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Faecal nitrogen, an index of diet quality in roe deer *Capreolus capreolus*?

Hélène Verheyden, Lise Aubry, Joël Merlet, Patrick Petibon, Béatrice Chauveau-Duriot, Nadine Guillon & Patrick Duncan

Indices of diet quality are useful tools for research on the ecology, behaviour and the management of wildlife. The quality of diets is an essential driver of the performance of individuals, which in turn drives the dynamics of populations. Dietary nitrogen (DN) is a good index of diet quality in some herbivores, and faecal nitrogen (FN) has proved a good predictor of DN in grazers, but there is some doubt as to its validity in browsers, since they eat food with significant amounts of tannins, which bind with proteins. In order to test the validity of FN to predict DN, we first test for seasonal and spatial variations of FN in a population of roe deer *Capreolus capreolus*. Secondly, we examine the relation between DN and FN according to the occurrence of condensed tannin in the diets of 41 individuals of this population. Third, using feeding trials with captive roe deer, we test if free condensed tannins in diets affect the relationship between DN and FN. The results show that FN broadly follows seasonal and spatial variations of food quality, with higher values in spring, and higher values in roe deer living in a farmed area with richer food resources compared to those of deer in forests. Both in wild and captive animals, DN increases with FN when the dietary condensed tannin is low or absent, but the relationship disappears when there is an appreciable amount of condensed tannin in the diet (> 2%). We conclude that FN is not suitable for predicting individual short term diet quality for browsers that ingest significant levels of tannin. FN may, nonetheless, reflect broad seasonal and spatial patterns of diet quality at the population level.

Key words: browser, *Capreolus capreolus*, food, indicator, nutrient, roe deer, ruminant, tannin

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Monitoring the diet quality of wild ruminants is useful for managing their populations, and for understanding some aspects of their behaviour and ecology, in particular the dynamics of populations in relation to the quality of their food resources. Diet quality encompasses energy value, protein, water, mineral and vitamin contents as well as anti-nutritional factors such as lignin, cutin, suberin, sili-

ca and secondary metabolites (Robbins 1983). As energy and/or protein are often the most limiting nutrients for wild herbivores, research has focussed on indices designed to estimate their concentrations in animal diets (Brown et al. 1995). However, direct measurement of diets in wild herbivores is difficult and faecal indices have been proposed to assess diet quality (Putman 1984). In particular, faecal nitro-

gen (FN) is recognised as an effective indicator for the assessment of spatial, seasonal or individual variations of dietary protein in a variety of grazers (see Leslie et al. 2008 for a review).

In large herbivores, FN is composed largely of metabolic nitrogen (both bacterial and endogenous) and a small amount of undigested plant nitrogen (Wehausen 1995, Schwarm et al. 2009). Metabolic FN increases with diet digestibility because the fermentation activity and turnover of gut bacteria increases. As a consequence, there is a linear relationship between digestibility and FN for grazers eating a variety of forages (see Holechek et al. 1982 for a review). As protein content and digestibility are positively correlated in plants (Robbins 1983), FN also correlates with dietary proteins (Holechek et al. 1982, Putman 1984).

Although useful to predict dietary quality of free ranging grazers, the use of FN to index diet quality of browsers is controversial due to the effect of tannins (Robbins 1983, Hobbs 1987, Osborn & Ginnett 2001), which occur naturally in many species of dicotyledons. Tannins are phenolic compounds that bind to proteins during the chewing and digestive processes. Hydrolysable tannins are degraded and then absorbed in the small intestine, but condensed tannins (CT) are largely indigestible. CT are present in the free form in the plant vacuole, and as a form bound to fibre or to plant proteins. When ingested, CT, mainly the free form, bind to proteins (plant and bacterial proteins and enzymes) in a pH-dependent manner along the digestive tract (Makkar 2003, Min et al. 2003). Tannins may also bind with some salivary proteins (Shimada 2006). These complexes are excreted in the faeces as tannin-protein (mostly endogenous) and tannin-lignin complexes (McSweeney et al. 2001). This leads to post-ingestive effects of tannins which are both positive and negative (Silanikove et al. 2001, Makkar 2003), notably a decrease of protein digestibility and inflation of nitrogen in the faeces (Robbins 1983, Kariuki & Norton 2008). Thus, FN may serve as a valid index of diet quality in browsers only in some circumstances, e.g. if the tannin intake is too low to alter the relation between dietary and faecal nitrogen, or if the effect of the tannins is quite constant across the diets concerned.

FN appears to vary in space and time in several wild ungulate species, both grazers (bighorn sheep *Ovis canadensis*; Blanchard et al. 2003), browsers (moose *Alces alces*; Leslie et al. 1989, white-tailed

deer *Odocoileus virginianus*; Page & Underwood 2006 and black-tailed deer *Odocoileus columbianus*; Leslie & Starkey 1985, 1987) and species that are likely to be mixed-feeders (such as sika deer *Cervus nippon*; Ueno et al. 2007 and Roosevelt elk *Cervus canadensis roosevelti*; Leslie & Starkey 1985, 1987). In these studies, FN follows the seasonal variations of the quality of the vegetation, with the lowest values in winter, then an increase to reach a peak in spring before decreasing in summer and fall (i.e. Leslie & Starkey 1985 and 1987). Variations in FN also occur between years (e.g. Blanchard et al. 2003) and locations (e.g. Osborn & Jenks 1998) in relation to climate, population density, food supplementation or habitat composition. Although such variations in FN seem to be linked to the quality of food resources, direct information on the relationship between faecal and diet quality in browsers is scarce (Leslie et al. 2008). In mule deer *Odocoileus hemionus* (browser), it has been shown experimentally that the crude protein content of natural diets was well predicted by FN (Hodgman et al. 1996), but the diets tested contained very few or no tannins. High tannin intake increased FN content relatively to dietary nitrogen (DN) in elk *Cervus canadensis nelsoni* (Mould & Robbins 1981), mule deer (Robbins et al. 1987) and white-tailed deer (Robbins et al. 1987, Osborn & Ginnett 2001), especially for high-nitrogen diets.

Roe deer *Capreolus capreolus* (browser) inhabit contrasting landscapes offering a wide range of food resources, from the tundra to the Mediterranean, and from forest to farmland (Andersen et al. 1998). This species is known to ingest tannins (Tixier et al. 1997, Verheyden-Tixier & Duncan 2000, Clauss et al. 2003), but diet composition is highly variable among habitats, ranging from highly nutritious grains, free of tannins, such as maize *Zea mays*, to pure browse containing tannins, such as bramble *Rubus fruticosus* (Tixier & Duncan 1996, Cornelis et al. 1999). To our knowledge, only two published papers (Kamler & Homolka 2005, Hewison et al. 2009) and one unpublished report (Blanchard 1998) have examined FN in roe deer. These studies show seasonal variations of FN corresponding to seasonal variations of plant protein contents and spatial variation of FN in relation to habitat. However, the relation between DN and FN has never been studied experimentally in this species.

Here we aim to assess whether FN is a good predictor of diet quality, expressed as DN, in the

natural diets of roe deer. First, we search for seasonal and spatial variations of FN in a population of roe deer. Secondly, we examine the relation between DN and FN in this population. Thirdly, using captive roe deer, we performed feeding trials with natural diets with variable concentrations of nitrogen and tannins, to test if free CT affects the relationship between DN and FN.

Material and methods

FN in the wild population

We studied a population of wild roe deer living in a rural landscape in the Comminges region of southwestern France (43°13'N, 0°52'E, see Benhaïem et al. 2008 for a detailed description). Our study area is part of the LTER-Europe Network (Available at: <http://www.lter-europe.net>), and is designated as one of its Long-Term Socio-Ecological Research (LTSER) platforms. Our study area comprise several habitat types: a central forest (13% of the area = forest sector) surrounded by an open sector composed of meadows (37%) and cultivated crops (31%; mainly wheat *Triticum* spp., maize, sunflower *Helianthus annuus*, soya *Glycine max* and sorghum *Sorghum bicolor*) mixed with small woods (14%) and hedges (6%).

Detailed sampling of the available vegetation was performed for other purposes in this area, particularly to study habitat selection. We used the results for the plants that are eaten by roe deer, to assess their nutritional quality. The vegetation was collected in both sectors so as to sample both forest and open landscape units in November (autumn), February (winter), April (spring) and August (summer) 2005 on 417-565 plots per season (plot size 25 × 25 cm quadrats up to a height of 120 cm) spread along regular transects. The plant material collected was grouped in categories (plant species or family and vegetative form: leaves, fruits, seeds and twigs and buds) and sectors, dried (60°C for 72 hours) and stored in a dark and dry room until the chemical analyses were done in 2009.

We collected faeces of roe deer by searching the ground in both open and forest sectors between September 2004 and June 2007. We obtained 159 samples in autumn (September-November), 156 in winter (December-February), 58 in spring (March-May) and 111 in summer (June-August).

In addition, we collected the faeces and the rumen contents of 41 wild roe deer shot during the hunting

season (July-January) in 2004, 2005 and 2006 in both sectors. The faeces and the rumen contents were stored in a deep freezer until analysed.

The botanical composition of the rumen was assessed by macroscopic determination of plant fragments following Maizeret & Tran Manh Sung (1984). After mixing each rumen sample, we collected a 100-g subsample, washed it in a sieve with a 5 mm and a 2 mm mesh. The fractions contained in the 5- and 2-mm mesh sieves were sorted by plant categories under a binocular microscope, then dried and weighed to the nearest 0.01 g. The results for each rumen were expressed as percent dry matter by weight (DM) by plant category. We estimated the nitrogen content of the rumen contents of each roe deer from values of the nitrogen content (see below) in each plant category and their proportions in the rumen, according to the following formula:

$$DN = \left(\sum_{i=1}^n NiDi \right) / 100,$$

where DN = dietary nitrogen concentration (% DM), Ni = nitrogen concentration in the plant category i (% DM) and Di = proportion of the plant category i in the rumen (% DM).

In addition, we classified each rumen sample as containing significant amounts of tannin or not, based on the concentration we measured in the plants used for the feeding trials and from the literature on the tannin contents in plants. In the literature, a concentration of CT > 5% DM is considered as high and concentrations ranging between 2 and 5% DM as moderate (Aerts et al. 1999, Barry & McNabb 1999). Low (1% DM) or trace (< 0.5% DM) concentrations are considered insufficient to affect protein digestion (Barry & McNabb 1999, Makkar 2003). For subsequent statistical analyses, we classified diets as containing no significant amount of CT when the proportion of plants with moderate or high tannin concentration amounted < 20% of the diet (approximately < 1% tannin in the whole diet). The remaining cases were considered as diets with significant amounts of tannins since they contained > 20% of moderately or highly tannic plants.

Feeding trials with captive animals

We used four captive and tame adult roe deer (one male: A and three females: B, C and D) raised in the 'Centre d'Études Biologiques de Chizé (CEBC)' in France and habituated to feeding trials. Before and

after the feeding trials, lasting from March to November 2005, the animals were raised together in an enclosure with both grass and trees. In addition to access to natural vegetation, including tannic plants, they were offered standard pellets for goats (Alicoop Proxima, 19.5% crude protein) *ad libitum*. During the feeding trials, the animals were kept separately in rooms (20 × 20 m) without access to natural vegetation. One additional captive roe deer, a tame adult female (E), raised on the INRA (Institut National de la Recherche Agronomique) farm of Gardouch (of the research laboratory 'Comportement et Ecologie de la Faune Sauvage (CEFS)' in Toulouse, France) was tested for two diets (sorghum and wheat) in autumn 2009. Before the trial, the female was raised in a 0.5 ha enclosure together with two other females. In this enclosure they fed on natural pasture (forbs and grass) and had *ad libitum* access to pellets for roe deer (Toulgrain, La Toulousaine SA, Baziège, France). During the trial, the female E was restrained to a

small part of the enclosure (15 × 10 m) where all vegetation had been removed.

Before the feeding trials, the animals were gradually habituated to feeding on experimental diets. During the feeding trials, they were offered the experimental diets and water *ad libitum* for seven days. The animals were weighed before and after the feeding trials and their intake was recorded daily; the quantity of food offered was adjusted so that only a small amount of food remained unconsumed each day. Fresh faecal pellets were collected each morning of the last four days of the trial, dried (48 hours at 60°C) and ground for analysis for tannin and nitrogen contents.

Eight diets known to be eaten by roe deer (Tixier & Duncan 1996, Tixier et al. 1997, Cornelis et al. 1999) and containing a range of tannin concentrations were tested (Table 1). Two diets contained only traces of CT: alfalfa *Medicago sativa* leaves (Barry & McNabb 1999) and wheat seeds (MacKown et al. 2008). Ivy *Hedera helix*, contained very

Table 1. Summary of the feeding trials performed with five captive roe deer. Diet composition, date, animal, intake, dietary nitrogen, faecal nitrogen and dietary free condensed tannin (% DM and levels: 0 when ≤1% DM, 1 when >1% DM) are shown.

Diet offered	Date, month and year	Animal	Intake g DM, mean (SD)	Dietary nitrogen % DM, mean (SD)	Faecal nitrogen % DM, mean (SD)	Dietary free condensed tannin % DM, mean (SD)	Tannin in diet levels 1/0
Bramble	23-29.03.2005	A	110 (12)	1.95 (0.23)	3.19 (0.60)	8.11	1
		C	95 (27)		3.12 (0.15)		
		D	110 (11)		2.99 (0.20)		
Maple-clematis	02-07.07.2005	A	175 (81) + 143 (45)	2.11 (0.11)	3.04 (0.20)	2.63 (0.33)	1
		B	65 (35) + 39 (4)	2.11 (0.15)	3.00 (0.14)	2.83 (0.50)	
		C	69 (43) + 57 (31)	2.10 (0.10)	3.42 (0.14)	2.65 (0.45)	
		D	219 (93) + 135 (33)	2.10 (0.13)	3.13 (0.07)	2.89 (0.26)	
Maple-oak	08-16.07.2005	A	177 (63) + 147(35)	2.13 (0.04)	2.55 (0.08)	5.82 (1.50)	1
		C	31 (29) + 130(33)	2.10 (0.06)	2.66 (0.15)	2.42 (1.65)	
		D	159 (84) + 143(31)	2.13 (0.04)	2.39 (0.07)	5.47 (1.84)	
Acorn	16-22.11.2005	A	176 (70)	0.8	3.28 (0.28)	4.06	1
		B	137 (31)		2.80 (0.12)		
		C	147 (70)		2.99 (0.08)		
		D	235 (70)		3.57 (0.47)		
Sorghum	23.11-03.12.2009	E	483 (193)	1.45	4.06 (0.42)	Moderate (Dykes & Rooney 2006)	1
Ivy	15-21.03.2005	C	113 (52)	1.39 (0.10)	2.34 (0.19)	0.87	0
		D	133 (15)	2.45 (0.19)			
Alfafa	02-07.04.2005	A	403 (4)	3.52 (0.14)	3.45 (0.36)	Trace (Barry & McNabb 1999)	0
		C	394 (7)		3.33 (0.16)		
		D	396 (11)		3.06 (0.15)		
Wheat	22.09-02.10.2009	E	319 (116)	1.81	3.05 (0.32)	Trace (McKown et al. 2008)	0

little CT (our study and Gonzalez-Hernandez et al. 2003). Four other diets contained moderate to high concentrations of CT (our study): leaves of bramble, acorn (seeds of oak *Quercus* sp.), a mixture of leaves of field maple *Acer campestre* and clematis *Clematis vitalba* and a mixture of leaves of maple and oak. We did not measure the concentration of CT in the sorghum diet because the experiment was done later and in a different laboratory than the previous feeding trials. However, the sorghum grain we used (a mixture of FR2036, FR2041, FR2572 and FR2102 French lines) has red glumes and pericarp/testa that are typical of sorghum containing moderate to high concentration of CT (Dykes & Rooney 2006, Liu et al. 2009). The leaves of trees and shrubs were fresh plants collected daily in an adjacent forest and offered each morning. Acorns collected during the previous winter and frozen were offered daily after thawing. Alfalfa was offered as hay and sorghum and wheat seeds came from the Toulgrain company. Samples of each food were dried at 60°C for 72 hours and ground for analysis for tannin and nitrogen contents.

Tannin and nitrogen analysis

Both plant and faecal samples were analysed for nitrogen content using the Dumas method with a CN gas analyser (LECO Corporation, St Joseph, Michigan, USA). CT content was determined in foliage and acorn diets using the procedure described by Terrill et al. (1992). The CT were separated into three fractions. First, free CTs were extracted with a mixture of acetone, water and diethylether. Secondly, protein-bound CTs were extracted with a boiling solution containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol. After these two extractions, the residue contained only fibre-bound CT. CT of these three fractions were then determined with a butanol-HCl colorimetric procedure. For preparation of standards, purified CT were extracted from the corresponding diet by chromatography on Sephadex LH-20.

Statistical analysis

We used linear mixed-effects (LME) models fitted by maximum likelihood to test the effects of season (four modalities) and sector (two modalities) on FN in the wild deer. FN may vary between years, but our sampling was unbalanced since not all seasons and sectors were sampled for all the years. Year was therefore set as a random factor to avoid the

variance between years being included in the error term.

We used a linear model to test the effects of dietary tannins (as a bimodal factor) on the regression of DN (dependent variable) on FN (independent variable) in the wild deer. The full model included the interaction term 'tannin*FN'.

For the analyses of the feeding trials, we used LME models to test the effect of dietary tannins (again as a binary factor) on the regression of DN (dependent variable) on FN (independent variable). The identity of the individuals was included as a random variable to prevent the variance between individuals being included in the error term. We tested for the significance of slopes (the null hypothesis was that the slope equalled zero) for diets with and without tannins. For diets without tannin, we tested the equality of the slopes of the regression between DN and FN for wild and experimental animals using the Fisher procedure (Fisher 1921). The statistical analyses were done on RGui version 2.9.1 software, (R Development Core Team 2007).

Results

Variation of FN in the wild roe deer

FN ranged between 1.5 and 5% DM. As expected, FN varied between seasons but differences were more pronounced in the open than the forest sector (interaction season*sector: L-ratio_{10,484} = 9.9, P = 0.020). FN was higher in the open sector than in the forest one, especially in spring and autumn, and peaked in spring (Fig. 1).

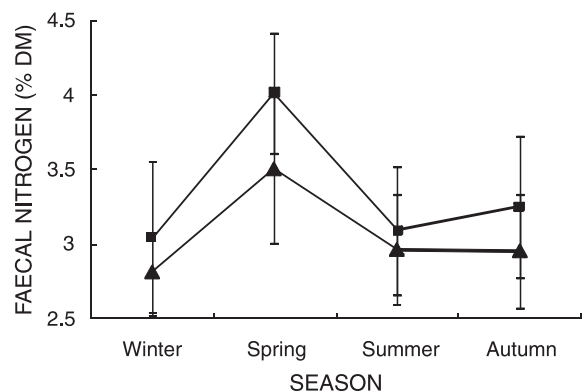


Figure 1. Variations of mean (\pm SD) faecal nitrogen values in roe deer, in relation to landscape (forest: ▲ and open landscape: ■) and season.

Table 2. Diet botanical composition (% of principal plants eaten), dietary nitrogen and faecal nitrogen (% DM) by season and dietary tannin level (0=low or absent, $\leq 1\%$ DM and 1=moderate or high, $> 1\%$ DM) in roe deer. Sample size is the number of individual roe deer. Diet botanical composition was assessed by analysis of the rumen content. The nitrogen content was estimated from the botanical composition of the rumen and the nitrogen content of the plant eaten (see section Methods). Au=Autumn, Wi=winter, Su=summer, ac=acorn, br=bramble, ast=Asteraceae, pri=privet, ros=Rosaceae and ger=Geraniaceae.

Season	Dietary tannin level 0/1	Diet botanical composition	Sample size	Dietary nitrogen, mean (SD)	Faecal nitrogen, mean (SD)
Au	1	> 84% ac	19	0.98 (0.07)	3.78 (0.49)
Au	0	70% soya, 18% br	1	399	397
Au	0	78% soya, 12% maize	1	430	394
Wi	1	> 91% ac	6	1.07 (0.02)	3.46 (0.40)
Wi	1	74% ac, 22% br	1	134	276
Wi	1	82% ac, 12% ast	1	126	461
Wi	1	> 86% br	3	1.99 (0.02)	3.51 (0.14)
Wi	1	64% br, 29% ivy	1	179	317
Wi	1	79% br, 12% pri	1	185	340
Wi	1	58% br, 13% pri, 10% ros	1	207	340
Wi	1	46% ros, 11% br, 11% ac, 11% ger	1	311	307
Wi	0	76% maize, 12% br	1	175	291
Wi	0	77% other legume, 8% br	1	262	304
Wi	0	71% alfafa, 8% br, 6% ast	1	429	301
Su	1	45% ros, 39% wheat	1	189	417
Su	1	88% br, 10% ivy	1	192	373

The relationship between DN and FN in wild roe deer

Using the analysis of rumen contents, we found 15 diets, with different proportions of 12 plant categories in the rums (Table 2). Using measures of the nitrogen content for each plant category, we estimated the DN, which ranged between 0.8 and 4.3% DM. The FN of the same animals ranged between 2.8 and 4.7%. From the results of our study, we know that acorn and bramble contained high levels of CT, but ivy contained very little (see Table 1). In addition, from the literature, we know that Asteraceae (Almeida-Cortez et al. 1999), Rosaceae (Gonzalez-Hernandez et al. 2003) and Geraniaceae (Okuda et al. 1992) contain moderate levels of CT. In contrast, the cultivated plants alfafa, wheat, soya and maize are free of tannin. Of the 41 shot deer sampled, the great majority had diets dominated by tannic foods, principally acorns and brambles. Only five (12%) had diets with low tannin levels (see Table 2). Tannins had an effect on the relationship between DN and FN (interaction FN*tannin: $F_{1,37} = 7.3$, $P = 0.010$; Fig. 2). DN was related to FN for diets without tannin (the slope was positive and significant: estimate = 1.38, SD = 0.5, $P = 0.014$), but not for tannic diets (slope estimate = -0.16, SD = 0.2, $P = 0.433$). The model explained 62% of the total variance (adjusted R^2).

The relationship between DN and FN in feeding trials

During the first feeding trial with ivy, two animals (A and B) ate abnormally little (< 30 g DM intake per day) and had diarrhoea, so we decided to stop these two trials. Animal A recovered quickly, but, animal B did not, so it was not used in the following trials ('bramble', 'maple-oak' and 'alfafa' feeding

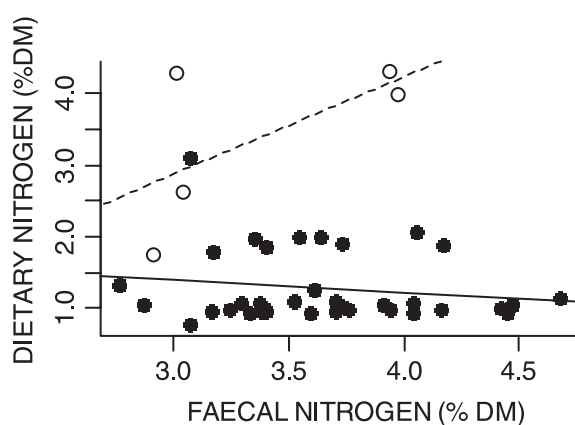


Figure 2. Relationship between dietary nitrogen (% DM) and faecal nitrogen (% DM) in wild roe deer; diets without significant levels of free condensed tannin (○) and diets with significant levels of free condensed tannin (●). The regression lines predicting dietary nitrogen with faecal nitrogen for diets with (solid line) and without (dashed line) free condensed tannin are shown.

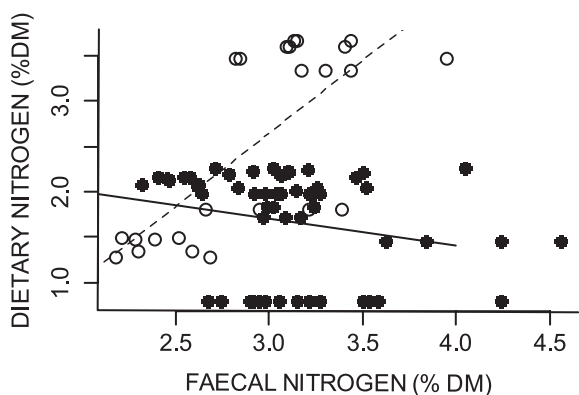


Figure 3. Relationship between dietary nitrogen (% DM) and faecal nitrogen (% DM) in tame roe deer fed with experimental diets: diets without significant levels of free condensed tannin (○) and diets with significant levels of free condensed tannin (●). The regression lines predicting dietary nitrogen with faecal nitrogen for diets with (solid line) and without (dashed line) free condensed tannin are shown.

trials). DN in the different diets offered ranged from 0.8 to 3.5% DM and FN from 2.3 to 4.1% DM (see Table 1).

The effect of free CT on the relationship between DN and FN was highly significant (interaction tannin*FN: L-ratio = 52.5, Df = 3, $P < 0.0001$; Fig. 3). The slope of regression line predicting DN with FN was positive and significant for diets without tannin (estimate = 1.6, SD = 0.3, $P < 0.0001$), but non-significant for diets with tannins (estimate = -0.3, SD = 0.2, $P = 0.102$).

The slopes of the regressions between DN and FN for diets without tannin (1.38 for the wild animals and 1.60 for the experimental ones) were not significantly different ($t = 0.3913$, $df = 95$, $P = 0.6965$).

Discussion

As expected, we found seasonal and spatial variations of FN in a wild population of roe deer. First, FN peaked in spring, an expected response to higher digestibility and protein content in the growing vegetation. Secondly, FN was higher in the open, farmland sector compared to forest, which again was expected since cultivated plants, are selected for high protein and benefit from fertilisation.

As expected, DN was positively correlated with FN in the wild roe deer only for tannin-free diets (maize, soya, alfalfa and other legumes). For tannic diets, there was no significant relation between DN and FN. Similar patterns were obtained experimen-

tally with captive roe deer. Indeed the slopes of the relation between DN and FN were similar between wild and experimental deer (we cannot reject the null hypothesis of equal slope for diets without tannin). The data from both wild and captive animals clearly demonstrate that the relationship between DN and FN for individual roe deer is fundamentally changed when the animals eat diets containing significant amounts of CT. Because of the delay between food intake and excretion, we acknowledge that the faeces collected in the rectums of a hunted deer do not relate directly to its rumen content. However, roe deer have a short retention time (< 24 hours; Berhend et al. 2004) and use routinely only a few patches in their home range (Le Corre et al. 2008). Thus, we assume that the individuals had not changed their food resources fundamentally in the previous couple of days. Although our experimental design for the captive deer experiment involved only a small number of animals and a short habituation period, the similarity with the data obtained in the wild population is striking, suggesting that they are robust. Further, our results are consistent with other studies of other ruminant species (Mould & Robbins 1981, Robbins et al. 1987, Nunez-Hernandez et al. 1992, Osborn & Ginnett 2001).

It could be expected that for tannic diets, FN would increase with the amount of free CTs. We have not enough data to test this prediction. However, our data suggest that FN is rather independent of tannin concentrations in the diet. Indeed maple-oak and maple-clematis diets had similar DN but oak contained more free CT than clematis. We expected higher FN for maple-oak diet, but obtained the reverse. In the same way, maple-clematis and bramble diets had almost equal DN, and bramble contained three times as much tannin as maple-clematis, but the FN values were not very different for the two diets. Thus, we agree with Heil et al. (2002) that the protein precipitating activity of tannins appears not to be closely related to their crude concentration in plants. It is very likely that different types of CTs in the different plant species have very different specificities for particular proteins, in relation to their molecular structure. Kaitho et al. (1997) also found that FN increased with increasing levels of dietary tannins in Menz sheep, but that additional variations due to tannin types occurred. Osborn & Ginnett (2001) and Makkar (2003) also stress that the effect of tannin is likely to vary between plant and animal

species due to great diversity of tannin and the specificity of defenses in different herbivores.

The tannic diets producing greater FN are high energy diets (acorn and sorghum grain). This may be explained by stimulation of bacterial activity and the secretion of digestive enzyme in the rumen in high energy diets. Brown et al. (1995) also found interactive effects of dietary protein and energy on FN in white-tailed deer.

On a more general level, our results might appear to be contradictory, since we found seasonal and spatial variations of FN that follow expected variations of plant quality (as has also been found in other species; Leslie et al. 2008). We conclude that FN is not suitable for predicting individual, short term diet quality for browsers that ingest significant levels of tannin, but FN may, nonetheless, reflect broad seasonal and spatial patterns of diet quality at the population level in some circumstances. We suggest that this could be a matter of scale, both spatial and temporal scale. At the individual levels and on a short term basis, many factors related to the previous meal of a given individual may affect the measurement of FN at a given moment, such as DN and energy, the concentration of CT in the diet and the molecular type of tannin. However, at the population level and for longer time scales, daily and individual variations of diet composition will be buffered by persistent trends relative to food resource quality such as seasonal and habitat related factors.

To conclude, we suggest that using FN to predict individual diet quality in animals eating tannins is not usually possible because the relationship between DN and FN is affected by too many factors, like the nature of the tannins, which are not well understood at the moment. We suggest that FN, measured at the population level and in long term studies, may indicate important trends in temporal and/or spatial variation in diet quality in roe deer (Hewison et al. 2009). However, comparisons between sites (within the same population) must be done cautiously, because FN can respond very differently to different diet types, with different amounts and types of tannin. Thus, knowledge of the botanical composition of the diets will help to interpret FN variations correctly.

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