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Source: Folia Zoologica, 60(1) : 63-69

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v60.i1.a10.2011>

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# Needles in faeces: an index of quality of wild ungulate winter diet

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Received 8 June 2010; Accepted 15 October 2010

**Abstract.** Norway spruce is a wide-spread food resource and its utilisable biomass exceeds the needs of herbivores. Needles seem to be a generally ignored food component in temperate forests that is consumed only when there are no better food sources. It is used especially during winters with deep snow cover. The aim of this study was to test presumption of needles as nutritive poor component of ungulate diets through botanical diet analyses and chemical nutrition estimation (content of crude protein and metabolizable energy volume in faeces) and elaborate the calibration curve on indirect estimation of quality food resources for ungulates in environment (NIRS needle content in faeces). High content of spruce needles corresponded well with a low quality winter diet of wild ungulates and may reflect animal nutritional constraints. As a consequence, the content of spruce needles may be used as an easy index of animal performance in a particular environment in forested area with coniferous forests in temperate zone. Needle content can be determined from the faeces by near infrared spectrophotometer and this easy technique can be recommended as indicator of the food resources quality for ungulates.

**Key words:** ruminant diet, diet quality, diet indicator, spectroscopy

## Introduction

Quantity and quality of vegetation available to large herbivores is important information that must be taken into account in the environment management of Temperate Zone. Forage availability limits both populations of herbivores and their impact on vegetation. During vegetation period there is generally abundant food supply that supports high densities of herbivores, while during winter when food sources are depleted and snow cover limits the access of herbivores to ground vegetation, they suffer from poor food supply. Depending on the quantity of accessible food, herbivores during winter use food of low quality (conifer bark, needles, grasses). According to their feeding strategy grazers prefer grasses, twigs of broadleaved trees and generally avoid conifers (Homolka 1990, 1991, Gebert & Verheyden-Tixier 2001, Suter et al. 2004). Browsers generally depend on resources available in the shrub layer in winter. They consume mostly shoots of broadleaved trees

and in case of a scarcity their diet may contain large proportion of conifer needles (Homolka 1995, Myrsterud 2000, Gebert & Verheyden-Tixier 2001, Sauve & Cote 2007).

Winter food of free living herbivores is partly improved with supplemental feeding provided by hunters, but this feeding is often not able to prevent destruction of nutritionally attractive plant species and worse condition of animals (Mattfeldt 1984, Sams et al. 1998, Kaji et al. 2004). Winter is therefore the critical period from both the animal's viewpoint and also environment viewpoint and the situation culminates after snowfall (Homolka & Heroldová 1992, Cornelis et al. 1999). High-quality environment management therefore must be based on equitability of the amount of available natural food resources in the course of a year and sufficient regulation of the number of herbivores in all seasons. This is why we need detailed information on feeding preferences and behaviour of ungulates and applicable methods for monitoring their food supply.

Norway spruce (*Picea abies*) needles are a wide-spread food resource for ungulates in temperate forests and their utilisable biomass exceeds the needs of herbivores in most forested areas. As herbivores prefer other components of food supply, needles seem to be a food source that is generally avoided due to low quality (low energy content and high content of substances inhibiting food digestion) (Cederlund et al. 1980, Prieditis 1984). Therefore, the Norway spruce is often able to grow out on localities with intensive impact of herbivores where the reproduction of attractive broadleaved trees tends to be totally destroyed. Intensive consumption of needles by herbivores indicates unfavourable feeding conditions that generally lead to worse health and reproductive condition of animals and also signal severe browsing impact (Andersson & Koivisto 1980, Nygrén 1984, Padajga 1984, Wishart 1984). We hypothesized that monitoring of the content of needles in the diet of free living herbivores can be used as an easy indicator of winter diet quality and can be also applied in rational management of ungulates. The aim of the study was 1) to evaluate the needle content in large herbivore diets as an important substance of their food that could be used as indicator of their nutrition quality and 2) to develop a method for easy determination of the needle content in the ungulate faeces as an indicator of food resource quality.

The needle content in the diet can be determined by macroscopic analysis of rumen content or microscopic analysis of faeces (Homolka & Heroldová 1992, de Jong et al. 1995, Gebert & Verheyden-Tixier 2001). Both of these methods are very time consuming and do not make possible the use of abundant samples in diet analyses. Recent studies indicate that near infrared reflectance spectroscopy (NIRS) can be a valuable method to estimate variety of chemical components in different materials like soil, plant or animal tissues (Büning-Pfaue et al. 1998, Foley et al. 1998, Ludwig et al. 2002). NIRS has been successfully applied also to determine the quality of the domestic ruminant diet (Offer et al. 1998, Kays et al. 2000) and some studies used NIRS to estimate botanical composition and quality of the diet of wild ruminants (Purnomoadi et al. 1996, Volesky & Coleman 1996, Walker et al. 1998, 2002, Landau et al. 2006, Dixon & Coates 2009). We tested the application of NIRS for determination of the needle content in the faeces.

## Material and Methods

### *Diet analyses*

We used a total of 336 faecal samples of five ruminant species for estimation of the needle content in their

winter diet: red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), white tailed deer (*Odocoileus virginianus*), chamois (*Rupicapra rupicapra*) and mouflon (*Ovis aries*). Material originated from three areas of the Czech Republic and was collected for previous studies focused on feeding ecology of herbivores (Homolka & Heroldová 1992, Homolka 1995). Food supply on all used localities contained sufficient quantity of spruce needles.

Fresh faeces were collected seasonally on all localities and then air dried. We used three seasons in accordance with predicted changes in botanical composition of herbivore diet (autumn: October–November; winter1: December–January; winter2: February–March). The proportion of individual food components in the ungulate diet was estimated by the microscopic analysis of the undigested items in faeces. Reliable relationship between diet (rumen content) and faeces composition was confirmed by Homolka & Heroldová (1992). From the faecal samples collected, one pellet was removed and used to prepare a microscopic slide. The representation of various food components was estimated on the basis of their relative coverage in the microscopic field. In evaluating the overall character of the diet, the components were pooled to form primary forage classes: grasses, bramble, leaves and twigs of deciduous trees, blueberry, needles, forbs, seeds and others.

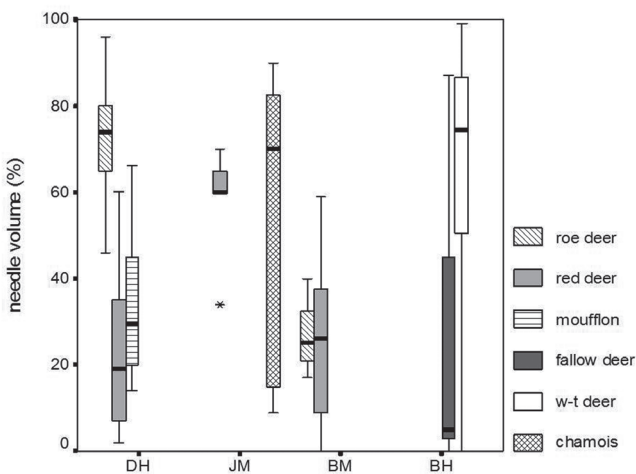
### *Quality of main diet component*

For comparison of the nutritional quality of spruce needles and other main diet component (> 5% in the diets) we calculated the metabolizable energy (ME), which we used as indicator of nutritional value. We picked samples of plants that the deer usually browse (leaves of bramble, terminal parts of shoots of deciduous trees and terminal parts of spruce twigs with needles) at foot hills in the Jeseníky Mountains in October 2006. The samples were dried in a ventilated drying chamber at 60°C to constant weight and analyzed for the content of Kjeldahl nitrogen, fat, fibre, acid detergent lignin, nitrogen free extract and ash with standard method (AOAC 1980). These values were used for calculation of ME by the procedure of Kamler & Homolka (2005).

### *NIRS estimating of the needle content*

Sixty five faecal samples were used as standards for testing possibility of NIRS to estimate needle content in diet. Measurements were carried out using a Nicolet Antaris, a near infrared reflectance spectrophotometer, in the 9860–4100 cm<sup>-1</sup> wavelengths range, and 50

scans from each sample was collected. For analysis, one pellet with flat area abraded on sand paper was used to simplify process of measurement. The pellet was measured lying direct on the cell of integrating sphere of the spectrometer. The spectra produced by the spectrometer represent the total chemical and physical properties of a sample. Chemical information appears at a specific point of the spectrum. Physical properties of a sample, such as particle size, are eliminated by mathematical corrections. Data were analysed using software TQ Analyst. Partial least square regression (PLS) was chosen for the calibration equation quantifying the relationship between NIR absorption and reference values (Martens & Naes 1991). PLS is recognized as a very powerful tool for developing models from spectroscopic data (Schenk & Westerhaus 1991) and is preferred in the studies analysing biological materials (Gillon et al. 1999, Xiccato et al. 1999). For validation of our equation we used standard error of cross validation (SECV). SECV is used to estimate the error of prediction for unknown samples by simulating the prediction process and leaving part of a data set out, thus developing the calibration model on the rest of data matrix, and making predictions for samples left out. This process is repeated several times so that each sample is left out once.



**Fig. 1.** Variability in the needle content in diet of five deer species in three season of the year in various areas (BH – Brdy Hills, BM – Beskydy Mountains, DH – Dražanská Highland).

## Results

### Needles in herbivore diet

Consumption of needles by large herbivores was common in snowy winter. At all three localities needles

formed a significant part of the herbivore winter diet. At the end of the vegetation season (autumn), needles appeared in the animal diet only sporadically (volume in the diets less than 1% on average). In winter, volume of needles in the ruminant diet increased to 40%. In general, concentrate selectors (roe and white-tailed deer) consumed more conifers than other species (mouflon, red and fallow deer) in average  $55.9 \pm 28\%$  and  $23.0 \pm 18\%$  resp. (Fig. 1).

The volume of needles in diet depends mostly on the overall structure of the food supply. If there is a food source of higher quality present, the animals prefer this source. It can be demonstrated on two following examples. In the locality BM, red deer and roe deer consumed relatively little amount of needles (27% resp. 24% of food volume) in the mountain area in the period of deep snow cover as they had enough beech branches broken off by icing to feed on (at steep slopes with low snow cover they reached grasses, ferns and blueberry). In the locality BH, the autumn food supply included bramble which formed a significant share of the white tailed deer diet (81%) (browser) as well as fallow deer (18%) (intermediate feeder). Snow that fell in the middle of the winter worsened the access of fallow deer to grasses and at the same time the supply of bramble lowered. The share of needles in the diets of both species increased significantly to 67% in white tailed deer and 24% in fallow deer. By the end of the winter, the reserves of bramble were depleted and almost the only food source of some nutritional value for white tailed deer were twigs of conifers that formed more than 80% of its diet (Fig. 1). When the snow melted, fallow deer could reach the grasses again and they regained the dominant position in its diet (85%). White tailed deer substituted bramble by needles during the winter ( $r = -0.957$ ;  $p < 0.001$ ;  $N = 32$ ), fallow deer substituted grasses as well as bramble ( $r = -0.898$ ;  $p < 0.001$ ;  $N = 33$ , resp.  $r = -0.795$ ;  $p < 0.001$ ;  $N = 33$ ).

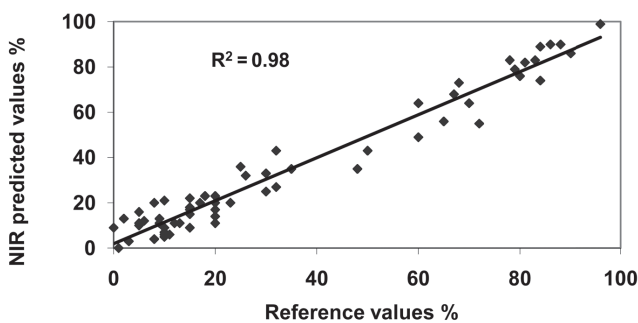
### Quality of needles and other main diet components

According to the amount of ME, needles were the lowest quality food among the nine studied components. Content of ME in needles was by half lower than in leaves of bramble and by third lower than in sprouts of broadleaves woody plants (Table 1). Low preference of spruce needles in the herbivore diet can be therefore explained by the nutritional quality. In contrast to that, the content of crude protein in the needles was comparable or higher than in other studied components (Table 1). The correlation between ME and crude protein in our diet component was low ( $r^2 = 0.307$ ,  $p = 0.0127$ ).



**Table 1.** Content of crude protein (CP % of dry matter =  $N \times 6.25$ ) and metabolizable energy (ME MJ/Kg % of dry matter) in the most important winter diet components.

	ME	CP
<i>Rubus fruticosus</i>	9.83	12.99
<i>Vaccinium myrtillus</i>	9.35	9.48
<i>Avenella flexuosa</i>	8.57	11.46
<i>Sorbus aucuparia</i>	8.28	6.23
<i>Calamagrostis villosa</i>	8.28	12.94
<i>Fraxinus excelsior</i>	7.98	6.09
<i>Salix caprea</i>	7.39	7.97
<i>Fagus sylvatica</i>	7.38	7.04
<i>Picea abies</i>	6.61	9.60



**Fig. 2.** Reference and NIR predicted values of needle content in the samples used for calibration.

#### Detection of needles by NIRS

A good relationship between values derived by the standard microscopic analyses and the predicted NIRS values for the content of needles was discovered. The needle content of reference samples ranged from 2 to 96% (Fig. 2). The standard error of calibration (SEC) of 6.41 and coefficient of determination ( $R^2$ ) of 0.98 were both indicative of good calibration statistics (Fig. 2). The calibration was validated by standard error of cross validation which was 18.8% for six used factors. This accuracy is comparable to the estimated precision of our standard microscopic technique. These results demonstrate that NIRS is a reliable technique for quantifying the content of spruce needles in faecal samples and we can processed native samples of pellets without any fitting.

## Discussion

#### Spruce needles in herbivore diets

Needles were an important component of the winter diet of the studied species and its content well corresponded with quality of their diet. Spruce needles were not

consumed during vegetation period and in winter they were presented in the diet as important food source after depletion of more attractive sources only. This low feeding attractiveness of needles is due to its low quality (Cederlund et al. 1980, Prieditis 1984). Consumption of needles also depends on the feeding type of a species (Hofmann 1989). The species that are adapted to utilization of grasses (red deer, mouflon) prefer grasses also in winter and needles form substantial part of their diet especially during snow cover. On the contrary, for the species accustomed to browsing on twigs consuming a minimal amount of grasses, needles formed up to 90% of their diet depending not only on snow cover but also on availability of other food resources (seeds, forbs, sprouts of broadleaved tree and shrubs).

Therefore, the presence of needles in diet is a good indicator of a food supply in the environment, with respect to feeding type of the ruminant, date of sample collection and snow cover height. High consumption of needles during a longer winter period shows impaired relations between the herbivores and the environment, disturbed either by changes of vegetation structure, or by high number of animals. The usual content of needles from localities with varied food supply was 30–45% of volume in winter. For example, Hodgman et al. (1996) tested the reliability of several faecal indicators for monitoring of the diet quality and intake in feeding trials with simulated diets. In three winter diets, the conifer browse formed only 0–20%. Low feeding preference of needles by herbivores correlates with quality of spruce that is lower than quality of all other main components of food supply. Low quality of needles was confirmed for example by Cederlund et al. (1980).

#### Diet quality

The quality of diet was evaluated according to the content of metabolizable energy. The energy content is considered as the main indicator of food supply quality for wild herbivores and also is considered as the main factor affecting food choice of herbivores (Berteaux et al. 1998). Many studies used nitrogen content in the diet or faeces as a simple and effective indicator of nutritional quality of ruminant diets (Latham et al. 1999). The applicability of nitrogen content is predominantly based on correlation between energy and nitrogen content in the diet rather than on lack of protein in the diet. The relationship between energy and nitrogen varies widely and therefore energy content reflects the value of food much more accurately than the content of nitrogen (Kamler & Homolka 2005). Our results support the importance of energy content. The content of nitrogen in our needle

samples was higher than in several components of higher feeding attractiveness for herbivores and the correlation between the nitrogen content and ME was weak ( $r^2 = 0.307$ ,  $p = 0.0127$ ). The ratio CP/ME was 1:0.68 in spruce, 1:0.99 in blueberry and 1:1.33 in rowan. Therefore, the content of nitrogen itself has only a limited value as an indicator of food supply quality despite the observed correlation.

#### *NIR estimation of needles*

There is no doubt that NIRS is an efficient tool for this type of analyses and further applications of this method are feasible. For testing the accuracy of our calibration we used the PLS regression method that is preferred for studying biological materials (Gillon et al. 1999, Xiccato et al. 1999). Our study verified the applicability of NIRS for the estimation of the content of spruce needles in the winter diet of free-living ruminants from their faeces and we verified this procedure for estimating quality of environment or suitability of the animal numbers. We would also emphasise other advantages such as speed and low costs of the analysis with sufficient accuracy of the estimation. For example, about 30 samples can be processed in an hour with only easy pre-preparation. This means, that the number of samples that can be processed is not limited which saves everyday laboratory cost and thus solves the usual major problem with limited possibility of analysed samples. Our procedure has a potential for being used on a large scale for monitoring winter nutritional conditions of ungulates. Furthermore, validity of the estimated parameters is not influenced by sample freshness (Pearce et al. 1993, Leite & Stuth 1994).

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#### **Conclusion**

Our findings verified low quality and dietary preference of spruce needles in comparison to other ungulate diet resources in winter. Proportion of spruce needles in the winter diet of large herbivores is therefore a good indicator of their diet quality (quality and quantity of food supply). Volume of needles in the diet can also be indirectly used for evaluation of suitability of herbivore density in most areas of the central Europe. There are three main advantages in using needles as a diet quality indicator: 1. Needles are abundant food source. Norway spruce represents 54% of forest stands in the Czech Republic. 2. Needles are neglected by herbivores and are used after depletion of other resource. Their content corresponds well with diet quality. 3. Needle content can be estimated from faeces or stomach using microscopic analysis or by NIRS. This variety of methods allows using the needle content indicator in studies of the environment quality on a large scale. Collection of herbivore faeces samples and their analysis is relatively easy. We developed simple applicable technique using near infrared spectrophotometer to determine the content of spruce needles from the faeces. Only one native pellet is necessary to determine a portion of needles in herbivore pellet. This procedure enables to analyse a large number of samples in a short time and saves cost on fitting herbivore pellets.

#### **Acknowledgements**

*This study was supported by the Grant Agency of the Czech Republic, Grant No. 206/03/P134.*

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