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SHORT COMMUNICATION

Short communication articles are short scientific entities often dealing with methodological problems or with byproducts of larger research projects. The style is the same as in original articles

Suitability of NIRS analysis for estimating diet quality of free-living red deer *Cervus elaphus* and roe deer *Capreolus capreolus*

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

In this study, we tested the efficiency of near infrared reflectance spectroscopy (NIRS) to assess nitrogen content in faeces of free-living ruminants. Faecal nitrogen (FN) content was analysed in 168 pellet groups from red deer *Cervus elaphus* and roe deer *Capreolus capreolus* in the growing season and in winter using both the standard Kjeldahl method and NIRS analysis. Estimates of nitrogen content obtained by the two methods did not differ ($P > 0.1$), and the correlation between FN values was significant ($P < 0.001$). FN content ranged within 1.10-4.58% of dry matter and, as anticipated, it was higher in the growing season than in winter in both species ($P < 0.01$). Faecal nitrogen values were also higher for roe deer than for red deer, although the difference was only significant in the growing season ($P = 0.007$). Our study confirmed that faecal nitrogen of free-living ruminants can be accurately determined with NIRS analysis. NIRS represents a low-cost analytical technique, which could replace conventional labourious methods and is highly promising for analyses of diet quality in free-living ruminants.

Key words: deer, diet quality, faecal nitrogen, near infrared reflectance spectroscopy, NIRS

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Information on the botanical composition and quality of herbivore diet is an essential component of studies of their feeding ecology. Studies of the diet of free-living ungulates are usually based on samples of faeces or rumen contents (Holechek, Vavra & Pieper 1982, de Jong, Gill, van Wieren & Burlton 1995, Cornelis,

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Casaer & Hermy 1999, Latham, Staines & Gorman 1999). Collection of rumen contents from killed animals is often limited to certain periods of the year and can be impossible in protected areas or in species that are not hunted. Therefore, it is important to develop reliable non-invasive techniques such as faecal analysis.

Faecal samples collected for studies of the botanical composition of diets may also be used to estimate diet quality (Sinclair, Krebs & Smith 1982, Putman & Hemmings 1986). This is due to the fact that there seems to be good correlation between diet quality (e.g. dietary nitrogen, dietary digestible energy, dry matter intake, and dry matter digestibility) and faecal chemical constituents (e.g. faecal nitrogen, faecal 2,6-diamnopimelic acid, and faecal fibre; Leslie, Starkey & Vavra 1984, Leslie & Starkey 1985, Jenks, Soper, Lochmiller & Leslie 1990).

Recent studies indicate that near infrared reflectance spectroscopy (NIRS) can be a valuable method to estimate the variety of chemical components in different materials like soil, plant or animal tissues (Büning-Pfaue, Hartman, Harder, Kehraus & Urban 1998, Foley, McIlwee, Lawler, Aragones, Woolnough & Berding 1998, Ludwig, Khanna, Bauhus & Hopmans 2002). NIRS has been successfully applied in agriculture to determine the chemical composition (quality) of pastures and other food (Offer, Percival, Dewhurst & Thomas 1998, Kays, Barton & Windham 2000). Some studies also used NIRS to estimate diet quality of ruminants from faecal samples. Diet crude protein, diet digestibility and also energetic values of the diet have been successfully estimated from faeces using this method (Lyons & Stuth 1992, Leite & Stuth 1994, Leite & Stuth 1995, Purnomoadi, Kurihara, Nishida, Shibata, Abe & Kameoka 1996). NIRS can also be used to estimate other characteristics of diet such as mineral content (Windham, Hill & Stuedemann 1991), energy content of the diet of non-ruminants (van Barneveld, Nuttall, Flinn & Osborne 1999) and botanical composition of the diet as well (Petersen, Barton, Windham & Hoveland 1987, Volesky & Coleman 1996, Walker, Clark & McCoy 1998, Walker, McCoy, Launchbaugh, Fraker & Powel 2002).

The potential for using NIRS in this way has been tested mostly on domestic animals so far; wider verification of the method for use in studies of diet quality in free-living ruminants has not previously been undertaken. In this study, we compare FN content obtained by the traditional Kjeldahl method and by NIRS to test the applicability of NIRS for this analysis in free-living ruminants. To verify the method on various types of material we used dung pellets sampled from red deer *Cervus elaphus* and roe deer *Capreolus capreolus* in the growing and winter seasons. Differences in feeding ecology between these two species exist, particularly when availability and quality of food is high, thus allowing a greater degree of selection; the diets of the same two species are more similar in localities (or seasons) where food resources are more limited (Homolka 1996). Red and roe deer are ruminants belonging to distinct feeding types (Hofmann 1989). 'Concentrate-selectors' such as roe deer, are generally more selective in their choice of diet as they require diet of higher quality (higher nitrogen, energy and lower fibre content) than do 'intermediate-feeders' such as red deer. The diet of the roe deer thus contains higher quality components like browse and forbs (de Jong et al. 1995, Homolka 1996, Raymond, Servello, Griffith & Eschholz 1996) and smaller proportions of grasses of low quality than does the diet of red deer (Homolka 1995, Merrill, Callahan-Olson, Raedeke, Taber & Anderson 1995, Gebert & Verheyden 2001).

In winter, free-living ruminants in the temperate zone face great limitation in food resources (Worden & Pekins 1995) and the quality of their diet is generally lower than in the growing season (Risenhoover 1989, Kucera 1997).

We therefore expected to find higher FN content in roe deer than in red deer and higher FN values for both species in summer than in winter.

Material and methods

Material for our study was collected in the Jeseniky Mountains in the Czech Republic. We collected 168 fresh faecal pellet groups in two seasons: the vegetation period running from May through September and the winter running from November through February (see Table 1). Samples were air-dried at 60°C for 48 hours, ground in a mill to pass a 1-mm screen, and divided into two subsamples. The first group of subsamples (reference values) were analysed for nitrogen using the standard Kjeldahl procedures (AOAC 1980) and the second group of subsamples were analysed using NIRS.

NIRS is based on the development of a calibration equation that reflects the relationship between the constituents in the sample and NIRS spectral information. Measurements were carried out using a FOSS NIR-System 6500, a near infrared reflectance spectrophotometer in the 1,100-2,500 nm wavelength range. The spectra produced by the NIRS instrument represent the total chemical and physical properties of a sample. Chemical information appears at a specific location in the spectrum. Physical properties of a sample, such as

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particle size, are eliminated by mathematical corrections. We used standard normal variate correction (SNV) which corrects each ith spectrum (row) in the data matrix separately by subtraction of the row mean and normalising in the row direction (Barnes, Dhanoa & Lister 1989). Data were analysed by linear regression using software NIR Calibration 1.0 (EFFICHEM, CZ). After defining the calibration data set, the software computes the calibration equation. Calibration equations quantify the relationship between NIR absorption and laboratory reference method. Partial least square (PLS) was chosen as the regression because it is generally considered to be the method of choice in multivariate calibration (Martens & Næs 1991). To examine the relationship between the results obtained using the two tested methods, we used the correlation coefficient and standard error of cross validation (SECV). SECV was used to estimate the error of prediction for unknown samples by simulating the prediction process by leaving part of the data set out, developing the calibration model on the rest of the data matrix, and making predictions for samples left out. This process was repeated several times so that each sample was left out once.

Before comparing the values obtained by the two methods, we tested the differences of reference faecal nitrogen (FN) values in subsamples (species, season). Both season and species affected the FN values (ANOVA: $F_{3,164} = 32.76$; $P < 0.001$); therefore, we tested the differences between the two methods used separately for seasons and species (t-test). To test if NIRS performed well for both feeding specialists, we calculated the equation of the regression curve separately for red and roe deer. Differences in the x-y regression slope were examined according to Armitage, Berry & Matthews (2001).

Finally, we tested the significance of differences in the reference FN values between species and seasons (two species in one season and one species in two seasons) using a t-test.

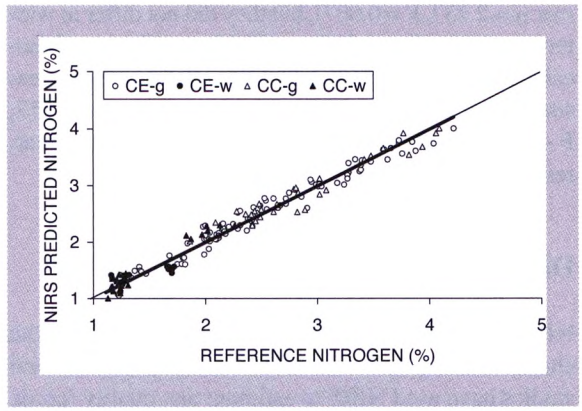


Figure 1. Reference nitrogen (%; obtained using the Kjeldahl method) and NIRS predicted nitrogen (%) in faeces of red deer (CE) and roe deer (CC) in the growing season (g = spring-summer) and winter (w) in Jeseníky Mountains ($y = 0.9964x$, $r^2 = 0.968$, SECV = 0.15). The thin line represents the ideal equation Y = X.

Results

In all samples, faecal nitrogen ranged within 1.10-4.58% of dry matter. We did not find any significant differences between the two methods when applied to paired samples whatever the species or season ($P > 0.1$ in all cases; Table 1). Values of the nitrogen content obtained by the two methods were closely correlated in pooled data sets ($r = 0.985$, $N = 168$, $P < 0.001$, SECV = 0.15; Fig. 1).

The calibration values determined separately for each of the species were also significantly correlated (red deer: $r = 0.985$, $P < 0.001$, SECV = 0.147; roe deer: $r = 0.986$, $P < 0.001$, SECV = 0.12), and the equations of the two regression curves were nearly identical (red deer: $y = 0.9642x + 0.0811$; roe deer: $y = 0.9509x + 0.1405$) and their slopes did not differ significantly ($t = 0.500$; $P = 0.597$).

In accordance with our hypothesis, the reference FN values were higher in roe deer than in red deer in sum-

Table 1. Number of samples (N) and nitrogen volume (%; $\bar{x} \pm$ SD) in red deer (CE) and roe deer (CC) faeces during the growing season (g = spring-summer) and winter (w) in our study (Jeseníky) found using the Kjeldahl method (lab) and NIRS analysis (NIR), significance of their differences (t-test) and comparison with literature data from Georgia (Leslie & Starkey 1985) and Nevada (Osborn & Jenks 1998), Sweden (Wahlström & Kjellander 1995) and Hampshire (Putman & Hemmings 1986).

	N	Jeseníky-lab	Jeseníky-NIR	Differences t	Differences P	Georgia	Nevada	Sweden	Hampshire
CE-g	96	2.5 ± 0.7	2.5 ± 0.7	0.001	0.999	3.1 ± 0.03			4.1**
CE-w	15	1.4 ± 0.1	1.5 ± 0.2	0.363	0.720	1.4 ± 0.04			2.4**
CC-g	30	2.9 ± 0.6	2.9 ± 0.6	0.018	0.986	3.4 ± 0.12*			3.5
CC-w	23	1.5 ± 0.5	1.5 ± 0.4	0.552	0.584	2.1 ± 0.12*	1.7 ± 0.04*	2.43	2.6

* *Odocoileus* spp.
** *Cervus nippon*

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mer ($t = 2.737$, $P = 0.007$), but they did not differ in winter ($t = 0.994$; $P = 0.330$; see Table 1). Values of faecal nitrogen were significantly higher in the growing season than in winter for both red and roe deer ($t = 13.557$, $P < 0.001$ and $t = 9.445$, $P < 0.001$ in red and roe deer, respectively).

Discussion

NIRS has been successfully used for analysis of the chemical composition of different materials. Several studies have used NIRS to estimate diet quality (crude protein, digestible organic matter or energy) in domestic and also free-living ruminants (Lyons & Stuth 1992, Purnomoadi et al. 1996). Our study verified the applicability of NIRS for estimation of faecal nitrogen in free-living ruminants. We did not find any differences in reliability of NIRS between deer species and seasons, and we confirmed advantages of NIRS.

We applied NIRS in estimation of faecal nitrogen, which is generally accepted as a reliable indicator of diet quality of free-living herbivores (e.g. Sinclair et al. 1982, Putman & Hemmings 1986, Hodgman, Davitt & Nelson 1996, Kamler, Homolka & Kráčmar 2003). Despite some doubts about its reliability (Hobbs 1987) NIRS is now widely used. Our results corresponded well with the expected differences in diet quality between seasons and deer species. We expected higher FN values in browsers (roe deer) than in intermediate feeders (red deer), and in the growing season than in winter. Roe deer usually consume a diet of less grasses and of a better quality than do red deer (Kuen & Bubenik 1980, Hearney & Jennings 1983, Latham et al. 1999). The fact that we found no differences in FN contents between the two species in winter may be due to the close similarity of red and roe deer diets in the period with snow cover (Homolka 1993).

The differences between the content of FN found in our samples and those reported by other authors (see Table 1) may have been caused by different quality of forage supply. A lower content of FN (2.43% DM) was found in roe deer from a forest environment in Sweden during summer (Wahlström & Kjellander 1995). On the other hand, the content of nitrogen detected in black-tailed deer *Odocoileus hemionus columbianus* (a feeding specialist like roe deer) in Georgia, USA, in a region with broadleaved forests was higher (3.4% DM; Leslie & Starkey 1985).

Our results showed the potential in using NIRS to estimate diet quality in free-living ruminants from faeces. We would also emphasise the advantages of NIRS, for instance speed and low cost of analysis. Furthermore, the number of samples that can be processed is not limited and the validity of the estimated parameters is not influenced by sample freshness (Pearce, Lyons & Stuth 1993, Leite & Stuth 1994). Our findings agree with the results of similar studies showing the potential of NIRS to estimate diet quality in ruminants (Leite & Stuth 1995, Cozzolino, La Manna & Martins 2002). We find the application of NIRS highly promising for faecal analyses of different characteristics of the diet of free-living ruminants. The real advantages of NIRS are its ability to estimate diet quality (digestible energy and protein) from faeces and also that it makes it possible to determine the botanical composition of the diet in wild ruminants from faecal material. Nevertheless, differences in botanical composition of the diet may influence the accuracy of NIRS, thus we recommend that further studies be carried out, especially in different feeding specialists.

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