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– A case study of the browser – grazer dichotomy

Heidi Rautiainen

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Abstract

Nutritional ecology implies the nutritional links between an animal and its environment. On an evolutionary time scale competition for food drives species formation by genetic adaptations to the environment and subsequent niche separation of species. On a short-term scale, animals have different strategies in order to meet their nutritional requirements, which ultimately influence their health and fitness. These strategies differ among and within species while the nutritional, chemical, and structural composition of the forage varies across seasons. As such, different individuals may select particular amounts or proportion they consume based on stomach anatomy, metabolic rate, body size, physical states and life history. Understanding adaptations to different forage is therefore an important part of our understanding of nutritional ecology of herbivores, especially in temperate climate where herbivores adapt both physiologically and behaviorally to seasonal variations in order to meet their nutritional requirements. The aim of this project was to investigate temporal variation in chemical composition of forage between and within two cervid species.

A total of 499 rumen samples collected from animals killed in the Koberg estate situated in southwestern Sweden were analyzed. Based on samples from fall, winter, spring and summer, the temporal variation of nutrient composition and interspecific differences between roe deer (*Capreolus capreolus L.*) and fallow deer (*Dama dama L.*) were investigated. The main results revealed that 1) the nutrient composition varied between species and across seasons so that roe deer selected higher proportion protein compared to fallow deer with highest proportions in spring 2) the nutrient composition varied across age and gender classes of fallow deer so that adult males selected lower proportion protein compared to female adults and juveniles in fall and 3) both species selected for a particular proportion of soluble carbohydrates, and that this proportion differed between gender and age classes within fallow deer so that adult females and subadult males selected for a particular proportion soluble carbohydrates compared to adult males. However, adult males selected for a stable proportion of protein in fall compared to adult females and subadult males. The results from this study can be used to understand inter- and intraspecific competition, to determine a correct carrying capacity of wildlife populations and to formulate balanced diets in order to avoid damages in agriculture and forestry.

Populärvetenskaplig sammanfattning

Näringsekologi omfattar sambandet mellan en organisms behov av föda och den omgivande miljön. Ur ett evolutionärt perspektiv är konkurrens om föda en viktig grund till artbildning via genetiska anpassningar och till miljö och föda, som i sin tur leder till att arterna kan sägas separeras i olika nischer – som en konsekvens av deras olika födointag. I ett kortare tidsperspektiv utvecklar det enskilda djuret dessutom olika strategier för att uppnå sitt födo- och energibehov, som in sin tur påverkar deras överlevnad och reproduktion. Ett viktigt mål inom näringsekologin som forskningsämne är därför att förstå vilka strategier djur har för att uppnå sina födobehov och hur det varierar mellan årstider. Syftet med detta arbete var därför att undersöka om olika evolutionära anpassningar av matsmältningssystemet leder till att det mindre rådjuret väljer en diet med mer protein, lösliga kolhydrater och mindre smältbara fibrer jämfört med den betydligt större dovhjorten. Utöver skillnader i födokvalité mellan arterna, så jämförs även skillnader mellan dovhjortar beroende på kön och ålder, där kalvar, hindar och unga handjur förväntades välja en högre foderkvalitet jämfört med de äldre hanarna, framförallt på grund av stora skillnader i kroppsstorlek.

I detta projekt analyserades totalt 499 vomprover tagna på rådjur och dovhjort skjutna under jakt, 2006-2013 på Koberg egendoms marker i Västergötland, under samtliga årstider. Samtliga prover analyserades med nära infraröd spektroskopi (NIRS) varav 140 analyserades våtkemiskt. Analyserna visade att näringsinnehållet varierade mellan 1) arterna (dovhjort och rådjur) och årstid så att rådjuret överlag selekterade en högre andel protein jämfört med dovhjort, medan andelen protein minskade för bägge arter från vår till vinter 2) kön och åldersklasser (dovhjort) så att hindkalvar och vuxna hindar valde en högre andel protein under hösten jämfört med äldre hanar, medan kalvar selekterade för att högre innehåll protein än vuxna hindar under vintern och jag visar dessutom att 3) rådjur och dovhjort valde föda så de konsekvent fick i sig en viss andel kolhydrater, men att denna andel var lägre för rådjur än för dovhjort. Detta skiljde sig från våra förväntningar baserat på tidigare litteratur, eftersom att rådjur anses selektera mer på lösliga kolhydrater jämfört med dovhjort. Dessutom selekterade vuxna hindar och subadulta hanar en viss andel lösliga kolhydrater jämfört med äldre hanar, medan äldre hanar valde föda med en viss andel protein under hösten. Kunskap om vilda djurs födobehov har relevans för såväl viltförvaltning som för jord- och skogsbruk. Resultaten från denna studie kan användas för att förstå födokonkurrensens betydelse mellan- och inom arter och för att beräkna en balanserad foderstat vid stödutfodring, som i sin tur kan användas för att minska skador på jord- och skog.

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Abbreviations

ADF	Acid-detergent fiber
ADF-N	Acid-detergent insoluble nitrogen
ADF-P	Acid-detergent insoluble protein
AA	Amino acids
AP	Available protein
BW	Body weight
CP	Crude protein
DM	Dry matter
ISO	International standardization operation
N	Nitrogen
NDF	Neutral detergent fiber
NIRS	Near-infrared reflectance spectroscopy
NPN	Non-protein nitrogen
TNC	Total non-structural carbohydrates
VFA	Volatile fatty acids
RMT	Right-angled mixture triangles

1 Introduction

Nutritional ecology can be described as “the study of animal nutrition that is based on ecology and evolution” (Raubenheimer & Simpson, 2016), focusing on the interactions between an animal and its environment (Raubenheimer *et al.*, 2012; Raubenheimer *et al.*, 2009; Parker, 2003). On an evolutionary time scale competition for food drives species formation by genetic adaptations to the environment (Raubenheimer *et al.*, 2012) and subsequent niche separation of the species (Codron & Clauss, 2010). The environment involves biotic (e.g., food availability) and abiotic components (e.g., seasonal variation in temperature, photoperiod; Raubenheimer *et al.*, 2009). On a short-term time scale, animals have different strategies in order to meet their nutritional requirements (Parker, 2003), which ultimately influence their health and fitness (Raubenheimer *et al.*, 2012). As an example, changes in the environment can have an impact on behavioral (foraging behavior) and physiological responses, phenotypic plasticity e.g., in gut morphology (Raubenheimer *et al.*, 2009). In addition, the variation in different spatiotemporal scales leads to a trade-off between quantity and quality of available forage in subsequent foraging decisions (Searle *et al.*, 2006; Senft *et al.*, 1987), especially in temperate climates where the availability and nutritional, chemical, and structural composition of forage varies seasonally (Marshal *et al.*, 2005; van Soest, 1994). Many herbivores in temperate climates therefore show behavioral and physiological adaptations to season in for example voluntary feed intake (VFI), metabolic rate, body growth and plasma hormone concentrations (Arnold *et al.*, 2004; Freudenberger *et al.*, 1994; van Wieren, 1992; Domingue *et al.*, 1991; Moen, 1973).

When discussing the nutritional requirements of ruminants, it is important to consider that the consumed feed of a ruminant will not only provide energy and nutrients for the animal, but a significant part of carbohydrates and dietary proteins are utilized by rumen bacteria (McDonald *et al.*, 2011), where carbohydrates represents the main source of energy for microbes (Bach *et al.*, 2005). The main source of energy for ruminants is volatile fatty acids (VFA’s), which are end products of microbial fermentation derived from digestible carbohydrates (cellulose and hemicellulose) and starch (van Soest, 1994; Russell *et al.*, 1992), whereas a lesser part of VFA´s are derived from dietary proteins (Bach *et al.*, 2005; Russell *et al.*, 1992). There are, however, differences in strategies among and within species in order to meet the nutritional requirements (Parker, 2003). As an example, different individuals may employ different nutritional strategies e.g., particular amounts or proportions they consume due to their metabolic requirements based on body size, metabolic rate, stomach anatomy, physical states and life history (Parker, 2003; van Soest, 1994; Estes, 1974; Jarman, 1974). Understanding adaptations to forage is an important part of our understanding of nutritional ecology of herbivores (Felton *et al.*, 2009), especially in temperate climates. The focus in this project is therefore to investigate the temporal variation in nutritional composition between roe deer (*Capreolus capreolus* L.) and fallow deer (*Dama dama* L.) as well as within fallow deer.

1.1 Interspecific adaptations to forage quality

Hofmann (1989) classified ruminants into three feeding types: i) concentrate selectors (browsers), ii) bulk and roughage feeders (grazers) and iii) intermediate feeders (see appendix, section 8.1). He considered that the nutritional requirements and digestive abilities are determined by both feeding strategies and digestive anatomy as a result from evolutionary adaptations. Accordingly, roe deer is classified as a browser (Hofmann, 1989; Hofmann, 1984) adapted to a faster-fermenting diet (Clauss *et al.*, 2009b), efficient post-ruminal digestion (Hofmann, 1989) and have a less efficient cellulose digestion compared to grazers (Clauss & Dierenfeld, 2008; Hofmann, 1989). Hence, they are expected to ingest readily digested food low in insoluble fiber and to be dependent on forage high on cell solubles such as dicotyledons (Tixier *et al.*, 1997; Hofmann, 1989).

As a true browser, roe deer resembles most closely to the original ruminant in evolutionary terms, whereas fallow deer is classified as an intermediate feeder and has by evolutionary adaptations an improved cell wall (e.g., cellulose) digestion and is therefore assumed to be able to handle more variable diets and seasons (Hofmann, 1989). Although Hofmann’s anatomical categorization have been supported to correlate well to feeding behavior and several of his predictions have been supported (Redjadj *et al.*, 2014; Clauss *et al.*, 2009a; Clauss *et al.*, 2009b; Knott *et al.*, 2004; Rowell-Schäfer *et al.*, 2001), his nutritional and physiological interpretations have also been criticized as being inadequate (Illius, 1997; Robbins *et al.*, 1995). As an example, Gordon and Illius (1994; 1996) and Robbins *et al.* (1995) concluded that they did not find support for the morphological adaptations to diet type among ruminants, but that feeding behavior rather is related to body size or forage characteristics. There are no strict grazers among any deer species in the world. However, roe deer and fallow deer represent two very different cases within the *Cervidae* family, with fallow deer resembling more closely to a true grazer. Although their feeding types are distinct (Hofmann, 1989), they often share the same general habitats, and in the temperate part of the world, both species are forced to adapt to seasonal variations (Obidziński *et al.*, 2013; Hofmann, 1989).

1.2 Sexual segregation hypotheses

Body mass is another aspect that can influence forage niche partitioning and digestive efficiency (Barboza & Bowyer, 2000; Main *et al.*, 1996; van Soest, 1994). According to the sexual-dimorphism-body-size hypothesis, genders would segregate because of differences in absolute body size between genders affecting nutritional requirements and hence selection for a certain nutritional composition (Mooring *et al.*, 2005; Ruckstuhl & Neuhaus, 2000; Main *et al.*, 1996). It predicts that larger males ingest high-fiber forage due to a longer retention time and higher absolute metabolic requirements (Main *et al.*, 1996), while gut capacity increase with absolute metabolic requirements (Barboza *et al.*, 2009). In contrast, it is proposed that females select highly digestible plant parts in order to reach their nutritional requirements due to gestation and lactation (Main *et al.*, 1996; Staines *et al.*, 1982). With the same reasoning, fawns and subadult males with high nutritional requirements for growth

(Smith *et al.*, 1975) would have to select for less fiber because of their small body size and shorter retention time compared to adult males.

The metabolic rate (and thus nutrient and protein requirement) is a function of metabolic body weight raised to the power of 0.75 according to the forage selection hypothesis (Miranda *et al.*, 2012), or body-size hypothesis (Jiang *et al.*, 2009) based on the Jarman-Bell principle (Demment & Van Soest, 1985; Geist, 1974; Jarman, 1974; Bell, 1971). Therefore, smaller animals have a higher energy requirement per unit body weight compared to larger animals (Welch, 1982). Thus, since larger animals have a relatively lower energy requirements, while rumen volume is isometrically related to body size (van Soest, 1996; Demment & Van Soest, 1985), larger animals consume high fiber diet compared to smaller animals (Main, 2008). The relatively larger rumen and longer retention time is suggested to facilitate a more efficient fiber digestion in large-bodied individuals (Müller *et al.*, 2013; Barboza & Bowyer, 2000). Hence, some common interpretations of the sexual-dimorphism-body-size hypothesis are that males of sexually dimorphic species should be more effective in fiber digestion and ingest forage high in fiber, while smaller individuals with higher metabolic rate per kilo body mass would need to compensate by selecting high digestible forage (e.g., higher protein) in order to meet their energy requirements (Müller *et al.*, 2013; Perez-Barberia *et al.*, 2007; Bonenfant *et al.*, 2004; Ruckstuhl & Neuhaus, 2000; Gross *et al.*, 1996; Clutton-Brock *et al.*, 1987).

Roe deer having a liveweight of 18-31 kg (Pettorelli *et al.*, 2002; Liberg & Wahlström, 1995), show a low level of dimorphism (Carranza, 1996), whereas fallow deer is highly sexually dimorphic with a liveweight ranging from 39 kg for adult females to 117 kg for males (McElligott *et al.*, 2001; Loison *et al.*, 1999; Carranza, 1996). If body size affects forage selection and nutrient composition, there should be intraspecific differences between dimorphic male and female fallow deer, but not in monomorphic roe deer. By considering variations in digestive capacity and retention time it is possible to make connections between nutrient intake and feeding strategies and to compare intra- and interspecific variation in nutrient intake across seasons. The aim of this project is to test predictions mainly derived from Hofmann (1989) i.e. that different feeding adaptations also have consequences for the choice of forage quality and as well as the differences in food choice derived from body size differences within species (Main *et al.*, 1996).

From these hypotheses it is predicted that:

1) Roe deer ingest forage high in protein and soluble carbohydrates and low in cellulose compared to fallow deer because of their distinct food niches based on their digestive anatomy, and

2) Both species show seasonal variation in nutrient composition with higher digestible forage (high solubles and low in fiber and lignin) in spring and summer compared to fall and winter because of the abundance of high digestible forage compared to winter.

Additionally, based on the sexual-dimorphism-body-size hypothesis it is predicted that:

3) There should be intersexual differences in nutrient composition in the highly dimorphic fallow deer, with males selecting higher proportion digestible fibre and lower protein compared to females, and

4) If body size affects forage selection and nutrient composition, female fallow deer, subadult males and fawns should select high quality forage compared to adult males.

2 Material and methods

2.1 Study area

The study is based on a total of 499 rumen samples collected by hunters on the Koberg estate situated the southwestern part of Sweden (58ºN, 12ºE) in Västra Götaland County. The samples were collected between 2006 – 2013 from roe deer (n = 61) and fallow deer (n = 438). The Koberg estate comprises approximately 95 km^2 of which the study area covers 81.1 km^2. The fallow deer density estimated by distance sampling (Buckland *et al.*, 2001) varied between 17.8 – 32.7 individuals/km^2 and roe deer varied between 0.78 – 1.71 individuals/km^2 during the study (Kjellander, unpublished data). The habitat is dominated by forest (79%) of which 53% consist of coniferous forest, 17% of clear-felled areas and younger forest, and 9% of mixed- and broad-leaved forest (Winsa, 2008). The remainder of the habitat is represented by: arable land and pastures (16%), lakes, ponds, parks and properties (3%) as well as mires and marshes (2%) (Kjellander *et al.*, 2012; Winsa, 2008). Supplementary feeding is provided from the 1st of November to the 30th of April, but may vary across years depending on availability of forage and/or snow cover. The amount and type of food varies among feeding sites, but silage is available for the animals during the whole supplementary feeding period and on most feeding sites, whereas sugar beets, carrots, potatoes etc. are provided occasionally. In addition, a significant part of the arable land is cultivated in order to provide forage for game animals (Grönberg, 2011).

2.2 Analyses of nutrient composition

All samples were stored in a freezer (at -20°C) after they were collected. For the sample preparation, all rumen samples were dried in 65°C to a water content of 4%. We estimated the proportion neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, available nitrogen, lignin and crude protein (CP) of all rumen samples by using *near-infrared reflectance spectroscopy* (NIRS) (Foley *et al.*, 1998). Because NIRS results are not related directly to concentration, the reflected spectrum is calibrated with known compositions of a subset of samples on which we performed wet chemical analyses (methods described below). We selected 140 samples (61 roe deer, 79 fallow deer) out of the total pool of samples for chemical analyses to obtain the dependent variable to be calibrated. The fallow deer samples selected for wet chemistry analyses ranged from highest to lowest values, in order to get the whole range of variation. The calibration models were created for each nutritional fraction by using Orthogonal projection to latent structures (OPLS) based on the spectral- and chemical concentration of nutritive fractions. The model (i.e. the relationship between the reflected spectrum and the known concentration) was verified by cross-validation (see appendix 8.6 for calibration and cross validation statistics). Once the relationships were validated, the model was used for predicting the nutrition contents of the remaining samples (499 – 140 = 359). The nutritional composition of forage depends on relative proportions of cell wall material and cell contents, of which the latter is rich in water-soluble carbohydrates and protein (McDonald *et al.*, 2011).

Lignin in the cell wall is of certain interest because of its resistance to microbial fermentation (McDonald *et al.*, 2011; Crampton & Maynard, 1938).

We performed wet chemistry analyses to determine content of dry matter (DM), ash, CP, lignin, NDF, ADF and acid-detergent insoluble nitrogen (ADF-N) of rumen samples (van Soest *et al.*, 1991), see table 1. ADF-N represents nitrogen attached to the ADF fraction that is unavailable for digestion and absorption (McDonald *et al.*, 2011) and was measured in order to estimate protein quality. By ADF-N available protein (AP) was calculated, which is more useful than crude protein for expressing protein content. AP was calculated as total N times a conversion factor of 6.25 subtracted by ADF-N (Licitra *et al.*, 1996). Sample preparations and chemical analyzes were performed by Agrilab AB, in Uppsala Sweden (Table 1). The proportion of cellulose (ADF – ADL) and hemicellulose (NDF – ADF) were then calculated. Total non-structural carbohydrates (TNC) was estimated by the formula: TNC = (100 – (NDF + AP + ash + fat)) (Irwin *et al.*, 2014). The TNC fraction includes starch, simple sugars, and soluble fiber. Because we did not perform assays of fat content specifically, I use the combined value of TNC + fat in the illustration of macronutrient contributions to the diets.

Table 1. *Methods for chemical analyses of the nutritive fractions including their components*

Fraction	Components	Method
DM	Dry matter	KLK 1965:1
Ash	Inorganic constituents e.g., minerals and silica	KLK 1965:1
CP (total N × 6.25)	Nitrogen from protein and non-protein nitrogen (e.g., amino acids, urea, ammonia)	Dumas method according to ISO (13878:1998)
NDF	Cellulose, hemicellulose and lignin i.e., cell wall material	van Soest *et al.* (1991)[1]
ADF	Cellulose, lignified nitrogen, lignin and silica	van Soest *et al.* (1991)[1]
ADF-N (ADIN)	N attached to the ADF fraction	ISO (13878:1998)
ADL	Crude lignin	van Soest *et al.* (1991)[1]

[1] The detergent system by Van Soest et al 1991 is described in Appendix 8.1.

2.3 Statistical analysis

2.3.1 Interspecific comparisons

All rumen samples were divided into the season the animal was killed by the following definition: spring (1st April – 31st May), summer (1st June – 30th September), fall (1st October – 15th December) and winter (16th December – 30th March). I used two-way ANOVA (type II or type III when appropriate for unbalanced data) followed by calculation of least-squares means with adjusted p-values by Tukey-Kramer method between roe deer (n = 59) and fallow deer (n = 285) across seasons. Interaction terms are not reported unless significant ($p < 0.05$). Residuals were tested for normality performed by Shapiro-Wilk test, and test for homogeneity of variance across groups were tested by Levene's test. For data where the assumption of normality was not met, logit transformation was used. For data still violating the assumption of homogeneity of variances, Kruskal-Wallis rank sum test was performed followed by Wilcox rank sum test. One outlier was excluded from the dataset (residual > 8*IQR), with a CP value of 666.38 g/kg DM, which was 33% higher compared to the second highest value of 499 g/kg DM. The reason for that extreme value is not known, but must obviously be an anomaly of some sort.

The proportion of crude protein, available protein, cellulose, hemicellulose, ADF-N (expressed in ADF-P i.e. ADF-N multiplied by conversion factor of 6.25) and lignin were used as dependent variables in the analyses. No rumen samples were available for roe deer fawns and thus they were excluded from the interspecific comparisons. Additionally, I created right-angled mixture triangles (RMTs) (Raubenheimer, 2011) in Excel (Version 14.6.7 – © 1998 – 2009) to illustrate the nutritional composition of their diet in a multidimensional context. The use of RMT's is a graphical approach of illustrating proportional data and has been recommended as a complementary geometric framework that can be used to understand how the animals regulate and differ in their nutrient intake (Raubenheimer, 2011; Simpson & Raubenheimer, 1993). In order to examine the interactions among nutrients and test the responses (e.g., intake) of an animal to variation in food composition across the two species (Raubenheimer *et al.*, 2012; Raubenheimer *et al.*, 2009), three RMT's per species were created, one for each macronutrient. Data was analyzed by using software RStudio (Version 1.0.44 – © 2009 – 2016 RStudio, Inc.) using the packages Rmisc (Hope, 2013), stats (R Core Team, 2016), base (R Core Team, 2016), lsmeans (Lenth, 2016) and car (Fox & Weisberg).

2.3.2 Intraspecific comparisons

Due to the limited data on roe deer, intraspecific analyses were restricted to fallow deer. Comparisons of the nutritive fractions were performed using two-way ANOVA across season, gender and age class. For intraspecific comparisons, fallow deer was divided into four age groups: fawns (≤ 0 years of age), subadults (1 – 2 years of age) and adults (≥ 4 years of age). Males were considered as adults at four years of age when a fully mature body mass and antler size has been attained (Visschers, 2014). Three year old male fallow deer were here considered as a too morphologically variable age class (Visschers, 2014) and was thus excluded from the intraspecific analyses. Additional tests were conducted between adult females (n = 128), subadult males (n = 95) and adult males (n = 39) in order to include summer in the analyses, as fawns were lacking in summer. Spring intraspecific comparisons were also excluded due to lack of data of females (n = 0) and a limited number of males (n = 5).

Table 2. *Number of rumens analyzed and used in this study, across species (roe deer and fallow deer), seasons and adults/fawns. Roe deer fawns were not available during any season. Based on animals killed in Koberg, southwestern Sweden, 2006 – 2013.*

	Fallow deer			**Roe deer**			**Total**
	Female	Male	Total	Female	Male	Total	

	Adult	*Fawn*	*Adult*	*Fawn*		*Adult*	*Adult*		
Spring	**-**	**-**	12	**-**	**12**	1	5	**6**	**18**
Summer	33	**-**	62	**-**	**95**	4	32	**36**	**131**
Fall	49	25	39	21	**134**	4	2	**6**	**140**
Winter	45	59	46	47	**197**	7	6	**13**	**210**
Total	127	84	159	68	**438**	16	45	**61**	**499**

3 Results

3.1 Interspecific variation in nutrient composition

Overall, there were statistically significant differences between species in terms of AP, ADF-P, cellulose, hemicellulose and lignin (Table 3). A two-way ANOVA was conducted to examine effects of species and season on different nutritive fractions. The main effect analysis showed a significant effect of season ($F_{3;\,338} = 91.285$, $p \leq 0.0001$) and species ($F_{1;\,338} = 115.691$, $p \leq 0.0001$) in terms of AP. The Tukey-Kramer test revealed that AP was significantly higher for roe deer compared to fallow deer in spring ($p = 0.0002$), summer ($p \leq 0.0001$) and winter ($p = 0.0017$), but there were no significant differences between species in fall ($p = 0.42$). Similarly, the main effect analysis showed a significant effect of season ($F_{3;\,338} = 6.391$, $p = 0.0003$) and species ($F_{1;\,338} = 16.045$, $p \leq 0.00001$) in terms of ADF-P. Roe deer had significantly higher proportions of ADF-P than fallow deer in summer ($p = 0.0158$), but not in spring, fall and winter ($p > 0.4$).

Table 3. *Overall nutrient composition (mean ± standard deviation) of roe deer and fallow deer of one year of age or older. Statistical tests performed by Welch two-sample t-test[1] indicated by t-values, significance level by p and degrees of freedom (df). Based on rumen samples from animals killed in Koberg, southwestern Sweden, 2006 – 2013.*

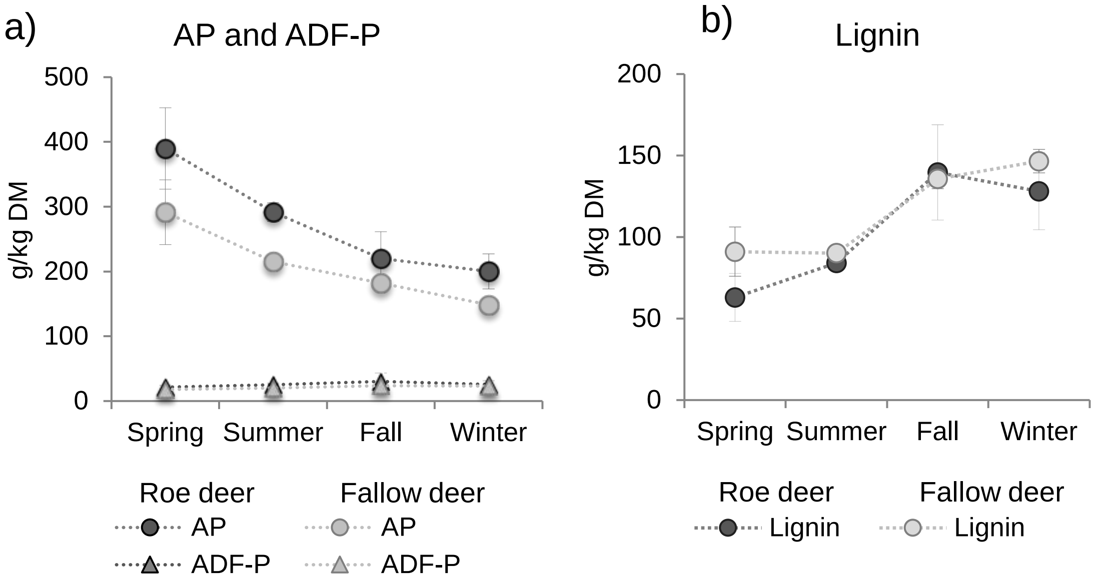
Nutritive fraction **g/kg TS**	**Roe deer** N = 60	**Fallow deer** N = 286	**t**	**df**	**p**
Crude protein	299.90 ± 69.90	209.05 ± 53.65	-9.49	74.21	< 0.00001
Available protein	274.54 ± 69.94	186.89 ± 54.85	-9.14	74.95	< 0.00001
ADF-P	25.36 ± 8.07	22.16 ± 7.49	-2.83	81.66	0.0059
Cellulose	191.97 ± 59.71	217.89 ± 49.07	3.19	77.22	0.002
Hemicellulose	162.49 ± 47.34	209.50 ± 33.25	7.32	71.69	< 0.00001
Lignin	97.12 ± 33.36	122.16 ± 37.17	5.18	92.42	< 0.00001

[1] Welch two-sample t-test uses Satterthaite-Welch adjustment for degrees of freedom (Satterthwaite, 1946).

Table 4. *Proportion (mean ± SD) of crude protein (CP), available protein (AP), protein bound to ADF (ADF-P), cellulose, hemicellulose and lignin in spring, summer, fall and winter for roe deer and fallow deer of one year of age or older. Subscript a, b and c (*$^{a,\ b\ or\ c}$*) indicate significant differences between seasons within species (*$p \leq 0.03$*), calculated using post-hoc Tukey-Kramer or Wilcox rank sum test. Based on rumen samples from animals killed in Koberg, southwestern Sweden, 2006 – 2013.*

	Sample size	CP	AP	ADF-P	Cellulose	Hemicellulose	Lignin
Roe deer							
Spring	6	411 ± 58 ^{a}	389 ± 60 ^{a}	21 ± 3 ^{a}	140 ± 51 ^{a}	175 ± 26 ^{a}	63 ± 14 ^{a}
Summer	35	317 ± 43 ^{b}	292 ± 42 ^{b}	25 ± 7 ^{a}	184 ± 41 ^{b}	213 ± 43 ^{b}	84 ± 14 ^{a}
Fall	6	250 ± 50 ^{c}	220 ± 39 ^{c}	30 ± 12 ^{a}	203 ± 55 ^{bc}	205 ± 23 ^{b}	140 ± 28 ^{b}
Winter	13	226 ± 47 ^{c}	200 ± 45 ^{c}	26 ± 10 ^{a}	233 ± 80 ^{c}	214 ± 27 ^{c}	128 ± 39 ^{b}
Fallow deer							
Spring	12	309 ± 73 ^{a}	291 ± 78 ^{a}	18 ± 5 ^{ab}	144 ± 21 ^{a}	205 ± 34 ^{a}	91 ± 24 ^{a}
Summer	95	235 ± 46 ^{b}	215 ± 44 ^{b}	20 ± 7 ^{a}	204 ± 30 ^{b}	185 ± 54 ^{b}	90 ± 18 ^{a}
Fall	88	206 ± 42 ^{c}	182 ± 44 ^{c}	24 ± 8 ^{b}	208 ± 48 ^{b}	157 ± 27 ^{b}	135 ± 29 ^{b}
Winter	91	172 ± 32 ^{d}	148 ± 33 ^{d}	23 ± 8 ^{ab}	252 ± 47 ^{c}	79 ± 45 ^{b}	146 ± 35 ^{c}

A disordinal interaction in lignin content was found between species and season ($F_{3; 338} = 2.6528$, $p = 0.048$; Figure 1b). The Tukey-Kramer test showed significant differences between roe deer and fallow deer in spring ($p = 0.048$) in terms of lignin, but not in summer, fall or winter ($p \geq 0.34$, in all cases). Kruskal-Wallis rank sum test revealed that there were significant differences in cellulose between seasons ($\chi^2 = 94.277$, df = 3, $p \leq 0.00001$) and species ($\chi^2 = 14.55$, df = 1, $p = 0.0001$; Figure 1c). The proportion cellulose was significantly higher in fallow deer compared to roe deer in summer ($W = 2330$, $p = 0.0005$), but no significant differences were found between species in spring, fall or winter ($W \geq 46$, $p \geq 0.42$; Figure 1c). Similarly, there were significant differences in hemicellulose between seasons ($\chi^2 = 44.0$, df = 3, $p \leq 0.00001$) and species ($\chi^2 = 63.583$, df = 1, $p \leq 0.00001$; Figure 1d). Wilcox rank sum test revealed that there were significant differences in the proportion of hemicellulose between roe deer and fallow deer in spring ($W = 68$, $p = 0.0013$), summer ($W = 2976$, $p = \leq 0.00001$) and fall ($W = 420$, $p = 0.016$), but not in winter ($W = 700$, $p = 0.29$; Figure 1d).



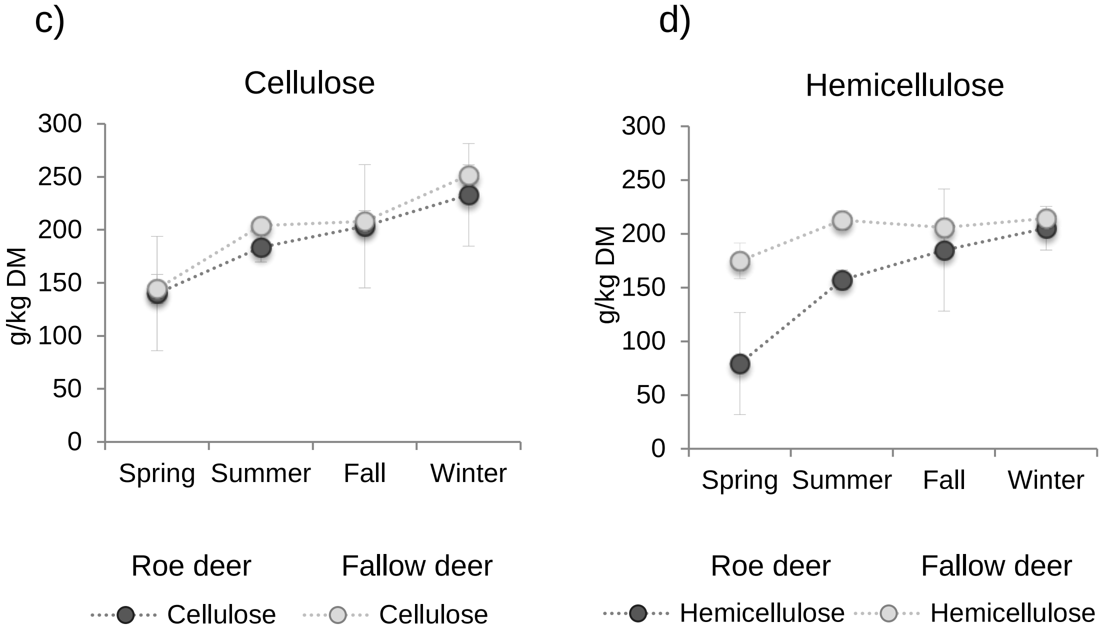


Figure 1. *Proportion of macronutrients in rumen samples of roe deer and fallow deer with comparisons between species. Dark grey marker points represents roe deer and light marker points represents fallow deer, with 95 % confidence interval. Proportion (g/kg dry matter) is represented on the primary Y-axis (dotted lines). Figure a) represents the proportion of AP and ADF-P, b) lignin, c) cellulose and d) hemicellulose. Based on rumen samples from animals killed in Koberg, southwestern Sweden, 2006 – 2013.*

3.2 Comparative nutrient regulation

Food composition of roe deer and fallow deer analyzed by using RMT’s illustrates that samples are clustered more tightly along the implicit axis representing non-structural carbohydrates and fat in summer (Figure 2a). Fat is included on this axis as we cannot separate fat from TNC using the subtraction method (Figure 2a). The RMT’s show that adult fallow and roe deer maintain a stable proportion of total non-structural carbohydrates (TNC) and fat compared to protein (Figure 2b) and digestible fibre (Figure 1c). Both species allow available protein and digestible fibers to vary in summer as indicated by the slope of the trendline and the low coefficient of determination (r^2). In winter, roe deer show a larger variation in non-structural carbohydrates and protein compared to fallow deer, although samples size for roe deer in winter was low (n = 13).

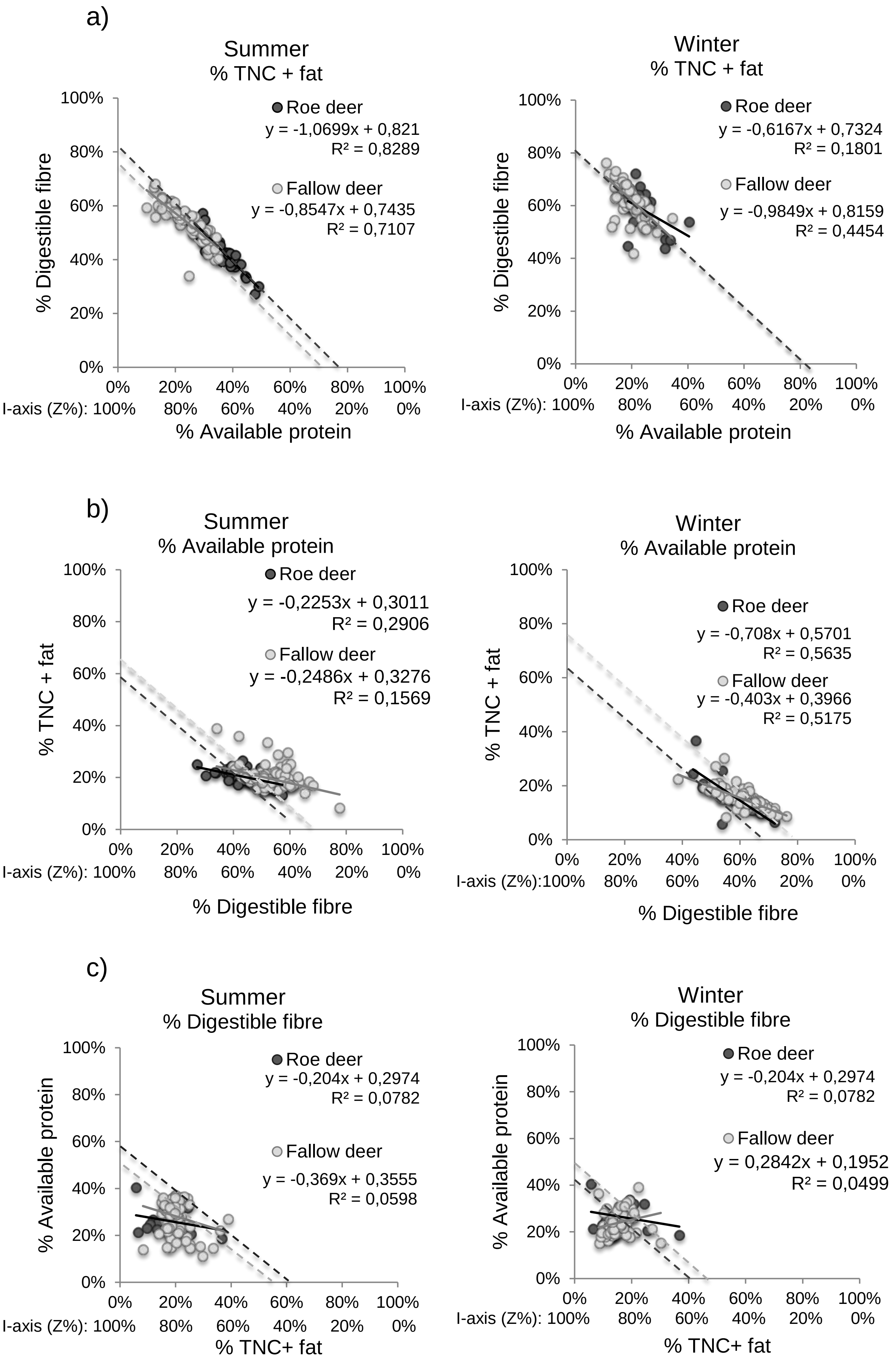

a)
Summer
% TNC + fat
Roe deer
y = -1,0699x + 0,821
R² = 0,8289
Fallow deer
y = -0,8547x + 0,7435
R² = 0,7107
% Digestible fibre
100%
80%
60%
40%
20%
0%
0%
20%
40%
60%
80%
100%
I-axis (Z%): 100%
80%
60%
40%
20%
0%
% Available protein
Winter
% TNC + fat
Roe deer
y = -0,6167x + 0,7324
R² = 0,1801
Fallow deer
y = -0,9849x + 0,8159
R² = 0,4454
% Digestible fibre
I-axis (Z%): 100%
% Available protein
b)
Summer
% Available protein
Roe deer
y = -0,2253x + 0,3011
R² = 0,2906
Fallow deer
y = -0,2486x + 0,3276
R² = 0,1569
% TNC + fat
I-axis (Z%): 100%
% Digestible fibre
Winter
% Available protein
Roe deer
y = -0,708x + 0,5701
R² = 0,5635
Fallow deer
y = -0,403x + 0,3966
R² = 0,5175
% TNC + fat
I-axis (Z%):100%
% Digestible fibre
c)
Summer
% Digestible fibre
Roe deer
y = -0,204x + 0,2974
R² = 0,0782
Fallow deer
y = -0,369x + 0,3555
R² = 0,0598
% Available protein
I-axis (Z%): 100%
% TNC+ fat
Winter
% Digestible fibre
Roe deer
y = -0,204x + 0,2974
R² = 0,0782
Fallow deer
y = 0,2842x + 0,1952
R² = 0,0499
% Available protein
I-axis (Z%): 100%
% TNC+ fat

Figure 2. *A. Right-angled mixture triangles (RMT) showing the relative components of digestible macronutrients in rumen samples of roe deer (dark grey dots) and fallow deer (light grey dots), comparing between summer (left panels) and winter (right panels). X-axis = % available protein (of total amount of digestible macronutrient DM), Y-axis = % digestible fibre, and I-axis (the implicit axis) = % total non-structural carbohydrates (TNC) and fat. Each dot represents a nutrient mixture, where X+Y+Z = 100% (Raubenheimer et al., 2015; Raubenheimer, 2011). The foods the animals consumed have a similar balance of non-structural carbohydrates (and fats, although this is a small contribution) compared to other macronutrients shown in the slope of the solid regression line falling parallel to the implicit axis, with the* R^2 *value indicating how strongly conserved this regulation is. For example, the food eaten by roe deer has a very similar proportion of TNC (slope= -1.067) and intake of these macronutrients appears highly conserved (*R^2 *value = 0.82). B. X-axis = % digestible fibre, Y-axis = % TNC + fat, and I-axis = % available protein. C. X-axis = % TNC + fat, Y-axis = % available protein, and I-axis = % digestible fibre. The foods the roe deer eat are not so similar in terms of their protein and fibre content (shown by the regression lines not falling parallel with the isolines in B and C) and this regulation is not well conserved (low* R^2 *values). Differences between ungulate species and seasons can also be interpreted in a similar way.*

3.3 Intraspecific variation of nutrient composition of fallow deer

For intraspecific comparisons, the statistical analyses were divided into two separate analyses. In the first, fawns were included but the analyses were restricted to fall and winter, as fawns were lacking in summer. In the second, additional tests were conducted between adult females, subadult males and adult males in order to include summer.

3.3.1 Between gender, age class and season

A two-way ANOVA was conducted to examine the effect of gender, age class and season on the proportion of AP and ADF-P. Non-parametric tests were performed on cellulose, hemicellulose and lignin. A significant disordinal interaction was found between the effects of gender, age class and season on the proportion AP ($F_{4;\,311}$ = 6.0753, p = 0.0001). The post-hoc Tukey-Kramer test showed that adult males had significantly lower AP in their rumen compared to female fawns (p = 0.02) and adult females (p = 0.015) in fall. In addition, male fawns selected significantly higher proportion AP (p = 0.001) compared to adult females; and female fawns tend to select higher proportion AP compared to adult females in winter (p = 0.05). Main effect analysis reveled no significant differences in terms of ADF-P between seasons ($F_{1;\,311}$ = 0.001, p = 0.975) or gender and age class ($F_{4;\,311}$ = 0.441, p = 0.779). Kruskal-Wallis revealed significantly higher level of cellulose in winter (χ^2 = 44.446, df = 1, $p \leq 0.00001$) and hemicellulose (χ^2 = 6.6418, df = 1, p = 0.009), but not between gender and age classes. In addition, Kruskal-Wallis revealed significant differences in terms of lignin between gender and age classes (χ^2 = 10.618, df = 4, p = 0.03), but not between seasons (χ^2 = 3.258, df = 1, p = 0.07). Wilcox rank sum test revealed that adult females had a higher proportion lignin compared to adult males (W = 434, p = 0.00089), subadult males (W = 911, p = 0.008), male fawns (W = 1648, $p \leq 0.00001$) and female fawns (W = 756, p = 0.00018) in winter. No significant differences were found between adults and fawns in fall ($p \geq 0.19$).

Table 5. *Proportion (mean ± standard deviation) of crude protein (CP), available protein (AP), nitrogen bound to ADF (ADF-P), cellulose, hemicellulose and lignin in summer, fall and winter for gender and age classes of fallow deer from year 2006 – 2013. Fawns are not represented in the statistical analysis in summer, as they were lacking in summer. Subscript a, b and c (*$^{a,\ b\ \mathrm{or}\ c}$*) indicate significant differences between seasons within species (*$p \leq 0.029$*), calculated using post-hoc Tukey-Kramer or Wilcox rank sum test.*

	Sample size	CP	AP	ADF-P	Cellulose	Hemicellulose	Lignin
Female fawns							
Fall	25	223 ± 39 ^{a}	199 ± 39 ^{a}	25 ± 5 ^{a}	221 ± 42 ^{a}	194 ± 18 ^{a}	130 ± 19 ^{a}
Winter	59	185 ± 34 ^{b}	162 ± 35 ^{b}	23 ± 8 ^{a}	251 ± 45 ^{b}	208 ± 27 ^{b}	137 ± 33 ^{a}
Male fawns							
Fall	21	205 ± 42 ^{a}	179 ± 42 ^{a}	25 ± 6 ^{a}	236 ± 48 ^{a}	203 ± 22 ^{a}	139 ± 27 ^{a}
Winter	47	195 ± 36 ^{a}	170 ± 34 ^{a}	25 ± 9 ^{a}	237 ± 47 ^{a}	209 ± 30 ^{a}	126 ± 33 ^{a}
Adult females							
Summer	33	243 ± 34 ^{a}	221 ± 34 ^{a}	22 ± 6 ^{a}	205 ± 27 ^{a}	206 ± 34 ^{a}	94 ± 14 ^{a}
Fall	49	218 ± 42 ^{a}	196 ± 42 ^{b}	23 ± 5 ^{a}	209 ± 48 ^{a}	201 ± 17 ^{a}	133 ± 22 ^{b}
Winter	45	165 ± 31 ^{b}	140 ± 32 ^{c}	25 ± 7 ^{a}	261 ± 41 ^{b}	211 ± 20 ^{b}	161± 28 ^{c}
Subadult males							
Summer	54	232 ± 53 ^{a}	214 ± 49 ^{a}	18 ± 6 ^{a}	204 ± 24 ^{a}	217 ± 49 ^{a}	84 ± 16 ^{a}
Fall	12	197 ± 44 ^{ab}	174 ± 46 ^{b}	23 ± 6 ^{b}	216 ± 48 ^{a}	213 ± 21 ^{a}	137± 31 ^{b}
Winter	29	176 ± 24 ^{b}	152 ± 26 ^{b}	23 ± 8 ^{b}	252 ± 41 ^{b}	212 ± 24 ^{a}	143 ± 29 ^{b}
Adult males							
Summer	6	218 ± 45 ^{a}	191 ± 45 ^{a}	26 ± 8 ^{a}	196 ± 23 ^{a}	216 ± 24 ^{a}	119 ± 16 ^{a}
Fall	22	185 ± 28 ^{a}	159 ± 35 ^{a}	25 ± 12 ^{a}	207 ± 53 ^{a}	210 ± 32 ^{a}	138 ± 39 ^{a}
Winter	12	190 ± 44 ^{a}	166 ± 46 ^{a}	24 ± 7 ^{a}	248 ± 50 ^{b}	208 ± 32 ^{a}	124 ± 36 ^{a}

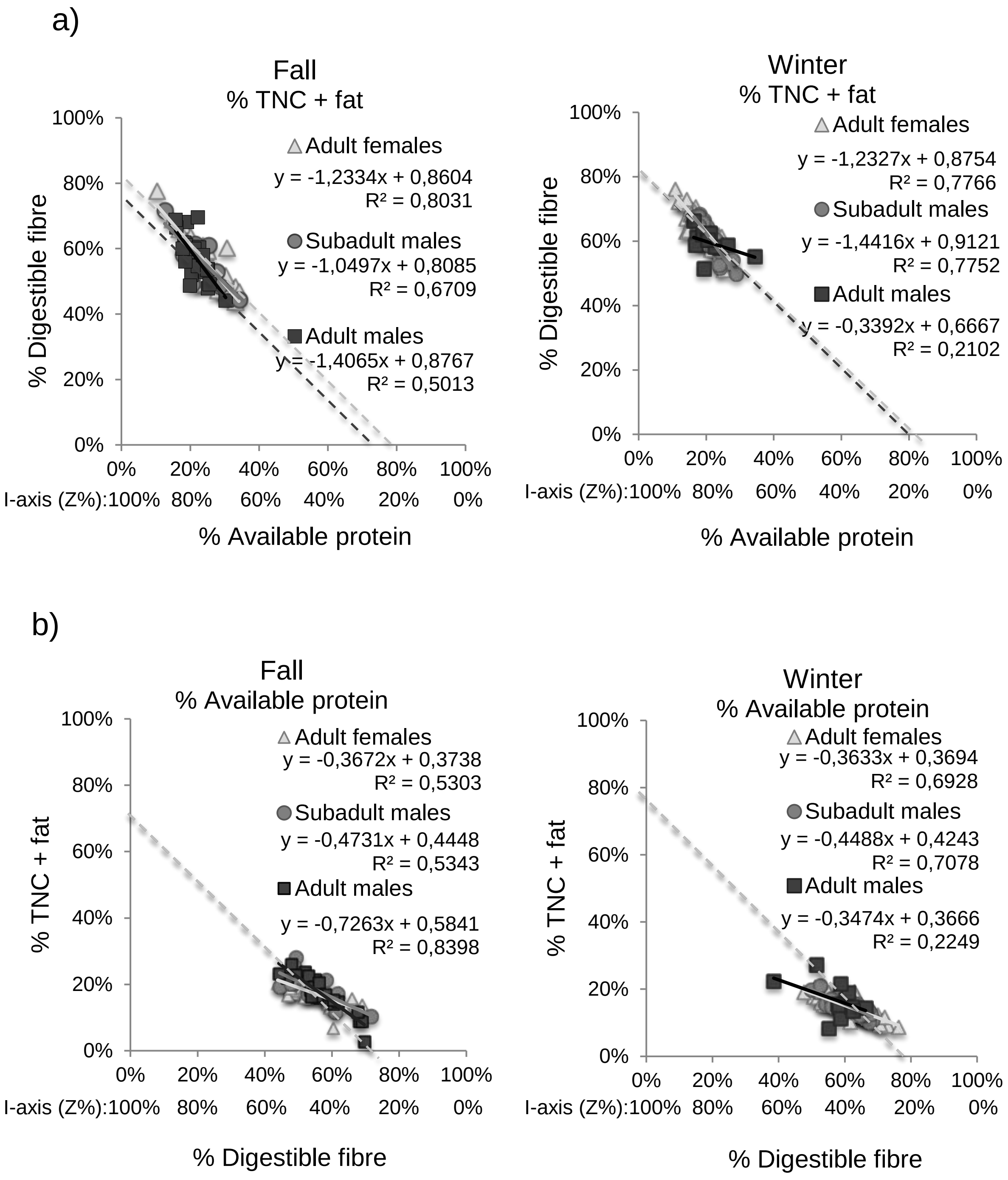
3.3.2 Between adult females, subadult males and adult males

A two-way ANOVA was conducted to examine the effect of gender, age class and season on the proportion of AP and cellulose. A significant disordinal interaction was found between gender and age class and season in terms of AP ($F_{4;\,253} = 4.2234$, $p = 0.0025$). Tukey-Kramer post hoc test reveled only significant difference in terms of AP between adult females and adult males ($p = 0.01$) in fall, but not between gender and age classes or gender within other seasons ($p \geq 0.5$). The main effect analysis of cellulose showed no differences between gender and age class ($F_{4;\,253} = 0.4172$, $p = 0.66$), but there was significant differences between seasons ($F_{2;\,253} = 42.2275$, $p \leq 0.00001$; Table 5). Kruskal-Wallis test showed significant differences of ADF-P between seasons ($\chi^2 = 15.975$, $df = 2$, $p = 0.00033$) and between gender and age classes ($\chi^2 = 15.712$, $df = 2$, $p = 0.00039$), and Wilcox rank sum test revealed that subadult males selected significantly lower ADF-P compared to adult males ($W = 65$, $p = 0.017$) and adult females ($W = 1165$, $p = 0.017$) in summer. No significant differences were found between adult males and adult females in summer, fall or winter ($W \geq 67$, $p \geq 0.19$).

Kruskal-Wallis test showed no significant differences of hemicellulose between seasons or between gender and age classes ($\chi^2 < 1.2$, $df = 2$, $p > 0.21$, in both cases). However, significant differences were found between seasons ($\chi^2 = 139.56$, $df = 2$, $p \leq 0.00001$) and gender and age classes ($\chi^2 = 29.663$, $df = 2$, $p \leq 0.00001$) in terms of lignin. Wilcox rank sum test revealed that adult males had significantly higher proportion lignin in their rumen contents compared to and adult females ($W = 21$, $p = 0.001$) and subadult males ($W = 17$, $p = 0.0003$) in summer. In addition, adult females had significantly higher proportion lignin compared to subadult males ($W = 1311$, $p = 0.0002$) in summer. Additionally, significant differences were found between adult females and adult males ($W = 434$, $p = 0.0009$) and adult females and subadult males ($W = 911$, $p = 0.004$) in winter, with highest content in females and lowest in adult males. No differences were found in fall between gender and age class in terms of lignin ($W \geq 119$, $p > 0.29$).

3.4 Regulation of macronutrients within fallow deer

The analysis of food composition of adult fallow deer (adult female, subadult male and adult male) using RMT's illustrates that the samples for adult females and subadult males are clustered more tightly along the implicit axis representing non-structural carbohydrates and fat in fall and winter compared to males (Figure 3a). Thus, the RMT's show that adult females and subadult males maintain a stable proportion of total non-structural carbohydrates (TNC) and fat compared to protein (Figure 2b) and digestible fibre (Figure 1c). However, adult males maintain a stable proportion of available protein in fall, as compared to adult females and subadult males (Figure 2b). In winter, however, the pattern is the opposite where adult females and subadult males maintain a more stable intake of protein as compared to fall, whereas adult males allow available protein to vary in winter. Both genders allow digestible fibers to vary in fall as indicated by the slope of the trendline and the low coefficient of determination (r^2). In winter, adult females and subadult males maintain a more stable intake of digestible fibre compared to fall, with the strongest pattern shown by subadult males.



c)

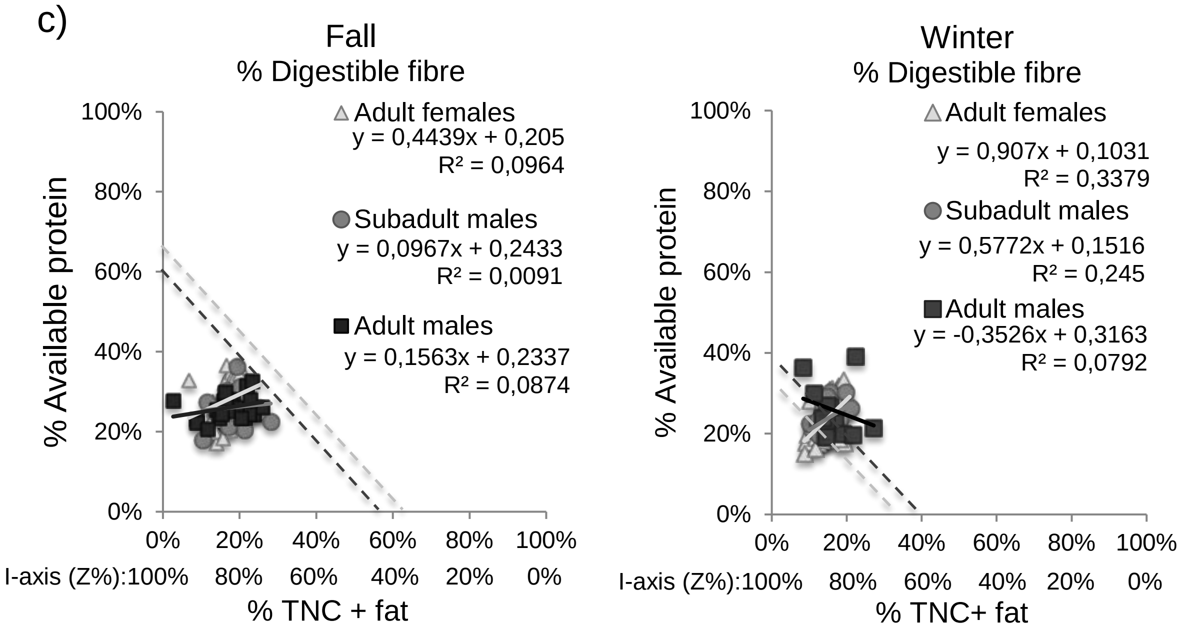


Figure 3. *Intraspecific comparisons of adult females between fall and winter using three component right-angled mixture triangles (RMT's) demonstrating regulation of different macronutrients (see figure 2). In figure 3a) total non-structural carbohydrates, b) available protein and c) digestible fibers are represented on the I-axis as Z (%).*

4 Discussion

This study revealed that 1) the nutritional composition of the diet varied across species and seasons in line with prediction 2 (P2), with greater variation in roe deer between seasons 2) both species selected for a particular balance between macronutrients and 3) the nutritional composition of the diet varied across age classes and gender in fallow deer. Specifically, I found that roe deer overall had significantly higher proportion of available protein (AP) in their rumen contents compared to fallow deer in line with prediction 1 (P1). I also found that both species selected for a particular proportion of soluble carbohydrates in summer, with the strongest pattern shown by roe deer (Figure 2a) in opposition to the prediction (P1). I found no differences in digestible fibre between age and gender classes within fallow deer, which do not fully support prediction 3 and 4 (P3 and P4). However, I found that female fawns and adult females selected a higher proportion AP compared to adult males in fall in accordance with the predictions (P3 and P4). In addition, adult female and subadult male fallow deer maintained a stable intake of AP in winter, whereas adult males maintained a stable intake of protein in fall (Figure 3b).

4.1 Seasonal variation in forage composition

The forage composition varied across seasons in line with the predictions (P2). The proportion of AP in the rumen samples varied across seasons, with the lowest mean in winter for roe deer (200 g/kg DM) and fallow deer (148 g/kg DM) and highest mean in spring during growing season (389 g/kg DM for roe deer and 292 g/kg DM for fallow deer). These variations reflect the variation in forage composition across seasons (Leslie *et al.*, 1984). In addition, the proportions of cellulose, hemicellulose and lignin increased over the seasons from spring to winter (Figure 1), suggesting to reflect the seasonal variation in forage composition and availability, as the lignification tend to increase with maturity, and thus reduce the digestibility and nutritive value of mature forage (van Soest, 1996; Jarman, 1974). The digestibility (rate of digestion) of dry matter of forage determines the nutritive value (quality) of forage, and depends on e.g., the chemical composition of dry matter (DM). The polysaccharides (cellulose, hemicellulose) and lignin increase with maturity resulting in decreased digestibility of the polysaccharides (McDonald *et al.*, 2011). Hence, neutral detergent fiber (NDF) is commonly considered as the primary chemical component determining the digestibility of forage (McDonald *et al.*, 2011). It is, however, important to consider other factors influencing the digestibility such as the physical structure of plant tissues (McDonald *et al.*, 2011), ration composition of nutrients and between and within animals species. As an example, the digestibility of fiber and organic matter also varies between ruminants (van Wieren, 1996; Milne *et al.*, 1977), which may be an effect of the variation of microbial populations between species, and thus transport and utilization of nutrients (Deutch *et al.*, 1998; Prins & Geelen, 1971). The digestibility may also be altered by environmental factors, decreasing in cold environments as a result of an increased passage rate through the rumen i.e. short retention time (Cheeke & Dierenfeld, 2013; Holand, 1994). In addition, excess of soluble carbohydrates may inhibit the microbial activity (Keunen *et al.*, 2002; Ekinci & Broderick, 1997; Clark *et al.*, 1992; Russell *et al.*, 1992) and, hence, reduce the digestibility of fiber, which will be discussed further in relation to the results (Section 4.2.3).

4.2 Interspecific comparisons of nutrient intake

4.2.1 Protein (nitrogen) content

Roe deer had significantly higher proportion of AP in their rumen contents than fallow deer in spring, summer and winter, in accordance with the predictions (P1). There was, however, no significant differences found in fall between species, which is likely to be an outcome of the highly skewed sample sizes of roe deer (n = 6) and fallow deer (n = 88) in fall. Another possible explanation could be that their diets are overlapping to a greater extent during this season than others. As an example, Obidziński *et al.* (2013) demonstrated that the fall-winter diet of roe deer and fallow deer overlapped by 60%, whereas in fallow deer select more graminoids and less ferns than roe deer in summer (Nichols *et al.*, 2016). Although the knowledge about protein requirements for cervids are limited (Dryden, 2011), it has been shown to be down at 5% of daily dry matter intake for maintenance and up to 25% for growth of fawns (Asleson *et al.*, 1996; Smith *et al.*, 1975). Selection of protein by cervids have been demonstrated by e.g., Moser *et al.* (2006); Mann and Putman (1989); Mereszczak *et al.* (1981), but absence of such selection has also been documented (Tixier *et al.*, 1997; Papageorgiou *et al.*, 1981). However, the obtained values in this study are higher than the protein content in forage with highest reported values of 220 – 290 g/kg DM (Verheyden-Tixier *et al.*, 2008; Moser *et al.*, 2006; Leslie *et al.*, 1984; Papageorgiou *et al.*, 1981). As an example, the crude protein (CP) content of forbs, which is part of their main diet in summer in addition to deciduous browse and shrubs (Nichols *et al.*, 2016; Cederlund *et al.*, 1980), has been shown to vary between 190 g/kg DM in winter to 270 g/kg DM in spring (Verheyden-Tixier *et al.*, 2008). In addition, the CP content varies between 80 – 195 g/kg DM in browse and 70 – 290 g/kg DM in ferns in all seasons with highest values in spring and lowest in winter (Verheyden-Tixier *et al.*, 2008). Our results are more similar to previous results obtained from rumen samples (Popovic *et al.*, 2009; Djordjevic *et al.*, 2006). In the latter two studies, the CP content of roe deer rumen samples in spring reached 360 – 374 g/kg DM as compared to approx. 411 CP g/kg DM of roe deer in our data set. However, these studies are based on data from Serbia and the higher protein content in this study may arise from differences in temperature and photoperiod in lower and higher latitudes with subsequent differences in plant growth and composition (Myneni *et al.*, 1997; Buxton, 1996; Klein, 1990), increasing the protein content in the higher latitudes in spring.

In order to interpret the contents of AP in the rumen contents, it is therefore necessary to consider the nitrogen (N) utilization of ruminants and the effect of other nutritive fractions present affecting the amount of N in the rumen contents. The proportion protein (in terms of CP or AP) present in the rumen is not a direct measure of the nutrient content of the ingested forage, but it also takes into account N derived from the urea, microbial protein as well as endogenous protein (Patton *et al.*, 2014).

Because the amount of microbial protein produced depends on nutrient availability, interaction with other nutrients and efficiency of the microbes (Patton *et al.*, 2014; Bach *et al.*, 2005), it is difficult to predict the amount of protein (amino acids) that are actually absorbed of an animal. In addition, the availability of protein can vary depending on solubility, structure (Cheeke & Dierenfeld, 2013; Stern *et al.*, 1978) and interaction of other nutrients such as carbohydrates (Bach *et al.*, 2005) affecting the degradation of protein. The higher proportion protein (as a part of cell contents) irrespective of body weight is in line with the prediction (P1) that roe deer is expected to ingest a higher proportion protein based on their metabolic adaptations (Table 6 – appendix; Hofmann, 1989).

The differences in nutritional requirements may also vary with reproductive strategies. As an example, females of species giving birth to several fawns have a higher energy and protein requirements compared females who give birth to fewer fawns (NRC (2007) and a lactating female has a considerably higher energy and protein requirements due to milk production (Barboza & Bowyer, 2000). Roe deer is a polytocous species i.e. usually give birth to litters larger than one (1-4 fawns) in late May to early June (Andersen & Linnell, 1997). In contrast, fallow deer are monotocous i.e. they usually give birth to only one fawn in mid to late June (Kjellander *et al.*, 2012; Birgersson & Ekvall, 1997). Thus, protein requirements of female roe deer would be expected to be higher than of fallow deer in especially summer.

The proportion of ADF-P was significantly higher for roe deer compared to fallow deer in summer. This can be explained by the higher intake of protein of roe deer, increasing the relative proportion of ADF-P. The ADF-P fraction represents the fiber-bound protein and hence unavailable for absorption (Licitra *et al.*, 1996) and may increase with maturity of the forage (McDonald *et al.*, 2011; Mitchell *et al.*, 1997). The increase in ADF-P for fallow deer in summer and fall (Table 4) may arise from an increase in ADF-P in grass during late summer (Mitchell *et al.*, 1997). However, if the forage has high tannin content ADF-P also measures tannin bound N (Ali *et al.*, 2016). Tannins have varying concentration and affinity for proteins across and within plant species (Chikagwa-Malunga *et al.*, 2009), which may decrease the fermentation rate and digestibility of the digesta (Gordon & Illius, 1996) if they are tightly bound to the N. On the contrary, condensed tannins in moderate levels can protect proteins from being hydrolyzed in the rumen (Min *et al.*, 2003) and hence have a beneficial effect on amino acid (AA) uptake in the small intestines by avoiding rumen fermentation. In addition, roe deer have been shown to secrete tannin-binding proteins with their saliva (Robbins *et al.*, 1995; Hofmann, 1989) and regulate their intake of tannins (Verheyden-Tixier & Duncan, 2000) which may allow them to ingest more tannins than fallow deer as long as the concentrations are not too high. The proportion tannins in the forage were, however, not measured in this study.

4.2.2 Digestible fiber and lignin

The proportion of hemicellulose and lignin was significantly higher for fallow deer compared to roe deer in spring. The content of lignin for roe deer is lower compared to previous studies where the lignin reached 17% of dry matter intake in February (Abbas *et al.*, 2013) compared to 13% in this study. In spring, male fallow deer where the sole representatives of the fallow deer data in this study, as compared to other seasons where all gender/age classes of fallow deer were included in the over all mean for fallow deer. This results in a skewed representation of fallow deer in spring. Therefore, the higher proportion of lignin for fallow deer in spring may be due to the tolerance for lower quality forage for adult males compared to roe deer based on e.g., their anatomical differences (Hofmann, 1989) or larger rumen (Demment & Van Soest, 1985). In white-tailed deer, it has been shown that they do not avoid lignin as long as the cell solubles where relatively high (Daigle, 2004), which could be another explanation of the higher lignin content of fallow deer in spring. It is, however, important to note that even though the lignified part of the forage is considered to be indigestible for a ruminant, it is required for a adequate rumination (van Soest, 1994).

The proportion of hemicellulose was significantly higher for fallow deer compared to roe deer in spring, summer and fall, and the intake of hemicellulose remained relatively stable for fallow deer across seasons. In the case of roe deer, hemicellulose was lowest in spring and increased gradually to the highest proportions in winter. This could be interpreted as roe deer avoiding hemicellulose in spring, but are forced to an increased intake as the forage matures, in accordance with the predictions (P1 and P2). As an example, roe deer has been shown to prefer feeding in the field layer (Liberg & Wahlström, 1995; Cederlund, 1983; Cederlund *et al.*, 1980), selecting high quality patches as well as plant species with high quality within patches, depending on seasonal availability (Moser *et al.*, 2006). Forbs (up to 90 %) and shrubs dominate their diet in summer and dwarf-bushes in winter (Nichols *et al.*, 2016; Obidziński *et al.*, 2013; Liberg & Wahlström, 1995; Kossak, 1983; Cederlund & Nyström, 1981; Cederlund *et al.*, 1980; Gębczyńska, 1980; Siuda *et al.*, 1969). The content of hemicellulose has been shown to vary between 170 – 180 g/kg DM in forbs and between 170 – 190g/kg DM in browse in all seasons (Verheyden-Tixier *et al.*, 2008). In graminoids, hemicellulose may range between 100 – 300 g/kg DM, increasing with maturity (McDonald *et al.*, 2011). In addition, it has been shown that roe deer in the boreal system select dwarf-shrubs in fall and the beginning of winter at a snow depth less than 50-60 cm but are forced to a diet dominated by twigs and bushes at greater snow depths (Cederlund *et al.*, 1980). In another case, roe deer increased their intake of grasses instead of twigs in fall and winter compared to summer (Abbas *et al.*, 2013), which may have even higher content of hemicellulose (up to 320 g/kg DM in winter; Poli *et al.*, 1996; Verheyden-Tixier *et al.*, 2008).

There was a significant difference between the two species in terms of cellulose in summer, of which fallow deer had higher proportion cellulose in their rumen than roe deer. The higher cellulose content of fallow deer in summer is in accordance with the prediction that roe deer would select a diet low in cellulose when food is abundant (Hofmann, 1989), and may be due to the higher intake of graminoids for fallow deer compared to roe deer in summer (Nichols *et al.*, 2016). The content of cellulose in graminoids has been shown vary between 196 – 380 g/kg DM (Verheyden-Tixier *et al.*, 2008; Poli *et al.*, 1996), whereas the protein content is highly variable in grass ranging from 80 g/kg DM in winter to 190 g/kg DM in spring (Verheyden-Tixier *et al.*, 2008; Poli *et al.*, 1996). There are several studies showing that the diet of fallow deer is frequently dominated by graminoids (Nichols *et al.*, 2016; Obidziński *et al.*, 2013; Marinucci *et al.*, 2005; Poli *et al.*, 1996; Putman *et al.*, 1993; Garcia-Gonzalez & Cuartas, 1992; Putman, 1986), but sometimes dominated by trees or shrubs (Morse *et al.*, 2009; Poli *et al.*, 1996; Garcia-Gonzalez & Cuartas, 1992; Bruno & Apollonio, 1991) or acorns (Morse *et al.*, 2009). In contrast, roe deer ingest less than 5.5% grass in all seasons (Obidziński *et al.*, 2013; Liberg & Wahlström, 1995; Kossak, 1983; Cederlund & Nyström, 1981; Cederlund *et al.*, 1980; Gębczyńska, 1980; Siuda *et al.*, 1969). However, the similar proportions of cellulose in both species in spring, fall and winter contradicts the prediction of higher proportion cellulose of fallow deer because their higher cellulolytic fermentation irrespective of body weight (Hofmann, 1989; Prins & Geelen, 1971) when accounting for seasonal variation. Nonetheless, the small sample sizes of roe deer may obscure any significant differences, as indicated by the high confidence interval. However, roe deer has previously been found to select for lower content of cellulose in the forage in all seasons (Tixier *et al.*, 1997). In addition, the content of cellulose of roe deer rarely reach 20% of the ingested forage (Deutch *et al.*, 1998; Holand, 1992). In our data set, the mean cellulose content of roe deer was highest in winter (23.6 – 25.1%), perhaps because they are forced to a diet higher in cellulose because of the physiochemical changes in forage, and/or because of intraspecific competition between roe deer and fallow deer in winter (Ferretti *et al.*, 2010; Ferretti *et al.*, 2008; Focardi *et al.*, 2006). In fact, recent studies have shown that roe deer decrease their use of artificial feeding sites at Koberg in the presence of competitors such as fallow deer (Ossi *et al.*, 2017; Cederholm, 2012).

4.2.3 Balancing of nutrient intake between species

Because fat is not a large component of the natural forage of deer (Felton *et al.*, 2016), the RMT's showed that adult fallow and roe deer maintain a stable proportion of total non-structural carbohydrates (TNC) (Figure 2a) compared to protein (Figure 2b) and digestible fibre (Figure 2c), with strongest pattern shown by the roe deer. Both species, however, allow available (Figure 2b) and digestible fibre (Figure 2c) to vary with food composition in summer (Appendix 8.4). Mixing of nutrient intake have previously been observed in moose (Felton *et al.*, 2016), white-tailed deer (Berteaux *et al.*, 1998), red deer (Ceacero *et al.*, 2015; Ceacero *et al.*, 2010; Ceacero *et al.*, 2009) and elk (Beck & Peek, 2005).

As carbohydrates represent the main source of energy for the microbes (Bach *et al.*, 2005; van Soest, 1994), the lower threshold TNC (Figure 2a) can be explained by the energy requirements (carbohydrates) of the rumen microbes (Felton *et al.*, 2016). A deficiency of starch can lead to reduced digestibility of fibers, reduced reproductive performance and metabolic disorders (van Vuuren *et al.*, 2010; van Knegsel *et al.*, 2005). The TNC's (starch and sugars) promote the microbial growth (Stern & Hoover, 1979) by increased uptake of N (Cameron *et al.*, 1991; Stern *et al.*, 1978) and thus improve the production of microbial protein and microbial AA supply in the small intestines (Bach *et al.*, 2005). In fact, the protein requirements of a ruminant are met to large extent by the microbial protein synthesis (Russell *et al.*, 1992), whereas a smaller amount originates from rumen undegradable proteins absorbed in the small intestines (Patton *et al.*, 2014; Bach *et al.*, 2005). However, if protein is deficient in the diet, ruminants are able to increase the recycling of urea, and are thereby able to compensate for the dietary loss and maintain the microbial protein synthesis (Russel *et al.*, 1992; Kristensen *et al.*, 2010; Appendix 8.5). Thus, when non-structural carbohydrates are not limiting, ruminants are less dependent on protein from their diet, as indicated by larger variation in AP than TNC for both species (Figure 2b). In contrast, excess of soluble carbohydrates may reduce pH, inhibit the microbial growth and activity (Keunen *et al.*, 2002; Ekinci & Broderick, 1997; Clark *et al.*, 1992; Russell *et al.*, 1992) and, hence, reduce the digestibility of fiber (Bach *et al.*, 2005; Russell *et al.*, 1992). This in turn may cause gastrointestinal disturbances and/or subacute ruminal acidosis (Keunen *et al.*, 2002; Olson *et al.*, 1999; Ekinci & Broderick, 1997). This may, as a consequence, set the upper threshold for TNC intake (Felton *et al.*, 2016; Appendix 8.4). In winter, the points of fallow deer were clustered more tightly along the vector for AP and non-structural carbohydrates than roe deer, indicating that roe deer are not able to regulate the intake of these macronutrients. This may be due to the competition between roe deer and fallow deer (Ferretti *et al.*, 2010; Ferretti *et al.*, 2008; Focardi *et al.*, 2006) so that fallow deer use the feeding sites to a greater extent compared to roe deer (Cederholm, 2012; Kjellander *et al.*, 2006).

4.3 Intraspecific comparisons

There may be some confusion regarding the two different hypotheses with the same predictions, but with different explanations, namely the body-size-dimorphism hypothesis and forage selection hypothesis of which the latter is based on the Jarman-Bell principle applied at intra-specific level (Miranda *et al.*, 2012). Both predict that larger males feed on high-fiber forages, whereas females select forage low in fiber in order to meet their energy requirements. However, the predictions of sexual-size-dimorphism hypothesis based on the Jarman-Bell principle are only valid if the interspecific allometry follows the same principle as the intraspecific allometry (Appendix 8.3).

4.3.1 Intraspecific variation of nutrient intake

Adult males had significantly lower AP compared to adult females and female fawns in fall, when comparing all gender and age classes across winter and fall. This is in accordance with the last prediction (v) i.e. larger males would have lower protein content compared to fawns and adult females because of the differences in body mass (Main *et al.*, 1996) and because fawns are expected to have a higher protein requirement for growth (Smith *et al.*, 1975). There were no significant differences in terms of hemicellulose and cellulose between gender and age classes, which do not fully support the predictions (P2 and P3) that males would select forage with higher proportion digestible fibers (cellulose and hemicellulose; e.g., Main *et al.*, 1996). The results differ from a previous study which concluded that females and fawns had a stronger selection for lignin and protein during the rut, whereas males selected for fiber (Miranda *et al.,* 2012).

In this study, the RMT's (Figure 3b) indicated that adult males actually select for a stable proportion of protein and there were no significant differences between gender and age classes during the fall in terms of lignin. In winter calves had significantly higher proportion AP compared to adult females. A recent study found that, in the case of roe deer, juveniles used feeding sites more compared to adults (Ossi *et al.*, 2017), which may explain the higher proportion protein for fawns in winter compared to adult females. In addition, adult females had significantly higher proportion lignin in their rumen contents compared to fawns, subadult males and adult males in winter. This shift to forage lower in lignin of subadult- and adult males compared to adult females in winter may be due to a greater use of artificial feeding sites by males compared to females in Koberg (Cederholm, 2012), allowing males to ingest more protein and less digestible fiber and lignin in winter from supplemental feeding while the quality (e.g., proportion protein decrease) of available forage for adult females is lowest during winter. In order to be able to include summer into the intraspecific comparisons, an additional analysis was performed on adult males, as fawns were lacking in summer. In addition to the previous analysis, the latter analysis revealed that adult males had significantly higher proportion lignin in their rumen contents compared to adult females and subadult males in summer. However, females were expected to select a diet higher in protein compared to adult males because of the greater energy expenditure of gestation and lactation (e.g., Main *et al.* 1996, Barboza *et al.,* 2009), but there were no significant differences in terms of protein of adult females compared to adult males even though approx. 40% of the females in our data set were lactating. This could, however, be due to the unbalanced data in summer and fall as well as smaller sample size for males increasing the confidence interval.

4.4 Balancing of macronutrient intake between gender and age class of fallow deer

The analysis of food composition of fallow deer using RMT's showed that adult females and sub-adult males maintained a stable proportion of macronutrients contributed by total non-structural carbohydrates than adult males in fall and winter, whereas males are more flexible (Figure 3a). In addition to the reasoning of maintaining a stable intake of TNC (section 4.2.3), a high intake of e.g., glucose is associated with reduced milk production and quality, as well as impaired metabolic adaptations to lactation (Larsen & Kristensen, 2009), which may be associated with the regulation of TNC by females. In contrast, deficiency of starch can reduce milk production (van Vuuren *et al.*, 2010). Adult males, however, regulated their intake of protein in fall more tightly compared to females and subadult males. For adult males (≥ 4 years old) there is a period of voluntary hypophagia (i.e. lack of feed intake) during the rut, whereas subadult males have a slight decrease in feeding activity (Apollonio & Di Vittorio, 2004). In Sweden, the mating season occurs between the second half of October to middle of November (Carlström & Nyman, 2005; Johansson, 2001). During starvation, animals end up in a negative energy balance i.e. a catabolic state involving mobilization of energy reserves (Herdt, 2000). The lower threshold of protein intake by adult males could be an attempt to provide enough AA's for the ammonia pool in order to avoid mobilization of AA's from muscles for the gluconeogenesis in the liver during prolonged starvation (Patton *et al.*, 2014), while ingesting enough protein in order to sustain an effective fibre utilization. This is because a diet deficient in N can lead to poor utilization of digestible fiber (Souza *et al.*, 2010) and subsequent starvation. The upper threshold could be due to the higher risk of urea poisoning when ingesting too high amounts of N during starvation (Edjtehadi *et al.*, 1978; Appendix 8.5). However, the mechanism behind a stable intake of proteins and if this is related to the late rut in fallow deer compared to roe deer (Mid July – end of August; Cederlund & Liberg, 1995), requires further investigation. In winter, the pattern was the opposite: females and subadult males selected for a stable protein intake, whereas males allowed protein to vary. This, again, could be due to the tendency of adult males to use the feeding sites to a greater extend than females and subadult males (Cederholm, 2012).

4.5 Improvements and considerations

It was predicted that (P3) there should be intersexual differences in nutrient composition in the highly dimorphic fallow deer, with males selecting low-quality forage compared to females but there would be no inter-sexual differences in the monomorphic roe deer. In this data set, we did not have enough data in order to compare the genders of the two species. By comparing the intraspecific differences within species, it would be possible to test if differences arise from differences of gender per se, or because of differences in body size. In addition to dietary factors affecting foraging decisions, there are non-dietary factors such as differences in predator avoidance between and within species (Christianson & Creel, 2009; Kie & Bowyer, 1999) as well as human impact (Hebblewhite & Merrill, 2011), which are not taken into account in this project. The study area is divided into two management areas where the northern (27.1 km^2) and southern part (54 km^2) is separated by a fenced road acting as a barrier for animal dispersal, one with high population density of fallow deer and the one with a lower density of fallow deer. This could be used to further investigate the niche differentiation and overlap of the two species and interspecific competition, because of the negative relationship between fallow deer density and roe deer density (Ferretti *et al.*, 2010; Focardi *et al.*, 2006), but also the intraspecific variation between genders.

4.6 Applied nutritional ecology

Relatively few studies are made on seasonality in digestive function and food selection, especially of deer species. It is sometimes stated that food selection is driven by protein in summer and energy in winter (Gray & Servello, 1995; Klein, 1990), which highlights the importance of further investigations of which factors influence food selection across seasons. Additionally, it is important to consider that forage quality is a relative term. Quality is often defined in relation to the relative proportion of cell contents (high digestibility) and cell wall material (low digestibility) (e.g., Hofmann 1989). In other words, it is commonly suggested that for ruminants “high quality” = low fibre and “low quality” = high in fibre forage (discussed in e.g., Felton *et al.,* 2016). At the same time, nutritional requirements and intake vary among and within animal species on an individual level. In addition, the term “concentrate selector” (Hofmann, 1989) is controversial (Clauss & Dierenfeld, 2008; Shipley, 2002) and therefore the term browser is commonly used. However, browse is not considered to contain more soluble carbohydrates than grass, but contains higher proportions of soluble fibers such as pectins (Clauss & Dierenfeld, 2008).

By using a multidimensional approach (Raubenheimer *et al.*, 2009), it is possible to see how animals regulate and balance their intake of macronutrients. In this project, it was seen that both roe deer and fallow deer selected for a particular balance of macronutrients, as well as across age- and gender classes within fallow deer. In another case, it was shown that moose increased their intake of twigs (i.e. browse) when restricted to an unbalanced pellet diet in relation to the self-selected diet composition under free-choice conditions, and it was suggested that this was done as an attempt to compensate for the disproportionately high energy content of the pellets (Felton *et al.*, 2016). In the case of roe deer in this study, instead of selecting for a high content of soluble carbohydrates, our results showed that they select for a particular balance of soluble carbohydrates: not too much and not too little. It can therefore be harmful to only consider forage with high digestibility as having “high quality” when composing supplementary feeding for wild or captive ruminants (Felton *et al.*, 2017; Felton *et al.*, 2016; Gattiker *et al.*, 2014; Clauss *et al.*, 2013). In addition, ruminants may compensate for an unbalanced diet derived from supplementary feed high in non-structural carbohydrates by increasing their intake of woody browse, and intensify the damages on forest (Felton *et al.*, 2017). In fact, browsing pressure on a normally avoided spruce has been shown to increase adjacent to artificial feeding sites in Koberg (Garrido *et al.*, 2014), possibly to compensate for an unbalanced diet.

5 Conclusions

In conclusion, the comparisons of diet nutrient composition showed that proportions of macronutrients varied across species and seasons and that there were differences also across gender and age classes of fallow deer. In addition, both roe deer and fallow deer selected for a particular balance of macronutrients, especially they appear to tightly regulate intake of soluble carbohydrates, with the strongest such pattern shown by the roe deer. This contradicts the prediction that roe deer select for a diet high in solubles, but instead carefully select for a particular balance of soluble carbohydrates. Additionally, adult males selected for a stable proportion of protein in fall, which may be associated with the period of hypophagia, but the mechanism behind such regulation remain for future investigations. Knowledge about different foraging adaptations and drivers behind food selection can be used to determine a correct carrying capacity of wildlife populations (Raubenheimer *et al.*, 2012), to formulate balanced diets in order to avoid damages on forest (Felton *et al.*, 2017) or to better understand inter- and intraspecific competition. Nutrition of wild herbivores is therefore integrated with the fields of wildlife management and forest management, and further investigations of the seasonal adaptations to forage are highlighted.

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8 Appendix

8.1 The detergent system of van Soest

Originally, the information about the food composition was based on proximate analysis of food (moisture, ash, crude protein, ether extract, crude fibre and nitrogen-free extractives) (McDonald *et al.*, 2011). However, this system has been criticized as being imprecise and archaic due to an underestimation of fibre and an overestimation of starch and sugars (McDonald *et al.*, 2011). Modern methods of analyzing the composition of feed are the detergent system of Van Soest for fibre analysis (van Soest *et al.*, 1991) and Near infrared reflectance spectroscopy (NIRS). The basic concept behind the detergent system is that constituents of plants can be divided into cell walls and cell contents. Cell wall constituents are insoluble in neutral detergent fiber, and are further divided into the fiber that is soluble in acid detergent (hemicelluloses and fiber-bound nitrogen, ADF-N) and the fiber that is insoluble in acid detergent (cellulose, lignin, lignified nitrogen and silica; van Soest *et al.*, 1991). In contrast, cell contents (lipids, sugars, pectin, starch, non-protein nitrogen and soluble protein) are soluble in neutral detergent fiber (van Soest *et al.*, 1991).

8.2 Interspecific differences in anatomy and metabolism

Roe deer are characterized by having a smaller reticulo-rumen (amount of tissue relative to body weight) and thus a smaller rumen capacity compared to fallow deer (Table 6; Prins & Geelen, 1971; Nagy & Regelin, 1975; Hofmann, 1989). Fallow deer have a longer retention time and thus suggested to be able to digest more fiber (Clauss *et al.*, 2008; Hofmann, 1989). In addition, roe deer have larger abomasum and smaller omasum relative to body weight than fallow deer (Nagy & Regelin, 1975). Their ability to digest cellulose is also considered to be low compared to grazers (Hofmann, 1989). Moreover, the rumen papillae are unevenly distributed and less prominent which results in a smaller total absorptive surface in fallow deer compared to roe deer (Clauss *et al.*, 2009b). Additionally, roe deer have larger salivary glands compared to that of fallow deer (Clauss *et al.*, 2008; Fickel *et al.*, 1998; Hofmann, 1989).

Table 6. *Characteristics of anatomy and metabolism in roe deer and fallow deer based on Hofmann's (1989) classification*

	Roe deer	**Fallow deer**
Anatomy		
Rumen[1]	Small	Larger
Omasum[2]	Less developed	Larger
Abomasum[3]	Large	Smaller
Salivary glands[4]	Large	Smaller
Rumen papillae[5]	Prominent	Less prominent
Small intestines[9]	Shorter	Longer
Large intestines	Longer	Shorter

Metabolism		
Fermentation rate[6]	High	Lower
Absorption of VFA[9]	Fast rate	Lower rate
Cellulolytic activity[7]	Low	Higher
Diet		
Composition[8]	High in solubles and low in insoluble fiber (e.g., cellulose)	Mixed. Avoid insoluble fiber, but is more adaptive to variation (in e.g., cellulose)

[1] Hofmann (1989); Nagy and Regelin (1975); Prins and Geelen (1971), [2] Hofmann (1989); Nagy and Regelin (1975), [3] Hofmann (1989); Nagy and Regelin (1975), [4] Fickel *et al.* (1998); Hofmann (1989), Clauss *et al.* (2008), [5] Clauss *et al.* (2009b), [6]Clauss *et al.* (2008); Hofmann (1989), [7] Hofmann (1989), Hofmann (2000) [8] Hofmann (1989), Hofmann (2000) [9] Hofmann (1989).

8.3 Intraspecific comparasions

As van Soest (1994) pointed out, the body weight to the power of 0.75 may not account for differences between sexes within species and should therefore not be used for intraspecific comparisons. The application of Jarman-Bell principle on intraspecific level is not fully supported (Luna *et al.*, 2013; Perez-Barberia *et al.*, 2008), and there are experimental studies that have failed to provide evidence that the Jarman-Bell principle is applicable for intraspecific comparisons because of the variation within gender (Weckerly, 1993) and because no differences in forage digestibility and/or quality were found between genders (Perez-Barberia *et al.*, 2007; Perez-Barberia & Gordon, 1999)

8.4 Carbohydrate metabolism in ruminants

The digestible fiber represents a main source of energy for the microbes in the rumen and is necessary for a functional rumen (Van Soest, 1994), although the digestive efficiency may vary between the species (Prins & Geelen, 1971). However, too high fiber intake in relation to other macronutrients increases the time of rumination and will not provide enough energy for the microbes (Van Soest, 1994), and therefore not the animal, which could lead to starvation. However, deficiency or excess of soluble carbohydrates are associated with several metabolic disorders (van Vuuren *et al.*, 2010; van Knegsel *et al.*, 2005; Keunen *et al.*, 2002; Olson *et al.*, 1999; Ekinci & Broderick, 1997). One of them is acidosis, which is considered to be particularly common in “browsing” ruminants (Clauss *et al.*, 2003), which could explain the more pronounced selection of a particular proportion of TNC for roe deer compared to fallow deer. In addition, acidosis is associated with a number of other disorders such as laminitis, decreased milk production and laminitis (Nocek, 1997; Underwood, 1992).

8.5 Nitrogen metabolism in ruminants

In ruminants, the ammonia is an important intermediate for microbial protein synthesis and excess is transported and converted to urea in the liver (Patton *et al.*, 2014; Hunnington & Archibeque, 2000). The urea is then excreted in the urine, or recycled to saliva and the rumen (Kristensen *et al.*, 2010). Thus, ruminants are able to meet their amino acid requirement as long as carbohydrates are not restricted. In terms of microbial protein, a notable part of the protein requirements for ruminants are met by microbial protein synthesis (Russell *et al.*, 1992). As an example, in dairy cattle, most protein (50 – 80%) reaching the small intestines for uptake is derived from microbial protein, whereas the remaining mainly originates from rumen undegradable protein (Patton *et al.*, 2014; Bach *et al.*, 2005; Storm & Ørskov, 1983) not available for absorption (such as ADF-P). However, high levels of starch in the diet can decrease the ruminal ammonia concentration (Olson *et al.*, 1999; Cameron *et al.*, 1991) and hence the microbial protein synthesis. During prolonged starvation, when glycogen stores are depleted, animals rely of hepatic glucose (Harvey & Ferrier, 2011). Animals are, however, able to use ketone bodies (instead of hepatic glucose) from the liver as energy for muscles and brain, and oxidized fatty acids as energy for the liver in order to spare glucose and muscle protein during prolonged starvation (Harvey & Ferrier, 2011). But in ruminants, this is associated with an increased of other metabolic disorders in ruminants such as ketosis (Herdt, 2000).

8.6 Acquisition of NIR spectra

Each pulverized rumen sample was thoroughly mixed before drawing approximately 40 g sample for scanning. The sample was filled into standard sample cup (diameter = 3.8 cm and depth = 0.9 cm) and covered with a piece of hard paper. NIR reflectance spectra, expressed in the form of log (1/Reflectance), were acquired with XDS Rapid Content Analyzer (FOSS NIRSystems, Inc.) from 780 to 2498 nm at an interval of 0.5 nm. Prior to collecting the NIR spectra of each sample, reference reflectance measurement was taken using the standard built-in reference of the instrument. For every sample, 32 monochromatic scans were made and the average value recorded. A total of 499 spectra were recorded, of which 61 samples belonged to roe deer and the remaining belonged to fallow deer. After examining the scores of the principal component analysis (PCA), 79 fallow deer samples were selected for wet chemistry analyses. The selected samples ranged from the highest to the lowest scores with an interval of ca. 2 points.

8.6.1 Calibration modelling

Prior to modelling, the data set was divided into calibration and validation sets (Table 5). The calibration set was composed of 105 samples for all nutritional fractions except lignin and NDF. For the latter two, the range of wet chemistry values was very narrow; hence 87 samples for lignin and 91 samples for NDF were used to develop the calibration models. Then calibration model was derived for each nutritional fraction by Orthogonal projection to latent structures (OPLS). Unlike Partial Least Squares (PLS) modelling approach, OPLS modelling is a two-step approach where it first removes spectral variations that have no correlation with the nutrition contents and then fit the calibration models based on predictive spectral variation. Path length difference and scattering are common sources of unwanted spectral variation due to packing inconsistency and particle size differences that should be filtered before developing the final model. To do this, the OPLS modelling approach used the information in the categorical response matrix Y (a matrix of dummy variables in our case) to decompose the X matrix (the spectral data) into three distinct parts: (1) the predictive score matrix and its loading matrix for X, (2) the corresponding Y-orthogonal score matrix and loading matrix of Y-orthogonal components, and (3) the residual matrix of X (Trygg & Wold, 2003). Components orthogonal to the response variable containing unwanted systematic variation were then subtracted from the original spectral data to produce a filtered descriptor matrix. The final discriminant model was then computed using the filtered predictive spectral variations only. The models were first fitted on the entire NIR region (780 – 2500 nm). Based on model performance, the calibration models were refined using selected NIR regions. All calibrations were developed on mean-centered data sets and the number of significant model components was determined by a seven-segment cross validation (a default setting). A component was considered significant if the ratio of the prediction error sum of squares (PRESS) to the residual sum of squares of the previous dimension (SS) was statistically smaller than 1.0 (Eriksson *et al.*, 2006). Finally, the computed models were validated using prediction sets and used for predicting the nutrition contents of the remaining samples.

To analyze absorption bands that influenced the models, a parameter called Variable Influence on Projection (VIP) was computed as follows.

$$VIP_{AK} = \sqrt{\left(\sum_{a=1}^{A}\left(w_{ak}^{2} * (SSY_{a-1} - SSY_{a})\right) * \frac{K}{(SSY_{0} - SSY_{A})}\right)}$$

VIP for *A* components and *K* variables is a weighted sum of squares of the OPLS weights (*w)* for a given component *a* and *k* variable, taking into account the amount of explained Y-variance (*SSY*) of *a* component, and SSY_0 and SSY_A are sum of squares of the response variable Y before and after extracting A number of components, respectively. Its major advantage is that there will be only one VIP-vector, summarizing all components and Y-variables, thereby enabling absorption bands that influence the calibration models to be identified. As a rule, predictors with VIP value greater than 1.0 have a strong influence on the model (Eriksson et al. 2006). All calculations were performed using Simca-P+ software (Version 13.0.0.0, Umetrics AB, Sweden).

Table 7. *Number of samples, range of values and means for nutritional contents used in calibration and validation data sets.*

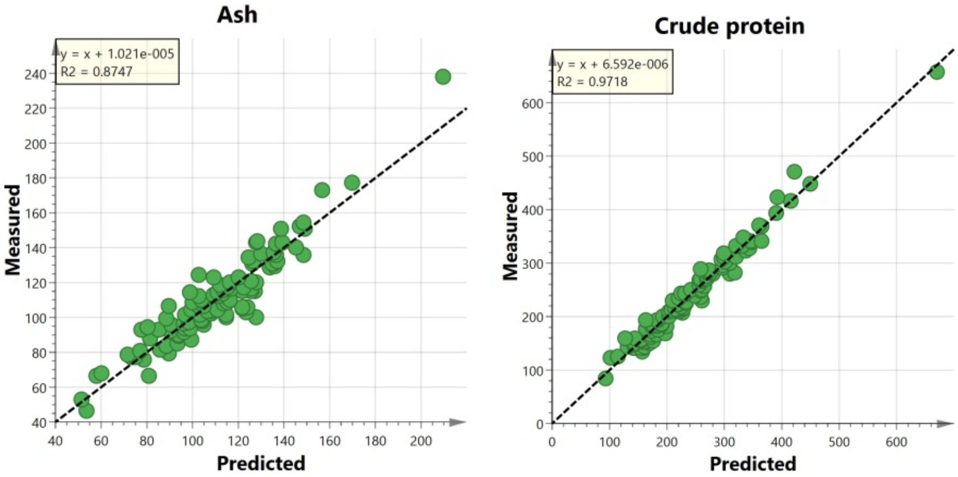
Nutritive fraction	Calibration data set			Validation data set		
	Mean	N	Range	Mean	N	Range
Ash*	111.5	101	46.5 – 237.9	110.7	32	57.01 – 107.5
Crude protein	247.8	105	84.4 – 656.4	243.3	35	134.5 – 500.2
Available N	39.2	105	13.4 – 102.9	38.4	35	21.3 – 77.03
ADF	329.1	105	122.7 – 551.3	324.8	35	152.2 – 473.4
NDF	514.4	91	154.1 – 752.4	512.8	30	233.5 – 693.6
Lignin*	111.2	87	41.7 – 222.4	115.03	32	43.8 – 201.9

* Note that 19 samples for ash and 21 samples for lignin were excluded during calibration due to the narrow range of the measured lab values. Excluding these samples substantially improved the prediction ability of the developed model. Values are rounded for simplicity of presentation.

Table 8. *Summary of model statistics developed for predicting nutrition content of rumen samples.*

Nutritive fraction	RMSEE*	R^2cv	RMSEP**	R^2test
Crude protein	15.2	0.972	20.6	0.951
Available nitrogen	2.48	0.969	3.51	0.934
ADF	17.4	0.961	18.2	0.947
Ash	10.1	0.875	12.5	0.796
NDF	29.4	0.941	27.4	0.924
Lignin	1.15	0.845	1.18	0.749

* RMSEE: root mean square error of estimation; ** RMSEP: root mean square error of prediction; R^2cv and R^2test: coefficient of determination for the regression between observed and predicted using cross validation and test sets, respectively.



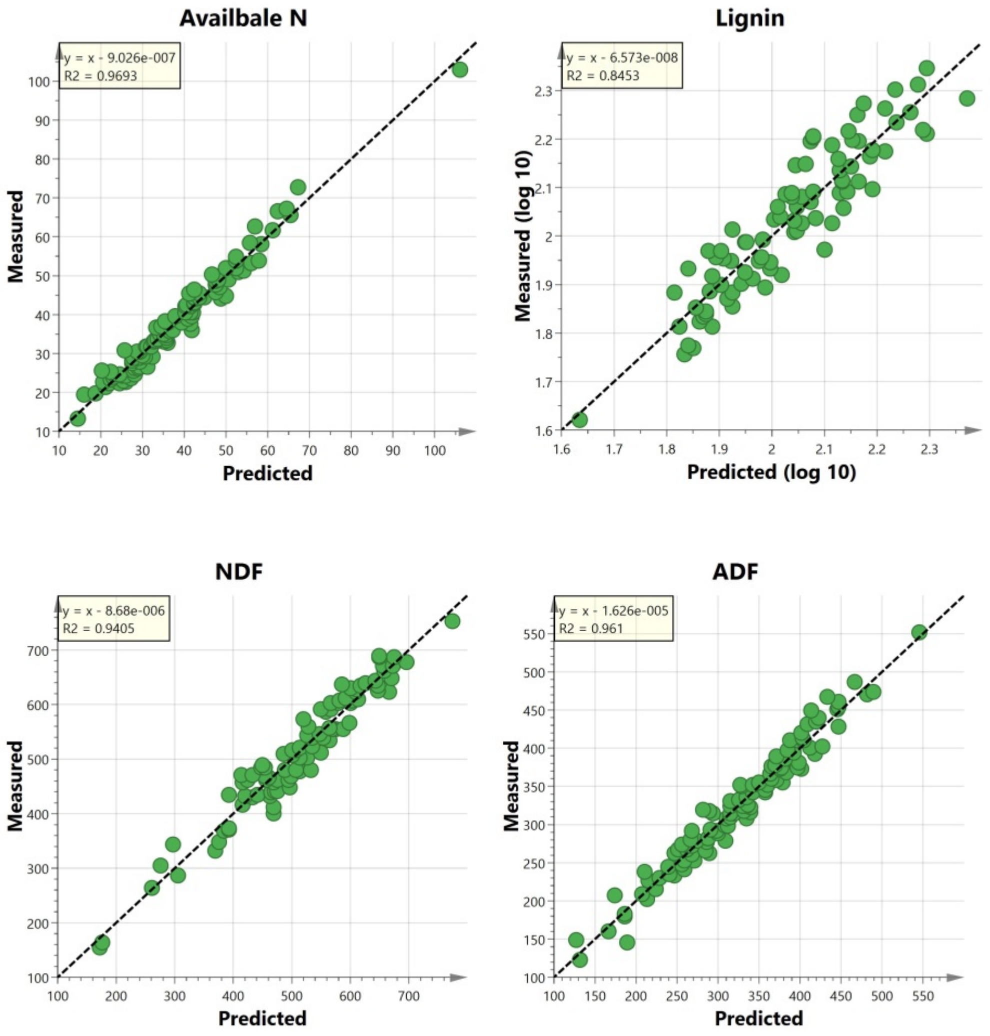
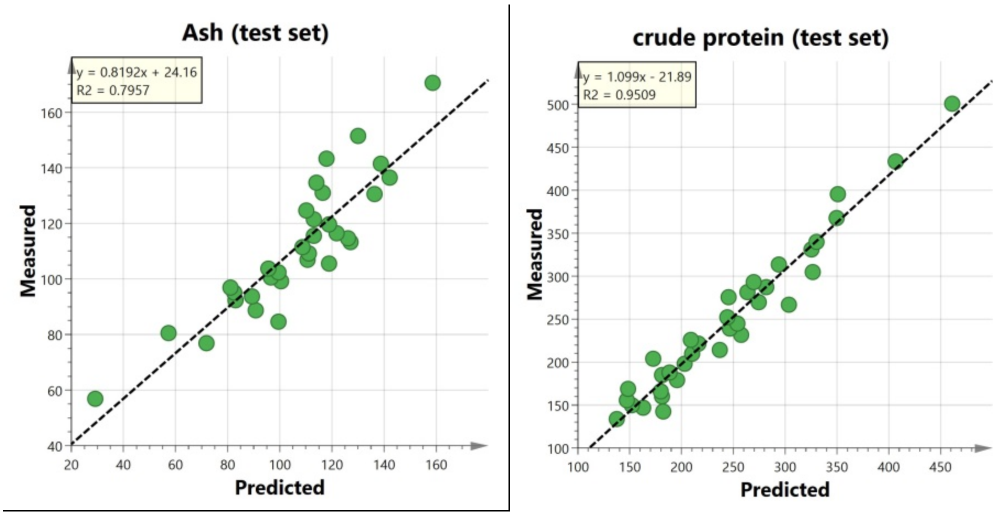


Figure 4. Predicted and measured nutrition contents of deer rumen samples based on cross validation of calibration data sets.



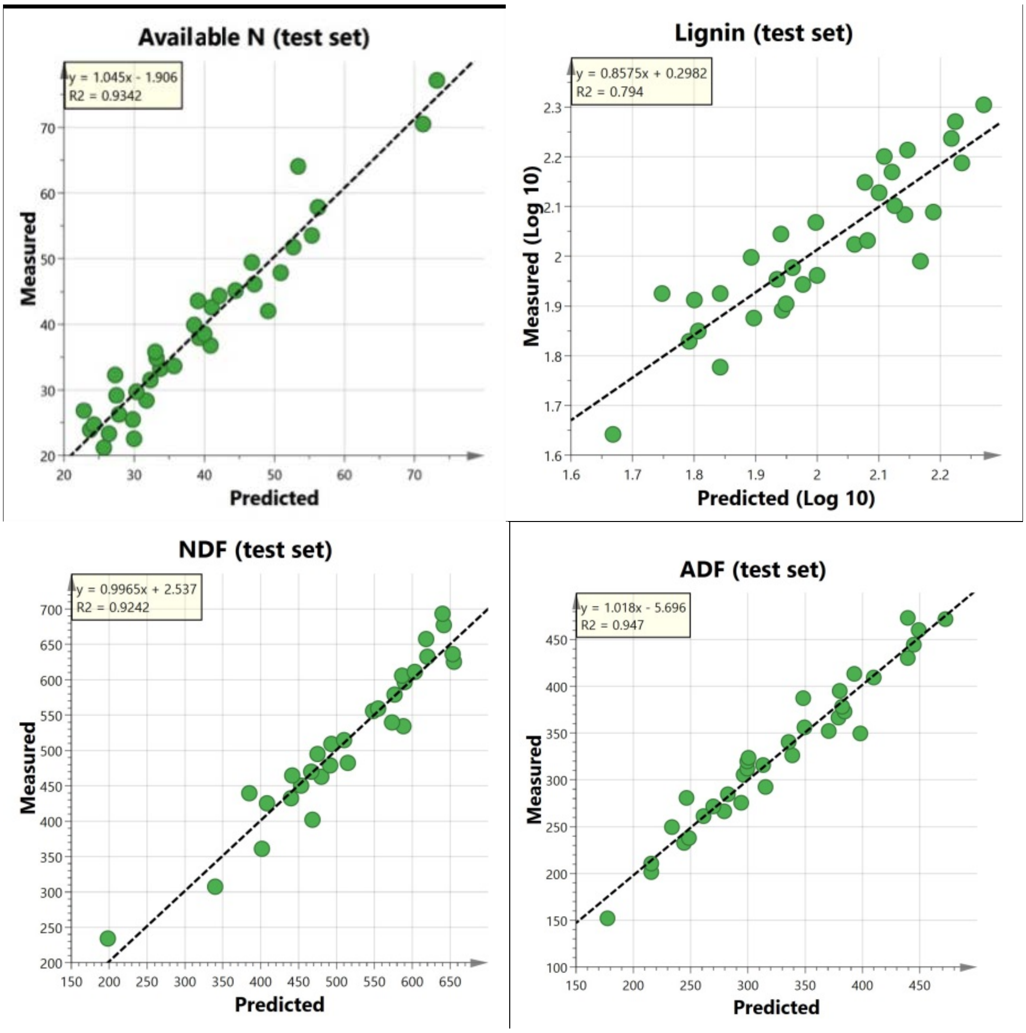


Figure 5. Predicted and measured nutrition contents of deer rumen samples for prediction test sets.

8.7 Comparative nutrient intake between species and seasons

A) **Spring** **Fall**

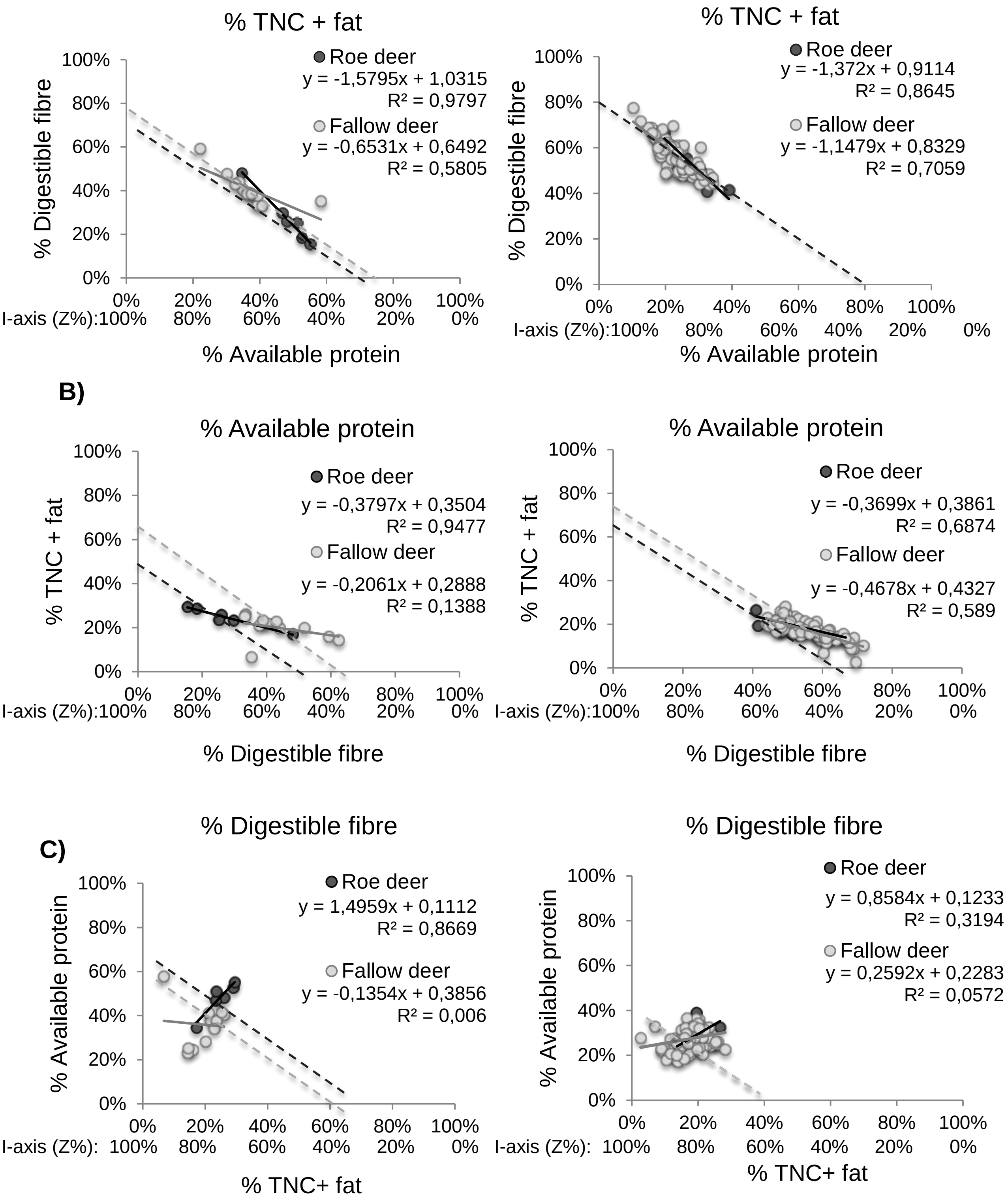


Figure 6. Three-component RMT's for roe deer and fallow deer representing a) total non-structural carbohydrates (TNC) and fat, b) available protein and c) digestible fibers in spring and fall.

8.8 Comparative nutrient intake within fallow deer in summer

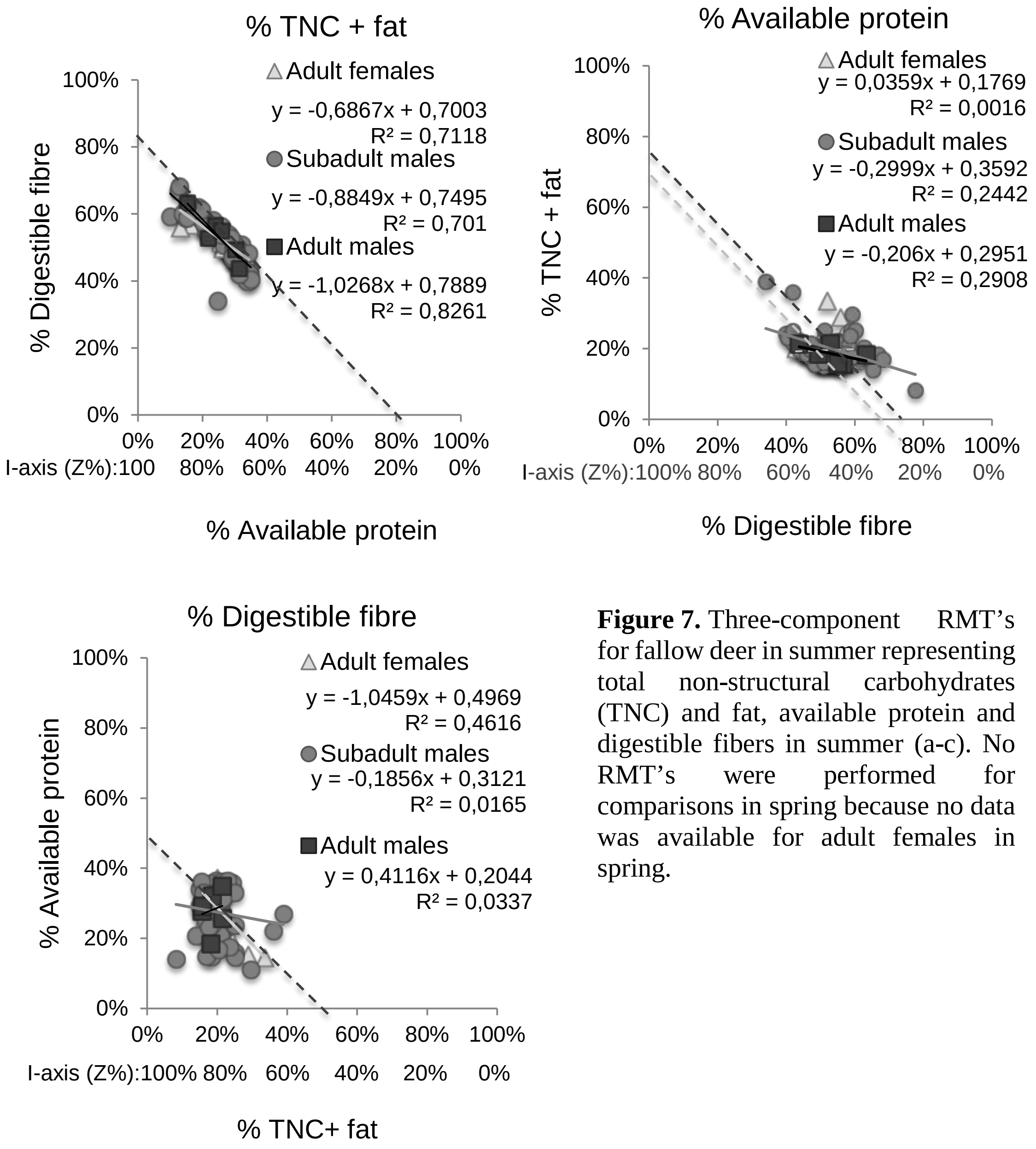


Figure 7. Three-component RMT's for fallow deer in summer representing total non-structural carbohydrates (TNC) and fat, available protein and digestible fibers in summer (a-c). No RMT's were performed for comparisons in spring because no data was available for adult females in spring.