon (Δ^{13}C) and nitrogen (Δ^{15}N) were calculated using the equation:

$$\Delta_{HAIR} = mean\ (\delta_{HAIR} - \delta_{DIET}) \, ,$$

where Δ_{HAIR} represents the isotope-specific diet-hair discrimination, δ_{HAIR} is the mean δ^{13}C or δ^{15}N value of the sections of the hair samples collected at the end of the experiment (day 140), and δ_{DIET} is the mean δ^{13}C or δ^{15}N value of the maize grain. Measurements of mean δ^{13}C and δ^{15}N values are reported as means ± SD.

Statistical analysis

Possible differences in δ^{13}C and δ^{15}N values in hair samples collected at the first sampling period (day 0-9, 34-42) and those taken at the end of the experiment (day 132-140) were evaluated using Paired Student´s t-tests. Measurements of mean δ^{13}C and δ^{15}N values are reported as means ± SD. Significance was tested at α=0.05 level. All statistical analyses were performed using R software, version 3.0.3 (R Development Core Team).

Results and Discussion

Achieving reliable dietary inferences based on isotopic data of serially sampled hair requires the conversion of spatial isotopic records into temporal records. Estimates of hair growth rate are therefore essential to increase the accuracy of such conversions (Schwertl et al., 2003). Table 3 shows the mean lengths and growth rates of the shoulder and rump hair of the individual wild boar determined in this study. Wild boar hair grew on average by 46.48 mm in 42 days, giving an average growth rate of 1.1 mm d^{-1} based on the average growth rates of shoulder and rump hair. The growth rates from these two body locations were only slightly different (i.e. 1.13 mm d^{-1} for shoulder hair and 1.02 mm d^{-1} for rump hair). The 10 mm sections of both shoulder and rump hair were analysed; if the body part- specific growth rates were applied separately, the 10 mm sections of shoulder hair would reflect 9 days of the experiment whilst the 10 mm sections of rump hair would reflect 10 days. Since the animals were on a constant diet throughout the experiment, this 1-day would not significantly affect the interpretation of gained results. It is important for researchers to consistently use the same sampling locations on bodies of their study animals when estimating diets using stable isotope analysis of hair. In studies involving reconstructing diets from isotopic signatures, there are always scenarios where a trade-off must be made between analytical precision, spatial (and thus temporal) resolution, as well as effort and cost. Therefore some assumptions may be compromised (Schwertl et al., 2003).

Table 3. The mean length and rate of growth of the shoulder and rump hair of individual wild boar.

	SHOULDER		**RUMP**	
	Mean length (mm)	**Growth rate (mm d^{-1})**	**Mean length (mm)**	**Growth rate (mm d^{-1})**
Individual **1**	47.57 ± 2.21	1.13	43.02 ± 3.46	1.02
Individual **2**	45.38 ± 4.77	1.08	42.38 ± 3.92	1.04
Individual **3**	49.98 ± 3.09	1.19	49.52 ± 4.17	1.17
Individual **4**	48.58 ± 3.04	1.15	46.42 ± 4.21	1.1
Mean ± SD	47.88 ± 1.94	1.13 ± 0.05	45.33 ± 3.30	1.02 ± 0.07

Values are reported as mean ± SD; n = 100 for individuals 1, 2, and 3 (i.e. n = 50 for shoulder and n = 50 for rump); n = 40 for individual 4 (i.e. n = 20 for shoulder and n = 20 for rump).

The hair growth rates estimated in this study were derived using recently shaved hair. Previous studies have shown, however, that no change in growth rate is associated with shaving (Lynfield and Macwilliams, 1970; West et al., 2004). There are also reports that nutrition, hormonal status, or environmental conditions may also influence rate of hair growth (Bradfield, 1981; Johnson, 1977; Mohn, 1958). The study animals were fed with a single item diet of low nutritional quality; it was concluded that estimates of hair growth rate may differ for free-ranging animals consuming diverse diets of moderate- or high- quality, thus providing opportunities for further investigation. The timing of the feeding experiment (June-October) was selected because it coincides with the availability of maize for free-ranging animals in Central Europe (June/July-November/December); previous studies have shown that, when available, maize is a major component of wild boar diet (about 87% of biomass intake; Amici et al., 2012; Herrero et al., 2006). As a result, it is possible that the estimated hair growth rates for captive wild boar in this study can reflect the growth rates of free-ranging individuals in agricultural areas. Thus these rates might be applicable in areas with large amounts of maize. To reliably and accurately interpret isotopic signatures of hair, the species- (body part-) specific hair growth rates estimations are required; this study hereby provides the first such estimates for wild boar (but in restricted conditions).

The determination of isotopic discrimination is an important consideration for the successful interpretation of isotopic signatures of hair (Caut et al., 2008b; Martínez del Rio et al., 2009). Table 4 shows the mean δ^{13}C and δ^{15}N values of the maize grain and the hair samples collected throughout the duration of the experiment; it also shows the mean discrimination factors for carbon and nitrogen (Δ^{13}C and Δ^{15}N) in wild boar hair. The measurements of isotopic discrimination for δ^{13}C and δ^{15}N in the wild boar hair were –2.3 ‰ and +3.5 ‰, respectively; this indicates a depletion in ^{13}C relative to the maize grain values, and enrichment in ^{15}N.

Table 4. Mean carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values of the maize samples (n=4), and the hair samples collected at the first sampling period (day 0-9, day 34-42) and at the end of the experiment (day 132-140), and mean discrimination factors (Δ) in wild boar (*Sus scrofa*) hair.

	Maize grain	**Hair (day 0-9)**	**Hair (day 34-42)**	**Hair (day 132-140)**	**Δ**
Carbon	–12.0 ± 0.03	–18.8 ± 0.7	– 14.3 ± 0.4	–14.3 ± 0.2	–2.3
Nitrogen	4.2 ± 0.2	7.8 ± 0.3	7.7 ± 0.1	7.7 ± 0.2	3.5

Values are reported as mean ± SD. The Δ^{13}C and Δ^{15}N values were calculated by subtracting the mean carbon or nitrogen isotope values of the hair samples collected at the end of the experiment and mean carbon or nitrogen isotope value of the maize grain. All values of δ^{13}C and δ^{15}N are presented in ‰.

The calculated mean Δ^{13}C in wild boar hair (– 2.3 ‰) is significantly below the values seen in hair from other mammalian omnivores (–1.6 to 4.3 ‰), but the mean Δ^{15}N value (3.5 ‰) lies within the range (– 0.5 to 4.1 ‰ ; Caut et al., 2008b; Hobson and Quirk, 2014; Kurle et al., 2014; Nardoto et al., 2006; Table 5). The observed value of Δ^{15}N agrees with the general assumption that a consumer's δ^{15}N changes predictably as trophic level increases and thus is enriched by ~3.4 ‰ relative to diet; this is mainly due to excretion of isotopically light nitrogen in urine (Peterson and Fry, 1987).

Table 5. Average values of trophic discrimination factors for stable isotopes of carbon (Δ^{13}C) and nitrogen (Δ^{15}N) in other mammalian omnivores.

Study species	**n**	**Δ^{13}C**	**Δ^{15}N**	**Source**
Domestic pig (*Sus scrofa*, breed *Seghers*)	5	0.2	2.7	(Nardoto et al., 2006)
Rat (*Rattus rattus*)	48	-1.6 to 1.1	-0.5 to 2.5	(Caut et al., 2008a)
Sprague-Dawley rat	24	2 to 4.3	2.3 to 4.1	(Kurle et al., 2014)
Striped skunk (*Mephitis mephitis*)	16	1.2 to 1.6	3.2 to 3.8	(Hobson and Quirk, 2014)

Values of Δ^{13}C and Δ^{15}N are presented in ‰.

The differences seen between published values for $\Delta^{13}C$ in the hair of other mammalian omnivores and the $\Delta^{13}C$ value estimated in this study could be a result of different diet types used in those other studies. Despite the lack of information on the effects of diet on $\Delta^{13}C$ values in mammalian hair, the previous studies suggest that the value of $\Delta^{13}C$ can be affected by diet type (e.g. Caut et al., 2009; DeNiro and Epstein, 1978, 1981; Lecomte et al., 2011). Before the feeding experiment was conducted, the study animals were fed with a pure C3 plant-based diet (mainly a wheat, barley, and oat mixture). However, the wild boar were switched to a pure C4 plant-based diet (maize grain) at the beginning of this experiment. Given the known deficiencies in essential amino acids in maize (Arentson and Zimmerman, 1995), the negative $\Delta^{13}C$ value may thus be attributed to the relatively low nutritional quality of the C4 maize grain. Thus, the animals feeding on C4 plants with low quality protein may have thus used more carbon from carbohydrates and lipids for tissue synthesis; as a result, the proportion of carbon derived from the maize grain was lower than the amount contained in the bulk diet (Arentson and Zimmerman, 1995; Codron et al., 2011; Murray et al., 1997). A similar pattern was recorded by Murray et al. (1997) in $\Delta^{13}C$ values of sheep wool, c. 3 ‰ depletion in ^{13}C relative to the pure C4 diet. It has also been shown that hair containing the root (as in this study) shows on average ~0.6 ‰ more depletion in ^{13}C than hair without the root; this is because the roots contain more lipids. Lipid-rich tissues are known to be depleted in ^{13}C relative to lipid-poor tissues (West et al., 2004). However, plucking rather than cutting hair avoids loss of recently grown hair, thus retaining the most recent isotopic information (Schwertl et al., 2003).

The estimated values of isotopic discrimination for wild boar hair in this study differ from those reported by Nardoto et al. (2006) for hair from domestic pig (*Sus scrofa*, breed "Seghers"). The estimated value here for $\Delta^{13}C$ is 2.5 ‰ lower, whilst for $\Delta^{15}N$ it is 0.8 ‰ higher. Several explanations exist for such differences. Firstly, differing diet compositions; their study individuals were fed with a diet composed of 25% soybean and 65% maize, with a 10% vitamin and mineral mix. The difference observed in $\Delta^{13}C$ and $\Delta^{15}N$ values is likely to be the result of different protein quality and amounts of essential amino acids of mixed C3- and C4- plant based diet vs. pure C4 diet (as used in the current study; Robbins et al., 2010). Secondly, the very long history of domestication has resulted in pronounced differences in the anatomy and physiology of domestic pigs compared to their wild ancestors; hence differences in their metabolic pathways may have resulted in divergent isotopic routing (Giuffra et al., 2000; Hemmer, 1990; Jones, 1998; Weiler et al., 1995). Thirdly, the age differences between the study animals; here, 3-year-old males were studied, whereas in Nardoto et al. (2006), only 152 day-old females were used. It is possible that the lower value of $\Delta^{15}N$ in their study is the result of age, since 152 day-old animals are still growing and therefore will have reduced $\Delta^{15}N$ (Kurle et al., 2014; Martínez del Rio et al., 2009). Growing individuals are believed to retain more ^{14}N in their body pool because they utilise more dietary protein for tissue synthesis rather than removing N as waste, resulting in lower $\Delta^{15}N$ values (Kurle et al., 2014; Martínez del Rio et al., 2009). Finally, the type of hair used in their study is not specified and the hairs used for analysis were sampled by clipping (i.e. without the root), which could also contribute to the differences seen in $\Delta^{13}C$ (West et al., 2004).

The results show that there was a significant difference in the mean values of $\delta^{13}C$ in the hair samples representing days 0-9 compare to those collected at the end of the experiment (day 132-140; $t = -16.28$, $n = 8$, $p < 0.0001$). In contrast, the mean values of $\delta^{15}N$ showed no difference between days 0-9 and 132-140 ($t = 0.832$, $n = 8$, $p > 0.583$). There were also no differences between days 34-42 and 132-140 for either $\delta^{13}C$ or $\delta^{15}N$ values in hair ($\delta^{13}C$: $t = -0.395$, $n = 8$, $p > 0.704$; $\delta^{15}N$: $t = 0.566$, $n = 8$, $p > 0.5888$). These findings suggest that at least 30 days is required for the $\delta^{13}C$ value of hair to be stabilized with diet. This is consistent with a previously published study on steers (*Bos taurus*) which estimated the delay between the two isotopic records of diet as being between 10-20 days (Zazzo et al., 2007). Also, Caut et al. (2008a) found a 40-day period for hair of shaved rats (*Rattus rattus*) to stabilize with diet. The constant value of $\delta^{15}N$ seen in the study animals throughout the experiment could be a result of a similar $\delta^{15}N$ signature of both the maize grain and the diet prior to the experiment. However, it may also suggest that the time for stabilization is shorter for $\delta^{15}N$, but this requires further investigation.

This feeding experiment was only conducted on four male wild boar of the same age which were fed with a single food item; therefore, further experimental studies are required to test for possible differences of TDFs ($\Delta^{13}C$ and $\Delta^{15}N$) as well as hair growth rates when a mixed diet of different nutritional quality is used. The range of food resources commonly used by free-ranging wild boar should also be addressed by future studies. The discrimination measurements (Δ^{13}C and Δ^{15}N) in this study were only determined in a single tissue (hair); therefore estimating these values also for other wild boar tissues with different metabolism is necessary. Estimates of discrimination for hair are, however, particularly valuable because hair can be sampled non-destructively. Also, the effect of age and sex on the discrimination values needs to be established, as well as the growth rate of wild boar hair (e.g. Kurle et al., 2004; Lecomte et al., 2011).

To sum up, estimations of average hair growth rate and diet-hair discrimination factors of δ^{13}C and δ^{15}N in hair of wild boar in this study provide a foundation for interpreting isotopic patterns of free-ranging individuals; once proportions have been established, they can be applied in estimations of problems such as crop damage, particularly in areas with large amounts of maize. However, a deeper understanding of the processes underlying the variation in isotopic discrimination of δ^{13}C and δ^{15}N requires further experimental studies under controlled conditions. Experiments such as this one are a stepping-stone for future studies focussing on the feeding ecology of wild boar, especially in areas of intense agriculture where wild boar populations continue to grow and spread, and where individuals are becoming more accustomed to food sources of human origin, particularly maize.

Chapter III

Carbon and nitrogen isotope discrimination between diet and hair of red deer: the effect of sex, age, body weight, and lactation[*]

Materials and Methods

Ethics statement

The experiment was carried out in accordance with the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague approved by the Animal Care and Use Committee of the Czech Ministry of the Environment (Permit number: 15106/ENV/14-825/630/14) and the Ministry of Agriculture (Permit number: 86779/2011-MZE). All care was undertaken to minimize stress and suffering to the animals according to deer farming standards. Collection of samples was done during routine handling procedure, when captive deer were weighted regularly.

Experimental design and sample collection

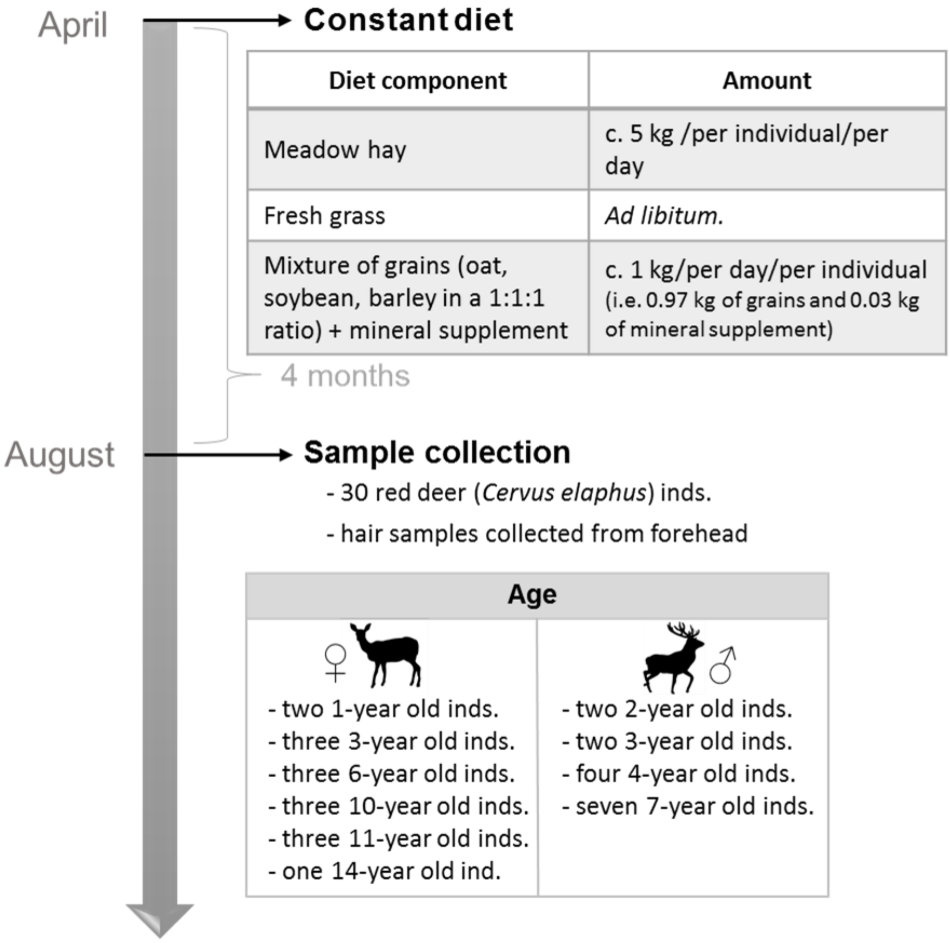
This experiment took place from April to August 2015 at an experimental deer farm at the Institute of Animal Science, Prague, Czech Republic. The experimental animals were 30 captive red deer, half male, half female (Figure 8). Thirteen females were lactating at the time of the sample collection in August. All individuals were kept in an outdoor enclosure (6 paddocks totalling 3.6 ha) throughout the experiment.

The deer grazed in paddocks since April and they were supplemented with meadow hay (ad libitum) plus a mixture of grains (oat, soybean, barley in a 1:1:1 ratio) as well as a small amount of Vitamix OSZ, a commercially-made mineral supplement (Figure 8). The grain mixture was ground small enough to pass through a 50-mm sieve; then it was carefully mixed to reduce selective consumption by the deer. Water was freely available. The deer were kept on this constant diet until sample collection in August. A four-month period was chosen because it had previously shown to be

---

[*] This chapter is based on a manuscript to be submitted to PLoS ONE journal: Holá, M., Ježek, M., Kotrba, R., Bartoš, L. Diet-hair discrimination factors of stable carbon and nitrogen isotopes in red deer (*Cervus elaphus*): the effect of sex, age, body weight, and lactation.

sufficient for isotope equilibration between the diet and hair (Caut et al., 2008a; Jones et al., 1981; Zazzo et al., 2007). A sample of hair was taken (plucked) from the forehead of each study individual. Experimental diet samples were collected twice during the experiment (Figure 8).



Diet component	Amount
Meadow hay	c. 5 kg /per individual/per day
Fresh grass	*Ad libitum.*
Mixture of grains (oat, soybean, barley in a 1:1:1 ratio) + mineral supplement	c. 1 kg/per day/per individual (i.e. 0.97 kg of grains and 0.03 kg of mineral supplement)

- 30 red deer (*Cervus elaphus*) inds.
- hair samples collected from forehead

Age	
♀	♂
- two 1-year old inds. - three 3-year old inds. - three 6-year old inds. - three 10-year old inds. - three 11-year old inds. - one 14-year old ind.	- two 2-year old inds. - two 3-year old inds. - four 4-year old inds. - seven 7-year old inds.

Figure 8. Experimental design of the feeding experiment with 30 individuals of red deer (1-14 years old females and 2-7 years old males). Experimental animals were fed with a constant diet of meadow hay, fresh grass, and a mixture of grains and mineral supplement.

Sample preparation and stable isotope analysis

Each of the hair samples was washed in 0.25M sodium hydroxide solution to remove lipids followed by two separate washes in purified water. The washed hair samples were then placed in clean screw top vials and oven-dried overnight at 60°C. The most recently grown hair sections (~ 5 mm) from the proximal end (hair root included) of the hair in the anagen growth phase with undamaged roots were used for isotope analysis (van Scott, 1957). The diet samples were dried overnight at 60°C, ground to a fine powder and then homogenized.

The δ^{13}C and δ^{15}N values of all samples were determined at the Iso-Analytical Ltd. (Cheshire, UK) using EA-IRMS (Elemental Analysis-Isotope Ratio Mass Spectrometry) coupled with Europa Scientific 20-20 IRMS. Isotopic analyses are expressed as ratios in delta (δ) notation relative to an international standard in parts per thousand (‰) according to the following equation:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \, ,$$

where δX denotes δ^{13}C or δ^{15}N, and R_{sample} represents the ratio of ^{13}C/^{12}C or ^{15}N/^{14}N, respectively. $R_{standard}$ is the international standard Vienna PeeDee Belemnite (V-PDB) for δ^{13}C and atmospheric N_2 for δ^{15}N. Replicate reference materials of either bovine liver IA-R042 or wheat flour IAR001 were analysed between a number of samples and each had a standard deviation <0.1 ‰ for both elements.

Data analyses

The diet-hair discrimination factors for carbon (Δ^{13}C) and nitrogen (Δ^{15}N) were calculated using the equation already used in the *Chapter I*:

$$\Delta_{HAIR} = mean\ (\delta_{HAIR} - \delta_{DIET}) \, ,$$

where Δ_{HAIR} represents the isotope-specific diet-hair discrimination factor, δ_{HAIR} is the mean δ^{13}C or δ^{15}N value of the hair samples, and δ_{DIET} is the mean δ^{13}C or δ^{15}N value of the experimental diet (i.e. meadow hay, fresh grass, and a mixture of grains and mineral supplement).

The possible effect of sex, age, body weight, and lactation on the Δ values of both isotopes was assessed using Generalized Linear Mixed Model (GLMM, PROC MIXED). The model was run as a fixed effects model with Δ^{13}C or Δ^{15}N values as a dependent variable with sex (female and male), age (1 to 14 years), body weight (50 to 230 kg), lactation in females (yes / no) nested within sex, and age nested within sex as fixed effects. Dependent variables were log-transformed (natural logarithm transformation) to improve normality of residuals and to reduce skewness. The significance of each fixed effect in the GLMM was assessed by the F-test. Least-squares means (LSMEANs) were computed for class variable sex. LSMEANs are, in effect, within-group means appropriately adjusted for the other effects in the model. If not specifically explained, non-significant factors ($P > 0.05$) were dropped from the model and will not be mentioned any further. Associations between the dependent variable and fixed effects were estimated by plotting predicted values against the fixed effect with predicted regression line. All data were analysed with the aid of SAS System version 9.4 (SAS Institute Inc.). Measurements of mean $\delta^{13}C$ and $\delta^{15}N$ values are reported as means ± SD.

Results and Discussion

As Table 6 shows, the range of δ^{13}C and δ^{15}N values of diet components fed to red deer was from –27.0 ‰ to –28.5 ‰ (with a mean of –28.2 ± 1.1 ‰) and from 1.0 ‰ to 4.0 ‰ (with a mean of 2.8 ± 1.4), respectively.

Table 6. Mean values (± SD) of isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N), and carbon (%C) and nitrogen (%N) content of the diet components fed to red deer over the duration of the experiment.

Diet component	**n**	**%C**	**%N**	**δ^{13}C (‰)**	**δ^{15}N (‰)**
Mixture of grains + mineral supplement	5	39.8 ± 0.4	3.9 ± 0.2	–27.0 ± 0.3	2.6 ± 0.2
Meadow hay	5	40.7 ± 0.4	1.2 ± 0.2	–29.3 ± 0.2	1.8 ± 0.9
Fresh grass	6	40.4 ± 2.0	3.5 ± 0.5	–28.5 ± 0.6	4.8 ± 0.7

All values of δ^{13}C and δ^{15}N are presented in ‰.

The Δ^{13}C and Δ^{15}N values of red deer hair samples ranged from +1.8 to –2.8 ‰ and 3.1 ‰ to 5.0 ‰, respectively. The mean values of isotopic discrimination for stable carbon and nitrogen isotopes in hair of red deer females, males and both sexes are shown in Table 7. Hair of both sexes were enriched in both δ^{13}C and δ^{15}N relative to the experimental diet values.

Table 7. Mean carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values, and diet-hair discrimination factors of carbon (Δ^{13}C) and nitrogen (Δ^{15}N) for females, males, and both sexes of red deer.

	δ^{13}C	**δ^{15}N**	**Δ^{13}C**	**Δ^{15}N**
Females	–26.1 ± 0.1	7.2 ± 0.4	2.2	4.4
Males	–25.8 ± 0.2	6.6 ± 0.3	2.5	3.8
Both sexes	–25.9 ± 0.2	6.9 ± 0.5	2.3	4.1

Values are reported as mean ± SD. The Δ^{13}C and Δ^{15}N values were calculated by subtracting the mean carbon or nitrogen isotope values of the experimental diet from the mean carbon or nitrogen isotope value of the hair samples. All values of δ^{13}C and δ^{15}N are presented in ‰.

The calculations of the mean $\Delta^{13}C$ and $\Delta^{15}N$ for red deer hair obtained here fall within the range of values reported for hair in other mammalian herbivores, i.e. – 0.34 ‰ to 3.5 ‰ for $\Delta^{13}C$ and 2.5 ‰ to 6.4 ‰ for $\Delta^{15}N$ (Darr and Hewitt, 2008; Sponheimer, 2003a, 2003b; Table 8). Since the measurements of isotopic discrimination are species-specific, the observed differences between discriminations values estimated here and other published values for hair of other mammalian herbivores could be a consequence of different studied species (e.g. Sponheimer, 2003a; McCutchan et al., 2003).

Table 8. The range of average values of trophic discrimination factors for stable isotopes of carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$) in hair of selected herbivores available from experimental studies.

Study species	**n**	**$\Delta^{13}C$**	**$\Delta^{15}N$**	**Source**
White-tailed deer (*O. virginianus*)	6	–0.34 to 1.21	4.69 to 5.80	Darr and Hewitt, 2008
Goat (*Capra hircus*)	4	3.2	2.6 to 5.0	Sponheimer, 2003a,b
Alpaca (*Vicugna pacos*)	2	3.2	3.6 to 6.4	Sponheimer, 2003a,b
Llama (*Lama glama*)	4	3.5	NA	Sponheimer ,2003a,b
Rabbit (*Oryctolagus cuniculus*)	4	3.4	NA	Sponheimer, 2003a,b
Cattle (*Bos taurus*)	7	2.6	2.5 to 3.9	Sponheimer, 2003a,b

Values of $\Delta^{13}C$ and $\Delta^{15}N$ are presented in ‰. NA: not available.

The measurements of isotopic discrimination for carbon in red deer hair obtained in this study still exceeds the commonly assumed values (0-1 ‰) of discrimination between diet and tissue (e.g. Post, 2002). The mean $\Delta^{15}N$ of 4.1 ‰ estimated here is higher than the frequently adopted value of 3.5 ‰ per trophic level (e.g. Peterson and Fry, 1987; Post, 2002). These findings emphasize the urgent need for species- and diet- specific discrimination factors to be determined before applying them to sensitive isotope-mixing models (Bond and Diamond, 2011).

Sex and age both contributed to the differences seen in isotopic discrimination for both isotopes. Neither Δ^{13}C nor Δ^{15}N values were affected by body weight and lactation in females. There was no difference between the Δ^{13}C values for female and male deer; thus this factor was dropped from the final model. The results thus indicate that within a single species, isotopic discrimination can be affected by demographic parameters, such as sex, and age, but not body weight and lactation. These findings are in accordance with the study of Lecomte et al. (2011) who reported that diet-tissue discrimination factors of stable carbon and nitrogen in mammals were affected by several factors related to the intra-population variability (i.e. age, sex and individual component).

The values for Δ^{13}C were dependent on both age ($F_{(1, 27)} = 10.82$, $P = 0.003$, not shown) and age nested within sex ($F_{(1, 27)} = 26.58$, $P < 0.0001$, Figure 9). Increasing age in males led to an increase in the Δ^{13}C values (Solution for fixed effects, $t = 4.36$, $P = 0.0002$). This was also apparent in females, although it was not significant ($t = 0.90$, NS).

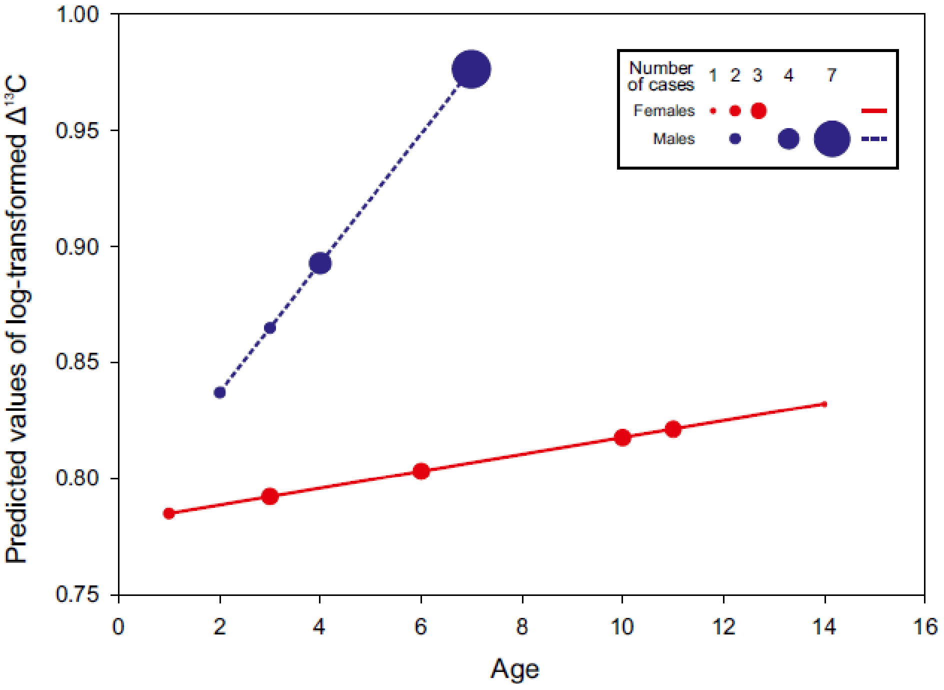


Figure 9. Predicted δ^{13}C values plotted against age for females and males.

Females had higher values of $\Delta^{15}N$ in comparison to males (GLMM, $F_{(1, 26)} = 42.53$, $P < 0.0001$, Figure 10). The values were dependent on age nested within sex ($F_{(2, 26)} = 10.50$, $P = 0.0005$, Figure 11). With females the $\Delta^{15}N$ values decreased with decreasing age (Solution for fixed effects, $t = -3.53$, $P = 0.0016$), while with males it was the opposite ($t = 2.93$, $P = 0.007$).

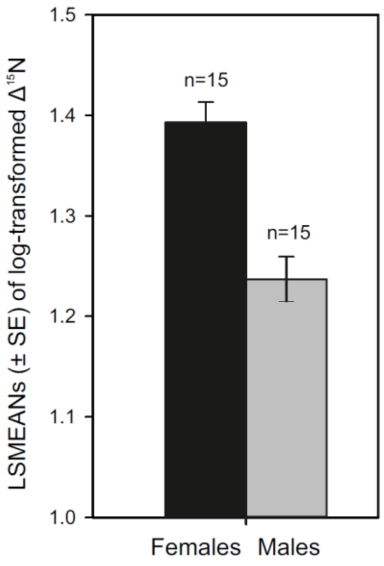


Figure 10. Least square means (± S. E.) of $\Delta^{15}N$ values according to sex of the deer.

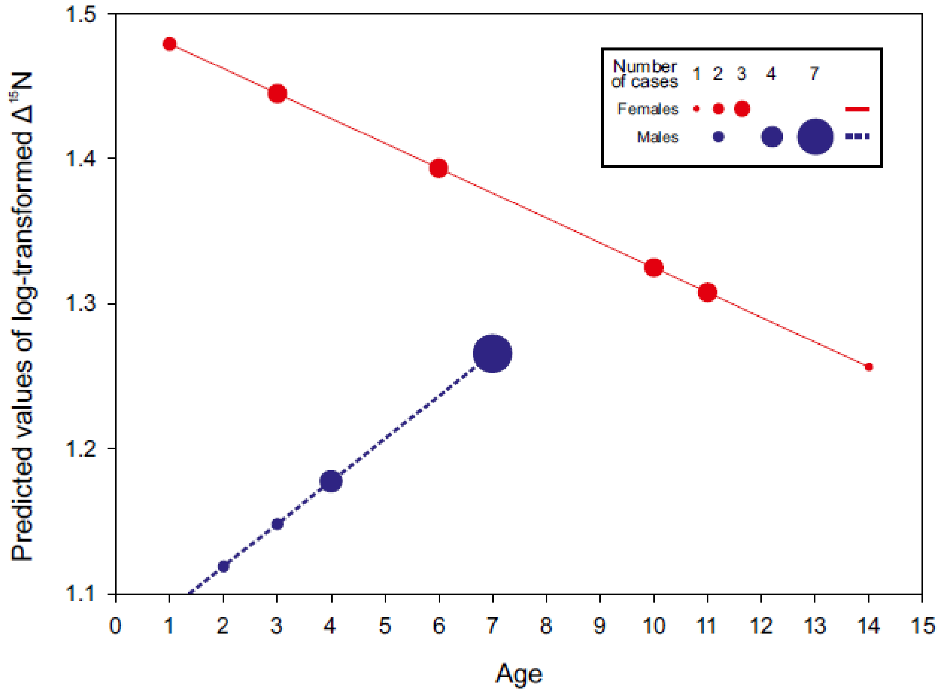


Figure 11. Predicted $\delta^{15}N$ values plotted against age for females and males.

Contrary to the observed results, Lecomte et al. (2011) have shown the opposite trend for $\Delta^{13}C$ and $\Delta^{15}N$ values in fur samples in females and males of arctic fox (*Vulpes lagopus*), with the values of $\Delta^{13}C$ being higher for females (by 0.3 ‰) and with the values of $\Delta^{15}N$ being higher for males (by 0.1 ‰). On the other hand, Kurle et al. (2014) have observed the same trend as here, with no differences in $\Delta^{13}C$ values between sexes of hair samples of Sprague-Dawley rats, whereas the $\Delta^{15}N$ values were higher for females than males (by 0.2-0.5 ‰).

The effect of sex on isotopic discrimination detected in previous experimental studies of wild animal trophic ecology has been usually attributed to variations in diet or foraging locations (Awkerman et al., 2007; Kurle and Worthy, 2001). Although there is a lack information on the effects of diet on $\Delta^{13}C$ and $\Delta^{15}N$ in mammalian hair, several studies have suggested that diet type can affect the values of both $\Delta^{13}C$ and $\Delta^{15}N$ (e.g. DeNiro and Epstein, 1978, 1981; Lecomte et al., 2011; Caut et al., 2009).

Sexual size dimorphism could also play a role in patterns observed in the $\Delta^{13}C$ and $\Delta^{15}N$ values of red deer hair in this study. Red deer is a classic example of species that exhibit sexual size dimorphism, with adult females being up to 50% lighter than adult males (Clutton-Brock et al., 1982). Larger males are better equipped to digest voluminous fibrous foods due to a greater volume of gastrointestinal tract and lower energetic requirements in comparison to females (Barboza and Bowyer, 2000; Clutton-Brock et al., 1982). An age effect observed here could be related to differential metabolic pathways or synthesis between younger and older individuals with differences between sexes being probably related to body size dimorphism (Cherel et al., 2005; Lecomte et al., 2011).

The effect of lactation was not confirmed in the analyses. This could be, however, caused by the fact that out of the fifteen females, there were only three non-lactating ones which could cause a low variability of collected data. Moreover, the three non-lactating females comprised two young individuals investing most of their energy into growth. Therefore, their energetic expenditures were comparable to those of lactating females which could steer the effect of lactation in the final model. The observed differences in $\Delta^{13}C$ and $\Delta^{15}N$ values between females and males could be thus possibly explained by the fact that most of the females were lactating at the time of sample collection. There are several possible explanations for the effect of lactation on the Δ^{13}C and Δ^{15}N values. Firstly, lactating females have higher energy requirements which may lead to nutritional stress and consequently to catabolism of body protein and thus to enrichment in δ^{15}N values. Such an effect of lactation on nutritional status of females has been previously reported in mammals (Kurle and Worthy, 2001; Parker et al., 2005; Popa-Lisseanu et al., 2015).

On the other hand, it has been suggested that Δ^{15}N values in animal tissue increase as dietary protein increases (Walter, 2014b). Similarly, Sponheimer et al. (2003b) have showed that captive herbivores consuming high-protein diets had higher Δ^{15}N values in tissues than the same animals consuming low-protein diets. Lactating females which have high energetic demands are able to remodel their gastrointestinal tracts in order to improve digestion and absorption of nutrients from diet (Barboza and Bowyer, 2000; Jenks et al., 1994; Zimmerman et al., 2006). Therefore, lactating females might be capable of absorbing more nitrogen in comparison with males consuming the same diet (Monteith et al., 2014). Also, females of white-tailed deer have been shown to feed selectively on diet with higher quality (i.e. containing more nitrogen) compared to males throughout the whole year (Beier, 1987). Furthermore, some studies focusing on effect of lactation on isotope values have showed a decrease in in either only Δ^{15}N, only Δ^{13}C, or both Δ^{13}C and Δ^{15}N values of lactating females (Habran et al., 2010).

To conclude, this experiment provides the first diet-hair discrimination factors of stable carbon and nitrogen isotopes ever reported for red deer. Furthermore, the results indicate that demographic parameters, i.e. sex and age in this case, affect isotopic discrimination. Nevertheless, further experimental studies are needed to assess the variability in diet-tissue discrimination factors and to quantify the sources of variation which need to be taken into account while designing and analysing ecological field studies. Experimental studies such as this one are urgently needed particularly for rapidly expanding species such as the red deer, which is being widely fed with supplemental food and also depend upon agricultural crops, since stable analysis may help to determine the contribution of these artificial food sources to the bulk diet.

Chapter IV

Use of near infrared reflectance spectroscopy to assess nitrogen and fibre fractions in red deer faeces and evaluation of winter food quality and its variability for red deer[*]

Materials and Methods

Study areas

Faecal samples of red deer were collected during winter 2013 in two study areas in the Czech Republic: (i) military training area Hradiště (Karlovy Vary region; hereinafter MTA Hradiště), and (ii) military training area Boletice (South Bohemia region; hereinafter MTA Boletice; Figure 12).

The MTA Hradiště (50°16′ N, 13°7′ E) is situated in the Doupovské hory Mountains and has a total acreage of 331 km^2. Forests cover 41% of the area, agricultural lands 8%, other land cover types 51%, and water surfaces 0.2%. Deciduous forests with Norway spruce (*Picea abies*), European larch (*Larix decidua*), and pine (*Pinus sylvestris, Pinus nigra*) cover 71%. Broad-leaved forests with beech (*Fagus sylvatica*), sycamore maple (*Acer pseudoplatanus*), and ash (*Fraxinus excelsior*) cover 29%. The predominant forest type is herb-rich beech forest. The other forest types are ravine forests, alluvial forests, oak-hornbeam forests, and thermophilous oak forests (Vojta and Kopecký, 2006).

The MTA Boletice (48°49′ N, 4°13′ E) has a total acreage of 219 km^2. Forests cover 60% of the area, agricultural land 10%, and shrubs and natural open areas 30%. Spruce forests with Norway spruce (*Picea abies*) and rowan (*Sorbus aucuparia*) are the dominating cover. Flowering beech forests and acidophilus beech forest mostly with European beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*), and fir forests with silver fir (*Abies alba*) are found at altitudes between 600 and 1000 m a.s.l. Large complexes of semi natural treeless areas occur at lower altitudes.

---

[*] This chapter is based on a manuscript to be submitted to Wildlife Biology journal: Holá, M., Ježek, M., Kušta, T., Červený, J.: Winter food quality and its variability for red deer in forest environment: overwintering enclosures vs. free-ranging areas.

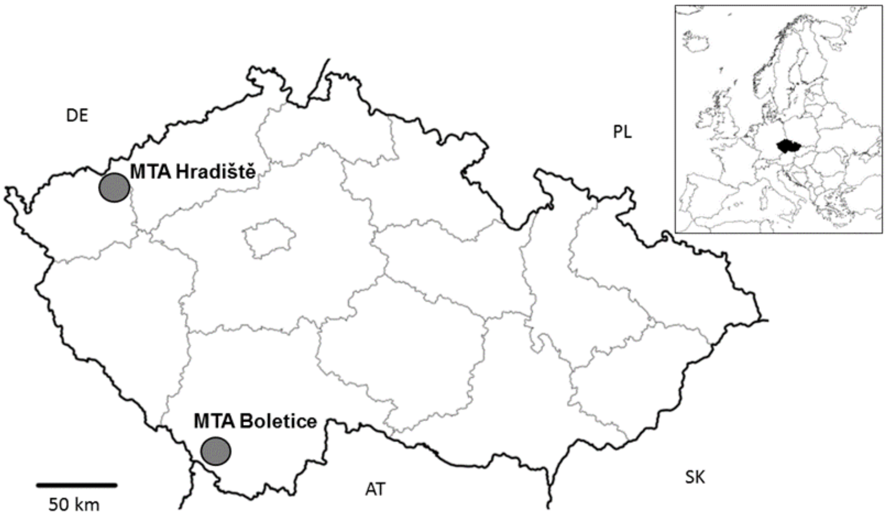


Figure 12. Location of the study areas (indicated by grey circles) within Europe and the Czech Republic.

The use of fenced overwintering enclosures for free-ranging ungulates is a common management practice in both study areas. The main purpose of these enclosures is to reduce damage to forest stands and to assist animal survival over harsh winter conditions. Animals are usually kept inside the enclosures from the beginning of December until the beginning of growing season (May). Regarding the overwintering enclosures in our study areas in 2013, the enclosure in the MTA Hradiště had a total acreage of 12 ha with 280 individuals of red deer (90%), and sika deer (10%; *Cervus nippon*), and the enclosure in the MTA Boletice had 8 ha with 35 individuals of red deer. Supplemental feed (i.e. corn and meadow hay) was regularly provided to free-ranging ungulates, including red deer, in the overwintering enclosures and neighbouring free-ranging areas in both study areas from November to May.

Sampling and chemical analyses

Fresh red deer faeces were randomly collected from fenced overwintering enclosures and neighbouring unfenced areas within each study area during winter 2013 (n = 149 in MTA Hradiště, n = 161 in MTA Boletice). All faecal samples were oven-dried to constant weight at 50°C and subsequently ground to pass 1 mm sieve. A subset of faecal samples (i.e. calibration (n=100), further used for NIRS interpolation of chemical constituents in the remaining samples, was assayed by standard chemical methods to determine the exact concentrations of FN, FADF, and FNDF. Total FN content was determined using an automated C/N analyser TruSpec (LECO Corporation, USA) after oxygen combustion in an oven at 950°C. Total FADF and FNDF concentrations were determined by standard methods of the Association of Official Analytical Chemists (AOAC, 1984).

Near infrared reflectance spectroscopy (NIRS): collection of reflectance spectra

The results of the chemical assays were then used to calibrate NIRS as described by Foley (1998). All faecal samples were scanned from 1 100 to 2 500 nm using a Thermo Nicolet NEXUS 670 scanning spectrophotometer and an OMNIC 7.4 software (Thermo Scientific^{TM}, USA). The spectrum of each sample was the average of 32 successive scans at a resolution of 4 cm^{-1}. Each spectrum was recorded as the logarithm of the reciprocal of reflectance (log 1/R). All samples were analysed at constant temperature and humidity and diagnostics tests were performed at the beginning of each test day in order to check the accuracy of wavelengths and the repeatability of measurements.

Calibration development and prediction of chemical constituents

The results of the chemical assays and all recorded spectra were transmitted to the TQ Analyst 7.4 software (Thermo Scientific^{TM}, USA) in order to develop a calibration models for N, ADF, and NDF. In order to reduce any possible effect of particle size variation on the NIR spectra, the scatter correction of standard normal variate (SNV) and detrend was applied to the spectral data, along with a number of possible combinations of derivative (1, 2), gap (4, 10), and smoothing (4, 10; Barnes et al. 1989). The calibrations were performed by partial least square (PLS) regressions (Shenk and Westerhaus, 1991). Cross-validation through a leave-one-out procedure was used to determine the optimum number of PLS factors included in the calibrations. During cross-validation, the assessed values were divided into one set for calibration and one for validation for each constituents using every seventh sample for the validation set (Shenk and Westerhaus, 1991). Outliers were detected by using the residual sample variance plot after the PLSR. The predictive ability of the PLS equations was evaluated on the basis of coefficient of determination (R^2) of the linear regression of predicted against measured values, the root mean square errors of calibration (RMSEC), the root mean square errors of cross-validation (RMSECV), and the ratio of performance deviation (RPD), which is the ratio of the standard deviation of the reference values and the root mean square errors of prediction (RMSEP). Good predictions are regarded as having an $R^2 \geq 0.80$ and an RPD ≥ 2. Predictions having $R^2 \leq 0.65$ and an RPD ≤ 1.5 are considered to be poor (Shepherd and Walsh, 2007).

Statistical analyses

The Kendall´s tau τ correlation coefficients were calculated in order to evaluate whether there was any relationships among FN, FADF, and FNDF in individual study areas.

The amounts of individual faecal indices of food quality within and outside the overwintering enclosures in individual study areas were compared by Student´s t-tests or Wilcoxon signed-rank test depending on the normality of the data.

To quantify the degree of variation in food quality in overwintering enclosures and neighbouring free-ranging areas, the coefficients of variation (i.e. CV, standard error divided by mean) for concentrations of FN, FADF, and FNDF were estimated. Significance was tested at $\alpha=0.05$ level. All statistical analyses were performed with the R software, version 3.1.1 (R Development Core Team).

Results and Discussion

Overall, a total of 310 red deer faecal samples, including 149 from the MTA Hradiště and 161 from the MTA Boletice, was analysed for concentrations of FN, FADF, and FNDF. The results obtained indicate that winter food quality and its variability for red deer differ between the overwintering enclosure and the neighbouring free-ranging area in the MTA Hradiště, whereas they are comparable in the MTA Boletice.

The developed calibration models (derived from the absorbance spectra of faeces) confirmed the high potential of near infrared reflectance spectroscopy for analysing a large number of samples and accurate determination of major faecal indices of food quality for red deer (Table 9). These models can now be used to predict FN, FADF, and FNDF in a large number of samples and with minimal costs. The suitability of NIRS for evaluating food quality for free-ranging deer with the use of faecal indices has already been confirmed by several authors (e.g. Kamler et al., 2004; Tellado et al., 2015).

Table 9. Predictive power of partial least square regression with cross-validation for modelling the relationship between spectral characteristics of faecal samples of red deer and concentrations of faecal nitrogen (FN), faecal acid detergent fibre (FADF), and faecal neutral detergent fibre (FNDF).

Constituent	N	R^2	RMSEC	RMSECV	RPD
N	100	0.99	0.03	0.18	2.8
ADF	100	0.98	1.21	4.42	2.9
NDF	100	0.99	0.58	3.55	2.4

N=number of samples used for calibration; R^2=the degree of correlation between the predicted values and the actual measured values; RMSEC=root mean square error of calibration; RMSECV=root mean square error of cross-validation; RPD=ratio of standard deviation of laboratory reference values and the root mean square error of prediction; RPD≥2 indicates good models.

Table 10 shows the concentrations of FN, FADF, and FNDF in red deer faeces collected within and outside the overwintering enclosures in the individual study areas. The use of FN as a proxy for food quality of herbivorous ungulates is based on the fact that there is a positive relationship between dietary N and FN (e.g. Hodgman et al., 1996; Leslie and Starkey, 1985). Nevertheless, it cannot always be assumed that dietary N is directly reflected in the faeces, particularly due to possible effects of secondary metabolites (e.g. Leslie et al., 2008; Palo and

Robbins, 1991). Therefore, conclusions based only on the FN levels should be strengthened by the use of multiple nutritional indices, such as fibre fractions in faeces (FADF and FNDF). These indices should be more sensitive to fluctuations in food quality than FN, especially when diets contain high amounts of secondary metabolites such as tannins (Hodgman et al., 1996; Leslie et al., 2008).

Table 10. The mean (±SD), minimum, and maximum values of faecal nitrogen (FN), faecal acid detergent fibre (FADF), and faecal neutral detergent fibre (FNDF) in red deer faeces collected from fenced overwintering enclosures and neighbouring unfenced areas within two study areas (MTA Hradiště and MTA Boletice) during winter 2013.

	ENCLOSURE				FREE-RANGING AREA			
FN	**μ±SD**	**n**	**Min**	**Max**	**μ±SD**	**n**	**Min**	**Max**
MTA Hradiště	2.17 ± 0,34	107	1.50	2.88	1.88 ± 0.19	42	1.34	2.28
MTA Boletice	2.21 ± 0.31	79	1.66	3.04	2.27 ± 0.31	82	1.75	3.26
FADF	**μ±SD**	**n**	**Min**	**Max**	**μ±SD**	**n**	**Min**	**Max**
MTA Hradiště	44.24 ± 5.13	107	28.10	55.40	47.84 ± 4.22	42	37.10	56.20
MTA Boletice	41.67 ± 4.02	79	33.70	52.30	45.78 ± 3.15	82	36.20	55.00
FNDF	**μ±SD**	**n**	**Min**	**Max**	**μ±SD**	**n**	**Min**	**Max**
MTA Hradiště	56.61 ± 5.62	107	38.80	73.80	60.710 ± 3.76	42	52.40	68.00
MTA Boletice	61.48 ± 7.67	79	48.10	75.00	60.80 ± 4.50	82	51.20	71.30

μ: mean, SD: standard deviation; n: number of collected faeces. All values expressed as % dry matter.

The Kendall´s tau τ correlation coefficient showed significant negative relationship between FN and FADF ($\tau_{\text{MTA Hradiště}} = -0.40$, $p < 0.5$; $\tau_{\text{MTA BOLETICE}} = -0.12$, $p < 0.5$), as well as FNDF ($\tau_{\text{MTA Hradiště}} = -0.65$, $p < 0.5$; $\tau_{\text{MTA BOLETICE}} = -0.78$, $p < 0.5$) in both study areas. The relationships between FADF and FNDF were positive ($\tau_{\text{MTA Hradiště}} = 0.67$, $p < 0.5$; $\tau_{\text{MTA BOLETICE}} =0.16$, $p < 0.5$). As expected, the results showed a strong negative relationship between FN and FADF, as well as FNDF. This is due to the FN levels being lower if the diet contains more indigestible compounds, such as fibre components, since FN is associated with indigestible fibre (Van Soest, 1994).

Regarding the differences in individual faecal indices between the overwintering enclosure and outside it in the MTA Hradiště, the FN contents were higher in the enclosure compared to the neighbouring area (Wilcox.t.: $Z=-4.582$; $p<0.0001$, Figure 13). The levels of

FADF and FNDF showed the opposite trend, with higher levels found in the free-ranging areas compared to the enclosure (*ADF*: Wilcox. t.: Z=4.040; p<0.0001, Figure 14; *NDF*: Student. t-test.: t=4.337; p<0.0001, Figure 15).

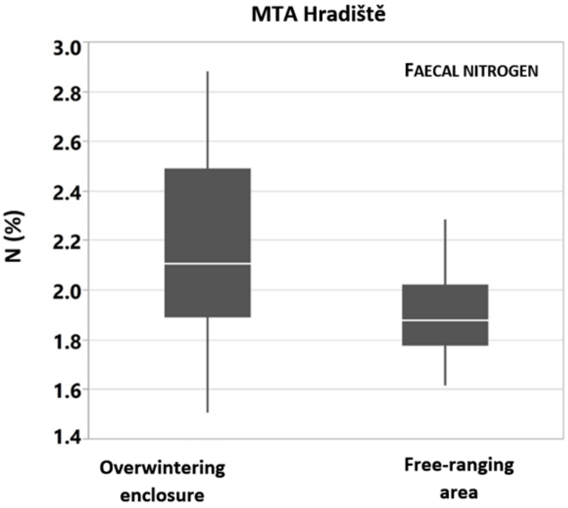


Figure 13. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal nitrogen* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Hradiště.

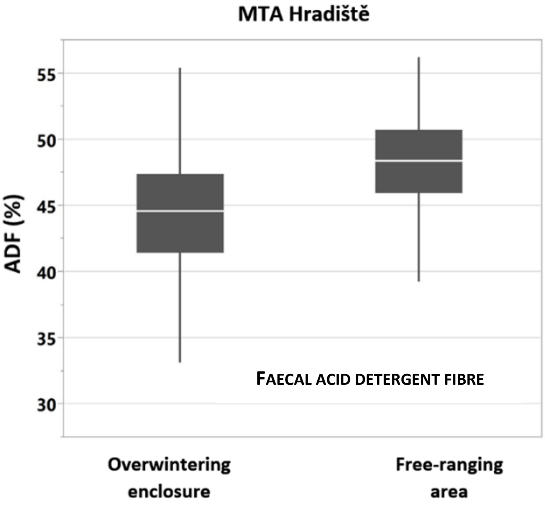


Figure 14. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal neutral detergent fibre* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Hradiště.

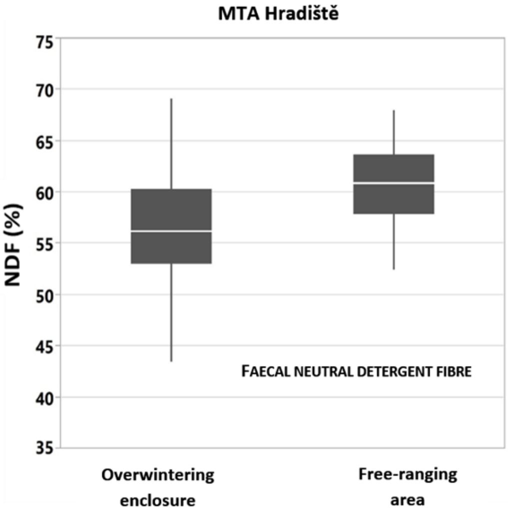
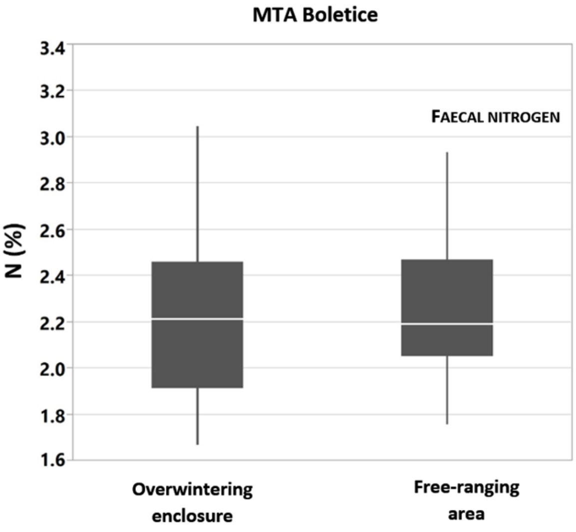


Figure 15. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal acid detergent fibre* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Hradiště.

Turning now to the MTA Boletice, the amounts of FN showed a different trend in comparison to MTA Hradiště. The FN levels in the MTA Boletice were similar in the overwintering enclosure and in neighbouring free-ranging area (Wilcox. t.: Z =-0.955; p<0.3394, Figure 16). Similarly, the concentrations of FADF and FNDF were comparable in the overwintering enclosure and outside it (*ADF*: Wilcox. t.: Z=-0.544; p<0.5861, Figure 17; *NDF*: Wilcox. t.: Z=-0.8928; p<0.3720, Figure 18).



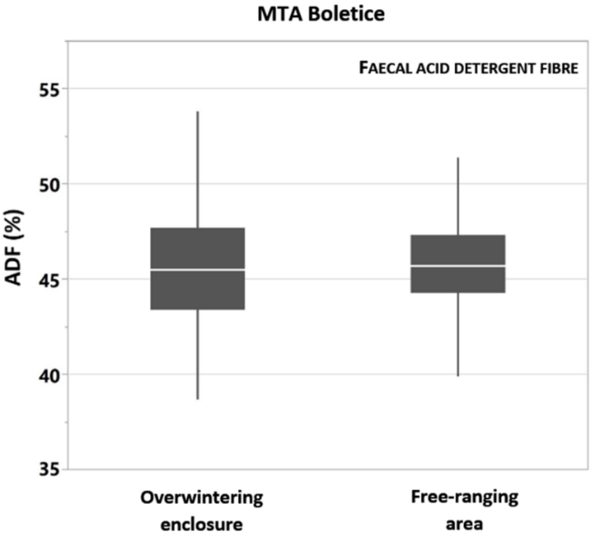


Figure 16. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal nitrogen* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Boletice.

Figure 17. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal acid detergent fibre* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Boletice.

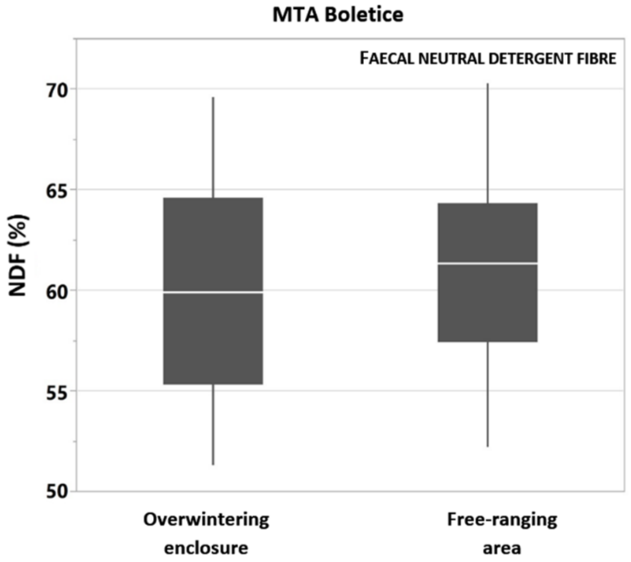


Figure 18. Box and whisker plots showing the median, minimum, maximum, upper and lower quartile for the concentrations of *faecal neutral detergent fibre* in red deer faeces collected in the overwintering fenced enclosures and neighbouring free-ranging areas in the MTA Boletice.

The observed differences in concentrations of faecal indices at individual study areas are most probably related to animal densities. The density of animals was significantly higher in the overwintering enclosure in the MTA Hradiště (i.e. animal density ranged from 22 to 24 individuals/1 ha) in comparison to the enclosure in the MTA Boletice, where the animal density was lower (i.e. 4 to 5 individuals/1 ha). Therefore, at high densities, there may be less plant species of higher quality for red deer as a consequence of over-browsing, thus favouring the growth of woody plant species which are less palatable for red deer (Suzuki et al., 2008). Such plant species have higher concentrations of tannins which are able to bind to plant proteins in the digestive tract of ruminants and thus reduce the levels of digestible protein and increase the excretion of FN (Palo and Robbins, 1991; Robbins, 1993). This could explain the higher FN values observed in the overwintering enclosure in the MTA Hradiště. Similarly Carpio et al. (2015) have found the highest FN values in areas with the highest red deer densities and tannin concentrations in faeces. On the other hand, other studies focusing on ungulate diets have observed a negative relationship between FN and population density, for example Sams et al. (1998) for white-tailed deer (*Odocoileus virginianus*), and Asada and Ochiai (1999) for sika deer (*Cervus nippon*). These authors have argued that as population density increases, competition for high quality plant species is higher and consequently the high quality plant species are rapidly depleted and the consumed food is of lower quality resulting in decreased FN levels (Asada and Ochiai, 1999; Sams et al., 1998).

Another possible explanation for the observed trends in faecal indices in the MTA Hradiště could be the fact that the red deer in the overwintering enclosure were to a larger extent dependent on supplemental foods provided by hunters, which is of better nutritional value and more palatable than winter plant species available outside the enclosure. As reported by Carpio et al. (2015), a positive relationship was found between FN and dietary N in plants on hunting estates with a supplemental food supply, whereas no relationship was observed in the absence of supplementary feeding.

The amounts of variation in faecal indices of red deer food quality showed interesting patterns in the individual study areas. In the MTA Hradiště, the amount of variation was higher in the overwintering enclosure compared to the free-ranging area for all studied faecal indices. In the MTA Boletice, on the other hand, the amounts of variation were comparable between the overwintering enclosure and outside it (Table 11).

Table 11. Coefficients of variation (%) for faecal indices of food quality for red deer in overwintering enclosures and neighbouring free-ranging areas in MTA Hradiště and MTA Boletice.

	MTA HRADIŠTĚ		MTA BOLETICE	
	ENCLOSURE	FREE-RANGING AREA	ENCLOSURE	FREE-RANGING AREA
FN	16	10	14	14
FADF	12	9	7	6
FNDF	10	6	8	7

Coefficient of variation (%) was estimated as the ratio of the standard deviation to the mean.

The different degrees of variation in food quality observed in this study may be again attributed to the differences in animal density. Increased population densities may lead to intensified competition for food resources and thus only highly socially ranked individuals are more successful at obtaining foods of high quality (Clutton-

Brock and Albon, 1985; Putman and Staines, 2004). The higher amounts of variation in food quality observed in the enclosure in the MTA Hradiště could hence be attributed to the differential access to high quality foods related to social rank (e.g. Appleby, 1980; Thouless, 1990). In the MTA Boletice, on the other hand, the amounts of variation were similar in the enclosure and outside it, most probably due to adequate population densities in the area and not intense competition for food resources.

To sum up, the results obtained suggest that the red deer food is of different quality and variability in the overwintering enclosure and neighbouring free-ranging area most probably due to high population density. Therefore, it is necessary to keep animals in overwintering enclosures at moderate densities and to provide high quality forage to all individuals in order to balance nutrition of both the individuals inside and outside the enclosures. Moreover, the analyses confirmed the high potential of NIRS for analysing large numbers of samples necessary for monitoring purposes of red deer diets. However, further studies are needed in order to provide deeper knowledge on red deer food quality and its variability in space and time.

Conclusions and Management Implications

In order to design effective management measures, it is essential to know where animals are and why. Where animals are is mainly determined by the spatiotemporal distribution of natural and supplementary food resources. Why animals are there is due to particular foods that animals prefer or are easily encountered, even though they may be of poorer quality. Foods that animals feed on determine their long-term survival and reproduction, which is vital for population persistence of all management-targeted species. It is therefore important to get detailed information on what animals are eating at the places they are and how easy it is to quantify it.

This doctoral thesis targets this important question of how to effectively study the diets of managed ungulate species with the use of traditional (i.e. stomach content analysis) and novel methods (i.e. stable isotope analysis, near infrared reflectance spectroscopy). Identifying the diet composition and dependence of wild boar on food sources of human origin (*Chapter I*) serves as a baseline for management actions employed to reduce human-wild boar conflicts. The results obtained in this chapter showed that wild boar are highly dependent on food sources of human origin (i.e. agricultural crops and supplemental foods provided by hunters) throughout the year. Therefore, wildlife management agencies need to target feeding practices and design restrictive measures for supplementary feeding and baiting of wild boar in the Czech Republic (i.e. define maximal possible amount of food and exact time periods for supplementary feeding, reduce non-target species at feeding sites). Nevertheless, more studies are needed in order to elucidate the possible effects of supplementary feeding and baiting practices on wild boar morphology, physiology and reproduction status. Considering the ongoing increase in wild boar numbers, the results obtained within this chapter are highly relevant in the context of emerging African swine fever and re-emerging tuberculosis among wild boar in Europe.

Trophic discrimination factors of carbon and nitrogen for hair tissues of wild boar and red deer determined in the experiments provide a stepping-stone for future studies interpreting isotopic patterns and applying stable isotope analyses on free-ranging individuals. Determining sources of variation (such as sex and age) in isotopic discrimination will help to understand why nitrogen and carbon discrimination factors vary and thus can help to predict their values (*Chapters II and III*). The results of the experiment conducted on red deer indicate that several sources of variation could co-occur in the same population. The results obtained provide a framework for quantifying multiple sources of variation in carbon and nitrogen isotope discrimination. The values of discrimination factors determined in these chapters are the first ever reported for wild boar and red deer. They can now be used in estimations of problems such as crop damage, especially in areas of intense agriculture, or may help to determine the contribution of supplementary feeding and baiting to the bulk diet of these two ungulate species. Determining the degree of dependence on supplemental feed for wild boar and red deer can improve the cost-effectiveness of ongoing management strategies as well as evaluate the consequences of modification of these management practice on animals, vegetation and interactions with other species. However, further experimental studies are needed in order to gain a deeper understanding of the processes underlying the variation in carbon and nitrogen isotope discrimination.

Developing the robust calibration models for nitrogen and fibre fractions in faeces (i.e. important food quality indices) enables an easy application of near infrared reflectance spectroscopy in determining the potential future consequences on population performance and persistence of red deer. The developed calibration models can now be used to predict nitrogen, acid detergent fibre, and neutral detergent fibre in a large number of samples and with minimal costs. According to the results obtained, winter food quality and its variability in overwintering enclosures and outside them appear to be affected by animal population density (*Chapter IV*) which needs to be taken into account by wildlife managers and hunters.

In addition, the results of this study may have implications in further studies where dietary analyses can be combined with movement studies to infer spatiotemporal movement patterns and resource selection simultaneously. The results of this thesis contribute towards a better understanding of feeding ecology of two important ungulate species in the Czech Republic, i.e. wild boar and red deer as well as provide a baseline for both further experimental studies as well as field investigations based on stable isotope analysis and near infrared reflectance spectroscopy. The results obtained will contribute towards efficient management of wild boar and red deer in the Czech Republic.

References

Alipayo, D., Valdez, R., Holechek, J.L., Cardenas, M., 1992. Evaluation of microhistological analysis for determining ruminant diet botanical composition. J. Range. Manage. 45 (2), 148-152.

Ambrose, S.H., 1991. Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. J. Archaeol. Sci. 18, 293-317.

Amici, A., Serrani, F., Rossi, C.M., Primi, R., 2012. Increase in crop damage caused by wild boar (*Sus scrofa* L.): the “refuge effect”. Agron. Sustainable Dev. 32, 683-692.

AOAC, 1984. Official methods of analysis. Association of Official Agricultural Chemists, Washington.

Appleby, M.C., 1980. Social rank and food access in red deer stags. Behaviour 74, 294-309.

Apollonio, M., Andersen, R., Putman R., (Eds). 2010. European Ungulates and Their Management in the 21st Century. Cambridge University Press, Cambridge, UK.

Arentson, R.A., Zimmerman, D.R., 1995. True digestibility of amino acids and protein in pigs with 13C as a label to determine endogenous amino acid excretion. J. Anim. Sci. 73, 1077-1085.

Asada, M., Ochiai, K., 1999. Nitrogen content in feces and the diet of Sika deer on the Bose Peninsula, central Japan. Ecol. Res. 14, 249-253.

Awkerman, J.A., Hobson, K.A., Anderson, D.J., 2007. Isotopic (δ^{15}N and δ^{13}C) evidence for intersexual foraging differences and temporal variation in habitat use in waved albatrosses. Can. J. Zool. 85 (2), 273-279.

Ballari, S.A., Barrios-García, M.N., 2013. A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. Mammal Rev. 44, 124-134. doi: 10.1111/mam.12015

Barboza, P.S., Bowyer, R.T., 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. J. Mammal. 81, 473-489.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. Appl. Spectrosc. 43 (5), 772-772.

Bartoń, K., 2013. MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn. (accessed 23.09.2015).

Bartoš, L., Kotrba, R., Pintíř, J. 2010. Ungulates and their management in the Czech Republic, in: Apollonio, M., Andersen, R., Putman, R. (Eds), European Ungulates and Their Management in the 21st Century. Cambridge University Press, Cambridge, UK, pp. 243-262.

Bassham, J.A., Benson, A.A., Kay, L.D., Harris, A.Z., Wilson, A.T., Calvin, M., 1953. The path of carbon in photosynthesis XXI. The cyclic regeneration of carbon dioxide acceptor. J. Am. Chem. Soc. 76 (7), 1760-1770.

Baskin, L., Danell, K., 2003. Ecology of Ungulates: A handbook of Species in Eastern Europe and Northern and Central Asia. Springer-Verlag, Berlin.

Bateman, A.S., Kelly, S.D., 2007. Fertilizer nitrogen isotope signatures. Isotopes. Environ. Health. Stud. 43 (3), 237-247.

Baubet, E., Bonenfant, Ch., Brandt, S., 2004. Diet of the wild boar in the French Alps. Galemys 16, 101-113.

Beier, P., 1987. Sex differences in quality of white-tailed deer diets. J. Mammal. 68, 323-329.

Belovsky, G.E., 1981. Food plant selection by a generalist herbivore: the moose. Ecology 62 (4), 1020-1030.

Ben-David, M., Flaherty, E.A., 2012. Stable isotopes in mammalian research: a beginner’s guide. J. Mammal. 93 (2), 312-328.

Ben-David, M., Schell, D.M., 2012. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia 127, 180-184.

Bleier, N., Lehoczki, R., Újváry, D., Szemethy, L., Csányi, S., 2012. Relationships between wild ungulates density and crop damage in Hungary. Acta. Theriol. 57 (4), 351-359.

Bond, A., Diamond, A.W., 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol. Appl. 21, 1017-1023.

Borowik, T., Cornulier, T., Jędrzejewska, B., 2013. Environmental factors shaping ungulate abundances in Poland. Acta. Theriol. 58 (4), 403-413.

Bradfield, R.B., 1981. Effect of undernutrition upon hair growth, in: Orfanos, C.E., Montagna, W., Stuttgen, G. (Eds.), Hair Research: Status and Future Aspects. Springer-Verlag, Berlin, pp. 251-256.

Břečka, J., Honzík, I., Neubauer, K., 2001. Stroje pro sklizeň pícnin a obilnin. Česká zemědělská univerzita v Praze, Praha.

Briedermann, L., 1970. Zum körper und organwaschstum des wildchweines in der Deutschen. Demokrtischen Republik. Arch. Forstwes. 19, 401-420.

Brookshire, E.N.J., Kauffman, B.J., Lytjen, D., Otting, N., 2002. Cumulative effects of wild ungulate and livestock herbivory on Riparian Willows. Oecologia 132 (4), 559-566.

Bugalho, M.N., Mayes, R.W., Milne, J.A., 2002. The effects of feeding selectivity on the estimation of diet composition using the n-alkane technique. Grass. Forage. Sci. 57 (3), 224-231.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd ed. Springer-Verlag, New York.

Calenge, C., Maillard, D., Fournier, P., Fouque, C., 2004. Efficiency of spreading maize in the garrigues to reduce wild boar (*Sus scrofa*) damage to Mediterranean vineyards. Eur. J. Wildl. Res. 50 (3), 112-120.

Carpio, A.J., Guerrero-Casado, J., Tortosa, F.S., Vicente, J., 2014a. Predation of simulated red-legged partridge nests in big game estates from South Central Spain. Eur. J. Wildl. 60 (2), 391-394. Res. doi: 10.1007/s10344-013 -0786-8.

Carpio, A.J., Castro–López, J., Guerrero–Casado, J., Ruiz–Aizpurua, L., Vicente, J., Tortosa, F.S., 2014b. Effect of wild ungulate density on invertebrates in a Mediterranean ecosystem. Anim. Biodivers. Conserv. 37, 115-125.

Carpio, A.J., Guerrero-Casado, J., Ruiz-Aizpurua, L., Tortosa F.S., Vicente, J., 2015. Interpreting faecal nitrogen as a non-invasive indicator of diet quality and body condition in contexts of high ungulate density. Eur. J. Wild. Res. 61, 557-562.

Caut, S., Angulo, E., Courchamp F., 2008a. Discrimination factors (Δ^{15}N and Δ^{13}C) in an omnivorous consumer: effect of diet isotopic ratio. Funct. Ecol. 22, 255-263.

Caut, S., Angulo, E., Courchamp, F., 2008b. Caution on isotopic model use for analyses of consumer diet. Can. J. Zool. 86 (5), 438-445.

Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443-453.

Cellina, S., 2008. Effects of supplemental feeding on the body condition and reproductive state of wild boar Sus scrofa in Luxembourg. PhD thesis, University of Sussex, Susex.

Cen, H., He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends. Food. Sci. Technol. 18, 72-83.

Cerling, T.E., Viehl, K., 2004. Seasonal diet changes of the forest hog (*Hylochoerus meinertzhageni* Thomas) based on the carbon isotopic composition of hair. Afr. J. Ecol. 42, 88-92.

Cerling, T.E., Wittemyer, G., Rasmussen, H.B., Vollrath, F., Cerling, C.E., Robinson, T.J., Douglas-Hamilton, I., 2006. Stable isotopes in elephant hair documents migration patterns and diet changes. Proc. Natl. Acad. Sci. USA. 103, 371-373.

Červený, J., Kamler, J., Kholová, H., Koubek, P., Martínková, N. 2013. Ottova encyklopedie Myslivost. Ottovo nakladatelství, Praha.

Cherel, Y., Hobson, K.A., Bailleul, F.R., Groscolas, R., 2005. Nutrition, physiology, and stable isotopes: New information from fasting and molting penguins. Ecology 86, 2881-2888.

Chytrý, M., 2013. Vegetation of the Czech Republic. Forest and scrub vegetation. Academia, Praha.

Clutton-Brock, T.H., Guinness, F.E., Albon, S.D., 1982. Red Deer: Behavior and Ecology of Two Sexes, University of Chicago Press.

Clutton-Brock, T.H., Albon, S.D., 1985. Competition and population regulation in social mammals, in: Sibly, R.M., Smith, R.H. (Eds.), Behavioural ecology. Oxford Blackwell Scientific, Oxford, pp. 557-576.

Codron, D., Codron, J., Sponheimer, M., Bernasconi, S.M., Clauss, M., 2011. When animals are not quite what they eat: diet digestibility influences ^{13}C-incorporation rates and apparent discrimination in a mixed-feeding herbivore. Can. J. Zool. 89, 453-465.

Côté, S.D., Rooney, T.P., Tremblay, J.P., Dussault, Ch., Waller, D.M., 2004. Ecological Impacts of Deer Overabundance. Annu. Rev. Ecol. Evol. S. 35, 113-147.

Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, M.E., Hobbie, E.A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo, L.H., Peñuelas, J., Reich, P.B., Schuur, E.A.G., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M., Wright, I.J., 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New. Phytol. 183 (4), 980-992.

Crawley, M.J., 1997. Plant Ecology. Second Edition. Blackwell Science, Oxford.

Crawley, M.J., 2007. The R book. John Wiley & Sons, Sussex.

Darr, R.L., Hewitt, D.G., 2008. Stable Isotope Trophic Shifts in White-Tailed Deer. J. Wildl. Manag. 72, 1525-1531.

Dardaillon, M., 1986. Seasonal variations in habitat selection and spatial distribution of wild boar (*Sus scrofa*) in the Camargue, southern France. Behav. Process. 13, 251-268. doi: 10.1016/0376-6357(86)90088-4

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42, 495-506.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45, 341-351.

Didion, M., Kupferschmid, A.D., Bugmann, H., 2009. Long-term effects of ungulate browsing on forest composition and structure. For. Ecol. Manage. 258S, S44-S55.

Dixon, R., Coates, D., 2009. Review: Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near. Infrared. Spectrosc. 17, 1-31.

Donohue, R.N., 2010. Aggressive behavior at supplemental feed sites and the effects of population density and supplemental feeding on annual survival and rate of population change in white-tailed deer. Texas A&M University, Kingsville.

Estell, R.E., 2010. Coping with shrub secondary metabolites by ruminants. Small Ruminant Res. 94, 1-9.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 40, 503-537.

Fernanda Cuevas, M., Ojeda, R.A., Dacar, M.A., Jaksic, F.M., 2013. Seasonal variation in feeding habits and diet selection by wild boars in a semi-arid environment of Argentina. Acta Theriol. 58 (1), 63-72.

Foley, W.J., McIlwee, A., Lawler, I., Aragones, L., Woolnough, A.P., Berding, N., 1998. Ecological applications of near infrared reflectance spectroscopy-a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. Oecologia 116 (3), 293-305.

Fournier-Chambrillon, C., Maillard, D., Fournier, P., 1995. Diet of the wild boar (*Sus scrofa* L.) inhabiting the Montpellier garrigue. J. Mountain Ecol. 3, 174-179.

Forsyth, D.M., Richardson, S.J., Menchenton, K., 2005. Foliar fibre predicts diet selection by invasive Red Deer *Cervus elaphus scoticus* in a temperate New Zeland forest. Funct. Ecol. 19, 495-504.

Frank, D.A., Groffman, P.M., Evans, R.D., Tracy, B.F., 2000. Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. Oecologia 123, 116-121.

Fry, B., 2006. Stable isotope ecology. Springer, New York.

Gebert, C., Verheyden-Tixier, H., 2001. Variations of diet composition of Red Deer (*Cervus elaphus* L.) in Europe. Mammal. Rev. 31, 3-4.

Giménez-Anaya, A., Herrero, J., Rosell, C., Couto, S., García-Serrano, A., 2008. Food habits of wild boars (*Sus scrofa*) in a Mediterranean coastal wetland. Wetlands 28 (1), 197-203.

Giuffra, E., Kijas, J.M.H., Amarger, V., Carlborg, O., Jeon, J.T., Andersson, L., 2000. The origin of the domestic pig: Independent domestication and subsequent introgression. Genetics 154, 1785-1791.

Gortázar, C., Herrero, J., Villafuerte, R., Marco, J., 2000. Historical examination of the status of large mammals in Aragon, Spain. Mammalia 64, 411-422.

Gortázar, C., Ferroglio, E., Höfle, U., Frölich, K., Vicente, J., 2007. Diseases shared between wildlife and livestock: a European perspective. Evol. Ecol. Res. 53, 241–256. doi: 10.1007/s10344-007-0098-y

Groot Bruinderink, G.W.T.A., Hazebroek, E., van der Voot, H., 1994. Diet and condition of wild boar, *Sus scrofa scrofa*, without supplementary feeding. J. Zool. 233 (4), 631-648.

Groot Bruinderink, G.W.T.A., Hazebroek, E., 1996a. Ungulate traffic collisions in Europe. Conserv. Biol. 10, 1059-1067.

Groot Bruinderink, G.W.T.A., Hazebroek, E., 1996b. Wild boar (*Sus scrofa scrofa* L.) rooting and forest regeneration on podzolic soils in the Netherlands. For. Ecol. Manage. 88, 71–80. doi:10.1016/S0378-1127(96)03811-X

Habran, S., Debier C., Crocker, D.E., Houser, D.S., Lepoint G., Bouquegneau, J.-M., Das, K., 2010. Assessment of gestation, lactation and fasting on stable isotope ratios in northern elephant seals (*Mirounga angustirostris*). Mar. Mammal Sci. 26 (4), 880-895.

Hacker, J.B., Minson, D.J., 1981. The digestibility of plant parts. Herbage Abst. 51, 459-482.

Hafeez, S., Abbas, M., Khan, Z., Rehman, E., 2011. Preliminary analysis of the diet of wild boar (*Sus scrofa* L., 1758) in Islamabad, Pakistan. Turk. J. Zool. 35 (1), 115-118.

Hahn, N., Eisfeld, D., 1998. Diet and habitat use of wild boar (*Sus scrofa*) in SW-Germany. Gibier Faune Sauvage/Game and Wildlife 15, 595-606.

Hanley, T.A., McKendrick, J.D., 1985. Potential nutritional limitations for black-tailed deer in a spruce-hemlock forest, southeastern Alaska. J. Wildl. Manage. 49, 103-114.

Hatch, M.D., Slack, C.R., Johnson, H.S., 1967. Further studies on a new pathway of photosynthetic CO2 fixation in sugarcane and its occurrence in other plant species. J. Biochem. 102 (2), 417-422.

Hemmer, H., 1990. Domestication: The Decline of Environmental Appreciation. Cambridge University Press, Cambridge.

Herrero, J., García-Serrano, A., Couto, S., Ortuño, V.M., García-González, R., 2006. Diet of wild boar *Sus scrofa* L. and crop damage in an intensive agroecosystem. Eur. J. Wildl. Res. 52, 245-250.

Herrero, J., García-Serrano, A., García-Gonzáles, R., 2008. Reproductive and demographic parameters in two Iberian wild boar *Sus scrofa* populations. Acta Theriol. 53 (4), 355-364.

Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K., Servheen, C., 1996. Use of stable isotopes to determine diets of living and extinct bears. Can. J. Zool. 74 (11), 2080-2088.

Hobbs, N.T., 1987. Fecal indices to dietary quality: a critique. J. Wildl. Manage. 51, 317-320.

Hobbs, N.T., 1996. Modification of ecosystems by ungulates. J. Wildl. Manage. 60, 695-713.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stables isotopes: a review. Oecologia 120, 314-326.

Hobson, K.A., Quirk, T.W., 2014. Effect of age and ration on diet-tissue isotopic (Δ^{13}C, Δ^{15}N) discrimination in striped skunks (*Mephitis mephitis*). Isotopes Environ. Health Stud. 50 (3) 300-306.

Hodgman, T.P., Davitt, B.B., Nelson, J.R., 1996. Monitoring mule deer diet quality and intake with fecal indices. J. Range Manage. 49 (3), 215-222.

Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443-457.

Holá, M., 2012. Spatial and Temporal Variation in the Quality of Summer Foods for Herbivores along a Latitudinal Gradient. MSc. Thesis, Faculty of Forestry, Dept. of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden, 33 pp.

Holá, M., Ježek, M., Kušta, T., Košatová, M., 2015. Trophic Discrimination Factors of Stable Carbon and Nitrogen Isotopes in Hair of Corn Fed Wild Boar. PLoS ONE 10 (4), e0125042. doi:10.1371/journal.pone.0125042.

Holechek, J.L., Vavra, M., Pieper, R.D., 1982a. Botanical composition determination of range herbivore diets-a review. J. Range Manage. 35 (3), 309-315.

Holechek, J.L., Vavra, M., Arthun, D., 1982b. Relationships between performance, intake, diet nutritive quality and fecal nutritive quality of cattle on mountain range. J. Range Manage. 35 (6), 741-744.

Iacumin, P., Davanzo, S., Nikolaev, V., 2005. Short-term climatic changes recorded by mammoth hair in the arctic environment. Palaeogeogr. Palaeoclimatol. Palaeoecol. 218, 317-324.

Iason, G., 2005. The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. Proc. Nutr. Soc. 64, 123-131.

Johnson, E., 1977. Environmental effects on the hair follicle, in: Jarrett, A. (Ed.), Physiology and Pathophysiology of the Skin. Academic Press, London, pp. 1237-1249.

Jenks, Jr., J.A., Leslie, D.M., Lochmiller R.L., Melchiors M.A., 1994. Variation in gastrointestinal characteristics of male and female white-tailed deer: implications for resource partitioning. J. Mammal. 75, 1045-1053.

Jones, R.J., Ludlow, M.M., Troughton, J.H., Blunt, C.G., 1981. Changes in the natural carbon isotope ratios of the hair from steers fed diets of C4, C3 and C4 species in sequence. Search 12, 85-87.

Jones, G.F., 1998. Genetic aspects of domestication, common breeds and their origin, in: Rothschild, M.F., Ruvinsky, A. (Eds.), The Genetics of the Pig. CAB International, Wallingford, pp. 17-50.

Kaczensky, P., Chapron, G., von Arx, M., Huber, D., Andrén, H., Linnell, J., (Eds). 2013. Status, management and distribution of large carnivores - bear, lynx, wolf & wolverine - in Europe. A Large Carnivore Initiative for Europe. Report Prepared for the European Commission. Brussels.

Kamler, J., Homolka, M., Čižmár, D., 2004. Suitability of NIRS analysis for estimating diet quality of free-living red deer *Cervus elaphus* and roe deer *Capreolus capreolus*. Wild. Biol. 10, 235-240.

Keuling, O., 2009. Managing wild boar - Considerations for wild boar management based on game biology data. PhD Thesis, Universität für Bodenkultur, Wien, 91 pp.

Krojerová-Prokešová, J., Barančeková, M., Šustr, P., Heurich, M., 2010. Feeding patterns of red deer *Cervus elaphus* along an altitudinal gradient in the Bohemian Forest: effect of habitat and season. Wildl. Biol. 16 (2), 173-184.

Kuijper, D.P.J., 2011. Lack of natural control mechanisms increases wildlife-forestry conflict in managed temperate European forest systems. Eur. J. Forest. Res. 130, 895-909.

Kurle, C.M., Worthy, G.A., 2001. Stable isotope assessment of temporal and geographic differences in feeding ecology of northern fur seals (*Callorhinus ursinus*) and their prey. Oecologia 126, 254-265.

Kurle, C.M., Koch, P.L., Tershy, B.R., Croll, D.A., 2014. The effects of sex, tissue type, and dietary components on stable isotope discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) in mammalian omnivores. Isotopes. Environ. Health. Stud. 50, 307-321.

Kušta, T., Keken, Z., Ježek, M., Kůta, Z., 2015. Effectiveness and costs of odor repellents in wildlife-vehicle collisions: A case study in Central Bohemia, Czech Republic. Transport. Res. D-TR. E. 38, 1-5. doi:10.1016/j.trd.2015.04.017

Lecomte, N. Ahlstrøm, Ø., Ehrich, D., Fuglei, E., Ims, R.A., Yoccoz, N.G., 2011. Intrapopulation Variability Shaping Isotope Discrimination and Turnover: Experimental Evidence in Arctic Foxes. PLoS ONE 6 (6), e21357. doi:10.1371/journal.pone.0021357.

Leránoz, I., 1983. Sobre la relación del jabalí (*Sus scrofa* L.) con la agricultura, en Navarraseptentrional. XV Congreso Internacional de Fauna Cinegética y Silvestre, Trujillo, España: 639-645.

Leslie, D.M., Starkey, E.E., 1985. Fecal indices to dietary quality of cervids in old-growth forests. J. Wildl. Manage. 49, 142-146.

Leslie, D.M., Bowyer, R.T., Jenks, J.A., 2008. Facts From Feces: Nitrogen Still Measures Up as a Nutritional Index for Mammalian Herbivores. J. Wildl. Manage. 72 (6), 1420-1433.

Litvaitis, J.A., 2000. Investigating Food Habits of Terrestrial Vertebrates, in: Boitani, L., Fuller, T.K. (Eds.), Research Techniques in Animal Ecology: Controversies and Consequences. New York Columbia University Press, pp. 165-190.

Lynfield, Y.L., Macwilliams, P., 1970. Shaving and hair growth. J. Invest. Dermatol. 55, 170-172.

MacAvoy, S.E., Arneson, L.S., Bassett, E., 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia 150, 190-201.

MACR, 2013. Ministry of Agriculture of the Czech Republic. Hunting statistics. http://eagri.cz/public/web/mze/lesy/myslivost/statistika (accessed 19.10.2015).

Martin, C., Pastoret, P.-P., Brochier, B., Humblet, M.F., Saegerman, C., 2011. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. Vet. Res. 42, 70. doi: 10.1186/1297-9716-42-70.

Martínez del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. 84 (1), 91-111.

Martens, H., Naes, T., 1991. Multivariate Calibration. Wiley, Chichester.

Massei, G., Genov, P.V., 2004. The environmental impact of wild boar. Galemys 16, 135-145.

Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. 11, 119-161.

McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102 (2), 378-390.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Technol. 106, 3-19.

Mobæk, R., Mysterud, A., Loe, E., Holand, Ø., Gunnar, A., 2009. Density dependent and temporal variability in habitat selection by a large herbivore; an experimental approach. Oikos 118 (2), 209-218.

Monteith, K.B., Monteith, K.L., Bowyer, R.T., Leslie, D.M., Jenks, J.A., 2014. Reproductive effects on fecal nitrogen as an index of diet quality: an experimental assessment. J. Mammal. 95 (2), 301-310.

Mohn, M.P., 1958. The effects of different hormonal states on the growth of hair in rats, in: Montagna, W., Ellis, R.A. (Eds.), The Biology of Hair Growth. Academic Press, New York, pp. 336-398.

Mould, E.D., Robbins, C.T., 1981. Nitrogen metabolism in elk. J. Wildl. Manage. 45, 323-334.

Moore, J.W., Semmens, B.X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 11, 470-480.

Murray, P.J., Witt, G.B., Moll, E.J., 1997. Wool, isotopes and diet selection in grazing sheep. Recent Advances in Animal Nutrition in Australia. 14, 253.

Mysterud, A., Stenseth, N.Ch., Yoccoz, N.G., Langvatn, R., Steinheim, G., 2001. Nonlinear effects of large-scale climatic variability on wild and domestic herbivores. Nature 410, 1096-1099.

Mysterud, A., 2010. Still walking on the wild side? Management actions as steps towards ‘semi-domestication’ of hunted ungulates. J. Appl. Ecol. 47 (4), 920-925.

Nardoto, G.B., Barboza de Godoy P., Sansigolo de Barros Ferraz, E., Balbaud Ometto, J.P.H., Martinelli, L.A., 2006. Stable carbon and nitrogen isotopic fractionation between diet and swine tissues. Sci. Agric. 63 (6), 579-582.

Nurmi, K., Ossipov, V., Haukioja, E., Pihlaja, K., 1996. Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (*Betula pubescens* ssp. *turtuosa*). J. Chem. Ecol. 22 (11), 2023-2040.

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. BioScience 38, 328-336.

Osborn, R.G., Ginnett, T.F., 2001. Fecal nitrogen and 2,6-diaminopimelic acid as indices to dietary nitrogen in white-tailed deer. Wildl. Soc. B. 29, 1131-1139.

Palmer, S.C.F., Mitchell, R.J., Truscott, A.M., Welch, D., 2004. Regeneration failure in Atlantic oakwoods: the roles of ungulate grazing and invertebrates. Forest Ecol. Manag. 192, 251-265.

Palo, R.T., Sunnerheim, K., Theander, O., 1985. Seasonal variations of phenols, crude protein and cell wall content in birch (*Betula pendula* Roth.) in relation to ruminant in vitro digestibility. Oecologia 65, 314-318.

Palo, R.T., Robbins, C.T., (Ed.) 1991. Plant Defenses Against Mammalian Herbivory. CRC Press. Inc. Boca Raton, Florida.

Parker, K.L., Barboza, P.S., Stephenson, T.R., 2005. Protein conservation in female caribou (*Rangifer tarandus*): effects of decreasing diet quality during winter. J. Mammal. 86 (3), 610-622.

Parker, K.L., Barboza, P.S., Gillingham, M.P., 2009. Nutrition integrates environmental responses of ungulates. Funct. Ecol. 23, 57-69.

Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variations. PLoS ONE 5 (3), e9672. doi:10.1371/journal.pone.0009672.

Peek, J.M., Schmidt, K.T., Dorrance, M.J., Smith, B.L., 2002. Supplemental feeding and farming of elk, in: Toweill, D.E., Thomas, J.W. (Eds.), Elk of North America: Ecology and Management, 2nd ed. Smithsonian Institute Press, Washington, USA, pp. 614-647.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293-320.

Pettorelli, N., Dray, S., Gaillard, J.-M., Chessel, D., Duncan, P., Illius, A., Guillon, N., Klein, F., Van Laere, G., 2003. Spatial variation in springtime food resources influences the winter body mass of roe deer fawns. Oecologia 137, 363-369.

Phillips, D.L., 2012. Converting isotope values to diet composition: the use of mixing models. J. Mammal. 93, 342-352.

Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261-269.

Pinheiro, J., Bates, D., 2000. Mixed-effects models in S and S-PLUS. Springer, New York.

Popa-Lisseanu, A.G., Kramer-Schadt, S., Quetglas, J., Delgado-Huertas, A., Kelm, D.H., Ibáñez, C., 2015. Seasonal Variation in Stable Carbon and Nitrogen

Isotope Values of Bats Reflect Environmental Baselines. PLoS ONE 10 (2), e0117052. doi:10.1371/journal.pone.0117052.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703-718.

Prins, H.H.T., Van Langevelde, F., 2008. Resource Ecology: Spatial and Temporal Dynamics of Foraging. Springer, Dordrecht.

Putman, R., Apollonio, M., (Eds). 2014. Behaviour and management of European ungulates. Whittles Publishing, Dunbeath.

Putman, R.J., Staines, B.W., 2004. Supplementary winter feeding of wild red deer *Cervus elaphus* in Europe and North America: justifications, feeding practice an effectiveness. Mammal Rev. 34, 285-306.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Mautz, W.W., 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68 (1), 98-107.

Robbins, C.T., 1993. Wildlife Feeding and Nutrition. Second Edition. Academic Press, San Diego.

Robbins, C.T., Felicetti, L., Florin, S., 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia 162, 571-579.

Rothman, J.M., Chapman, C.A., Hansen, J.L., Cherney, D.J.R., Pell, A.N., 2009. Rapid Assessment of the Nutritional Value of Foods Eaten by Mountain Gorillas: Applying Near-Infrared Reflectance Spectroscopy to Primatology. Int. J. Primatol. 30, 729-742.

Sahlsten, J., Bunnefeld, N., Månsson, J., Ericsson, G., Bergström, R., Dettki, H., 2010. Can supplementary feeding be used to redistribute moose *Alces alces*? Wildl. Biol. 16 (1), 85-92.

Sams, M.G., Lochmiller, R.L., Qualls, C.W., Leslie, D.M., 1998. Sensitivity of condition indices to changing density in a white-tailed deer population. J. Wildl. Dis. 34, 110-125.

Schley, L., Roper, T.J., 2003. Diet of wild boar *Sus scrofa* in Western Europe, with particular reference to consumption of agricultural crops. Mammal Rev. 33, 43-56.

Schneider, B.H., 1935. The subdivision of the metabolic nitrogen in the faeces of the rat, swine and man. J. Biol. Chem. 109, 249-278.

Schwertl, M., Auerswald, K., Schnyder, N., 2003. Reconstruction of the isotopic history of animal diets by hair segmental analysis. Rapid Commun Mass. Spectrom. 17 (12), 1312-1318.

Semmens, B.X., Ward, E.J., Moore, J.W., Darimont, C.T., 2009. Quantifying inter- and intra-population niche variability using hierarchical bayesian stable isotope mixing models. PLoS ONE 4, e6187.

Shenk, J.S., Westerhaus, M.O., 1991. New standardization and calibration procedures for near infrared reflectance spectroscopy. Crop Sci. 31, 469-474.

Shenk, J.S., Westerhaus, M.O., 1993. Near infrared reflectance analysis with single and multiproduct calibrations. Crop Sci. 33, 582-584.

Shepherd, K.D., Walsh, M.G., 2007. Infrared spectroscopy: Enabling an evidence-based diagnostic surveillance approach to agricultural and environmental management in developing countries. J. Near Infrared. Spectrosc. 15, 1-19.

Showers, S.E., Tolleson, D.R., Stuth, J.W., Kroll, J.C., Koerth, B.H., 2006. Predicting diet quality of white-tailed deer via NIRS fecal profiling. Rangeland Ecol. Manag. 59 (3), 300-307.

Singh, N.J., Grachev, I.A., Bekenov, A.B., Milner-Gulland, E.J., 2010. Tracking greenery in central Asia-the migration of the saiga antelope. Divers. Distrib. 16 (4), 663-675.

Škorpík, M., Andrejkovič, T., Jurmanová, E., Korecký, J., Krejčí, J., Lazárek, P., Padělková, L., Reška, M., Rothöckl, T., Růžička, M., Vančura, P., Vítek, P., 1993. Plán péče o národní park Podyjí a jeho ochranné pásmo. Správa Národního parku Podyjí. Znojmo.

Sponheimer, M., Robinson, T., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D., Ehleringer, J., 2003a. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. Can. J. Zool. 81 (5), 871-876.

Sponheimer, M., Robinson, T., Ayliffe, L., Roeder, B., Hammer, J., Passey, B., West, A., Cerling, T., Dearing, D., Ehleringer, J., 2003b. Nitrogen isotopes in mammalian herbivores: hair δ^{15}N values from a controlled feeding study. Int. J. Osteoarchaeol. 13, 80-87.

Stuth, J., Jama, A., Tolleson, D., 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crop. Res. 84, 45-56.

Šumava NP, 2015. Vegetation of the Šumava National Park. http://www.npsumava.cz/en/3277/sekce/flora/ (accessed 23.10.2015).

Suzuki, M., Miyashita, T., Kabaya, H., Ochiai, K., Asada, M., Tange, T., 2008. Deer density affects ground-layer vegetation differently in conifer plantations and hardwood forests on the Boso Peninsula, Japan. Ecol. Res. 23,151-158.

Tellado, S., Orpez, R., Muñoz-Cobo, J., Azorit, C., 2015. Fecal-FT-NIRS as a Noninvasive Tool for Assessing Diet Quality of Mediterranean Deer. Rangeland Ecol. Manag. 68 (1), 92-99.

Thompson, A.K., Samuel, M.D., Van Deelen, T.R., 2008. Alternative Feeding Strategies and Potential Disease Transmission in Wisconsin White-Tailed Deer. J. Wild. Manag. 72 (2), 416-421.

Thouless, C.R., 1990. Feeding competition between grazing red deer hinds. Anim. Behav. 40, 105-111.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57, 32-37.

Tremblay, J.P., Hester, A., Mcleod, J., Huot, J., 2004. Choice and development of decision support tools for the sustainable management of deer-forest systems. For. Ecol. Manage. 191, 1-16.

Tyrrell, L.P., Newsome, S.D., Veins, M., Bowden, R., Fogel, M.L., Murray, M., 2013. Vibrissae growth rates and trophic discrimination factors in captive southern sea otters (*Enhydra lutris nereis*). J. Mammal. 94 (2), 331-338.

Valentini, A., Pompanon, F., Taberlet, P., 2009. DNA barcoding for ecologist. Trends Ecol. Evol. 24 (2), 110-117.

Van Beest, F.M., Mysterud, A., Loe, L.E., Milner J.M., 2010. Forage quantity, quality and depletion as scale-dependent mechanism driving habitat selection of a large browsing herbivore. J. Anim. Ecol. 79 (4), 910-922.

van Scott, E.J., Reinertson, R.P., Steinmuller, R., 1957. The growing hair roots of the human scalp and morphologic changes therein following amethopterin therapy. J. Invest. Dermatol. 29, 197-204.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell University Press, Ithaca, New York.

Van Soest P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74 (10), 3583-3597.

Verheyden-Tixier, H., Duncan, P., 2000. Selection for small amounts of hydrolysable tannins by a concentrate-selecting mammalian herbivore. J. Chem. Ecol. 26, 351-358.

Vojta, J., Kopecký, M., 2006. Vegetation of secondary forests and shrubs in Doupovské hory hills. Zprávy Čes. Bot. Společ., Praha 21, 209-225.

Walter, W.D., Kurle, C.M., Hopkins, J.B., 2014a. Applications of stable isotope analysis in mammalian ecology. Isotopes. Environ. Health. Stud. 50 (3), 287-290.

Walter, W.D., 2014b. Use of stable isotopes to identify dietary differences across subpopulations and sex for a free-ranging generalist herbivore. Isotopes. Environ. Health. Stud. 50 (3), 399-413.

Warinner, C, Tuross, N., 2009. Alkaline cooking and stable isotope diet-tissue discrimination in swine: archaeological implications. J. Archaeol. Sci. 36, 1690-1697.

Weiler, U., Appell, H.J., Kremser, M., Hofäcker, S., Claus, R., 1995. Consequences of Selection on Muscle Composition. A Comparative Study on Gracilis Muscle in Wild and Domestic Pigs. Anat. Histol. Embryol. 24, 77-80.

Weisberg, P.J., Bugmann, H., 2003. Forest dynamics and ungulate herbivory: from leaf to landscape. Forest Ecol. Manage. 181, 1-12.

West, A., Ayliffe, L., Cerling, T., Robinson, T.F., Karren, B., Dearing, M.D., Ehleringer, J.R., 2004. Short-term diet changes revealed using stable carbon isotopes in horse tail-hair. Funct. Ecol. 18, 616-624.

White, T.C.R., 1993. The inadequate environment. Nitrogen and the abundance of animals. Springer-Verlag, Berlin.

White, M.A., 2012. Long-term effects of deer browsing: Composition, structure and productivity in a northeastern Minnesota old-growth forest. Forest Ecol. Manage. 269, 222-228.

Wilcox, J.T., Van Vuren, D.H., 2009. Wild pigs as predators in oak woodlands of California. J. Mammal. 90, 114-118.

Zazzo, A., Harrison, S.M., Bahar, B., Moloney, A.P., Monahan, F.J., Scrimgeour, C.M., Schmidt, O., 2007. Experimental determination of dietary carbon turnover in bovine hair and hoof. Can. J. Zool. 85 (12), 1239-1248.

Zeman, J., Hrbek, J., Drimaj, J., Plhal, R., Kamler, J., Adamec, Z., Heroldová, M., 2016. Wild boar impact to the natural regeneration of oak and acorn importance in its diet. Acta Univ. Agric. et Silvic. Mendel. Brun. 64, 579-585.

Zimmerman, T.J., Jenks, J.A., Leslie, Jr., D.M., 2006. Gastrointestinal morphology of female white-tailed and mule deer: effects of fire, reproduction, and feeding type. J. Mammal. 87, 598-605.

Supplementary Material

Table S1. Frequency of occurrence (%) of all diet items identified in wild boar stomachs collected from hunted individuals in four study sites in the Czech Republic during 2010-2012.

DIET ITEM	LATIN NAME	SPRING	SUMMER	WINTER
Corn	*Zea mays*	35%	26%	48%
Oat	*Avena sativa*	1%	0%	12%
Wheat	*Triticum aestivum*	21%	41%	30%
Barley	*Hordeum vulgare*	15%	2%	15%
Cereals unspecified		4%	31%	17%
CEREALS TOTAL		**55%**	**59%**	**76%**
Rape	*Brassica napus*	4%	2%	3%
Sunflower	*Helianthus annuus*	3%	0%	5%
Potatos	*Solanum*	0%	2%	0%
Beet	*Beta vulgaris*	0%	0%	3%
OILSEEDS AND ROOT CROPS TOTAL		**7%**	**2%**	**8%**
Fresh grass		46%	50%	30%
Hay		0%	2%	6%
GRASSES TOTAL		**46%**	**52%**	**36%**
Nettle	*Urtica dioica*	1%	0%	1%
Dandelion	*Taraxacum officinale*	18%	7%	2%
Plantain	*Plantago lanceolata*	3%	2%	1%
Herbs unspecified		4%	4%	1%
HERBS TOTAL		**23%**	**11%**	**5%**
Roots		39%	41%	45%
Rhizomes		11%	6%	6%
ROOT AND RHIZOMES TOTAL		**44%**	**43%**	**47%**
Leaves of oak	*Quercus robur*	0%	4%	7%
Leaves of beech	*Fagus sylvatica*	0%	4%	1%
Leaves of aspen	*Populus tremula*	0%	4%	1%
Leaves of maple	*Acer platanoides*	1%	2%	0%
Leaves of birch	*Betula*	0%	0%	2%
Leaves od alder	*Alnus*	0%	2%	0%
Leaves of hornbeam	*Carpinus*	1%	0%	0%
LEAVES OF TREES TOTAL		**3%**	**15%**	**9%**

Table S1_Part 2

DIET ITEM	LATIN NAME	SPRING	SUMMER	WINTER
Seeds of Fireweed	*Epilobium angustifolium*	1%	0%	0%
Seeds of Fallopia	*Fallopia*	7%	0%	6%
Seeds of Goosefoots	*Chenopodium*	3%	2%	2%
Seeds of Bindweed	*Convolvulus*	3%	9%	9%
Seeds of Bistorta	*Bistorta*	0%	0%	2%
Seeds of Common flax	*Linum usitatissimum*	1%	0%	0%
Seeds of Madder	*Galium*	8%	11%	7%
Seeds of Campion	*Silene*	1%	2%	0%
Seeds of Amaranth	*Amaranthus*	1%	2%	2%
Seeds of Sorrels	*Rumex*	0%	4%	2%
Seeds of Wood violet	*Viola*	1%	0%	1%
SEEDS OF HERBS TOTAL		**14%**	**28%**	**15%**
Seeds of Dog-rose	*Rosa canina*	3%	0%	1%
Seeds of Hawthorn	*Crataegus*	0%	0%	0%
Seeds of Sea buckthorns	*Hippophae*	0%	2%	0%
Seeds of Ash	*Fraxinus*	1%	0%	0%
Seeds of hornbeam	*Carpinus*	7%	0%	3%
Seeds of beech	*Fagus sylvatica*	1%	7%	6%
Seeds of oak	*Quercus robur*	25%	17%	17%
Seeds of Walnut trees	*Juglans*	0%	0%	0%
SEEDS OF TREES TOTAL		**34%**	**22%**	**25%**
Fruits of European blueberry	*Vaccinium myrtillus*	1%	7%	1%
Fruits of European raspberry	*Rubus idaeus*	3%	4%	0%
Fruits of Apple tree	*Malus*	4%	17%	16%
Fruits of Pear tree	*Pyrus*	0%	6%	1%
Fruits of Wild cherry	*Prunus avium*	3%	17%	1%
Fruits of Egg plum	*Prunus domestica*	1%	9%	0%
Fruits of Grape vine	*Vitis vinifera*	0%	0%	0%
FRUITS TOTAL		**11%**	**44%**	**18%**
Red deer	*Cervus elaphus*	0%	0%	0%
Roe deer	*Capreolus capreolus*	4%	2%	3%
Wild boar	*Sus scrofa*	0%	0%	2%
Wild hare	*Lepus europeus*	4%	0%	1%
Bank vole	*Clethrionomys glareolus*	1%	9%	1%
Yellow-necked mouse	*Apodemus flavicollis*	1%	0%	2%
Common vole	*Microtus arvalis*	1%	2%	3%

Table S1_Part 3

DIET ITEM	LATIN NAME	SPRING	SUMMER	WINTER
Eurasian shrew	*Sorex araneus*	0%	2%	0%
European water vole	*Arvicola terrestris*	0%	2%	3%
MAMMALS TOTAL		**13%**	**17%**	**14%**
FISH TOTAL	***Osteichthyes***	**0%**	**0%**	**3%**
BIRDS TOTAL	***Aves***	**6%**	**6%**	**2%**
European common frog	*Rana temporaria*	1%	0%	0%
Slow worm	*Anguis fragilis*	1%	0%	0%
AMPHIBIANS TOTAL	***Amphibia***	**3%**	**0%**	**0%**
Ants	*Formicidae*	0%	2%	0%
Caterpillars		0%	0%	0%
Larvae		48%	19%	37%
Long-horned grasshoppers	*Ensifera*	0%	2%	0%
Reddish worm	*Lumbricus terrestris*	25%	17%	2%
Beetles	*Coleoptera*	28%	37%	6%
Spiders	*Araneae*	1%	0%	0%
Molluscs	*Mollusca*	14%	7%	4%
INVERTEBRATES TOTAL	***Invertebrata***	**42%**	**50%**	**39%**
MUSHROOMS TOTAL	**Fungi**	**13%**	**4%**	**14%**
OTHERS TOTAL		**25%**	**15%**	**43%**