NIRS in Wild Rodents' Research

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The near-infrared spectroscopy (NIRS) method proved to be a useful tool to determine the amount of a particular food ingredient in the diet from faeces or chyme and to estimate the food quality.

Keywords: Near-Infrared Spectroscopy ; Wild Rodents ; Faeces

1. Brief Introduction to Near-Infrared Spectroscopy (NIRS)

Near-infrared spectroscopy is a spectroscopic method that uses the near-infrared region (i.e., 1000–2500 nm) from the electromagnetic spectrum . The principle of the method consists of different absorption or reflection of the near-infrared light of different wavelength incidents on the organic material. The reflected spectrum is given by the type and amount of chemical bonds in a particular material. However, since the individual peaks of a spectrum curve are not strictly bond-specific, it is usually necessary to refine the estimate by creating a calibration equation. This is obtained by applying mathematical methods (e.g., partial least squares regression) to the spectra obtained from samples of known composition (for more detail, see [1]).

The NIRS method enables fast, technologically easy, low-cost, and repeatable determination of the composition of a large number of organic material samples [2]. It requires a much smaller sample size than classic analysis. The method has been successfully used for chemical evaluation in nutritional studies of many animal species (ruminants [3], marine mammals [4], koalas [5], giant pandas [6] etc.). The NIRS method can be used to determine the content of basic nutrients (nitrogenous substances, fiber, and water content) [1], to determine the presence or proportion of a particular food [7], as well as to detect changes in the content of substances represented in minimal concentrations (e.g., the level of hormones [8][9] or fatty acids in milk [10]). For almost half a century, the method has been increasingly used, especially in agriculture and pharmacy (typically, where large quantities of very similar samples are routinely processed) [11]. Use in other areas (e.g., medicine, biology, and ecology) followed with some delay [12].

In recent years, with technological progress, possibilities of applying the NIRS method have significantly expanded and have not yet been fully exploited. For example, a miniaturization of sensors [13] has already allowed in-situ analysis of a sample instead of laboratory processing [14]. The use of NIRS for the analysis of liquids, or samples dissolved in liquids, has great potential in ecological and ecophysiological studies, but it has been used only rarely so far [11].

2. Specifics of Using the NIRS Method in Wild Rodents

2.1. Analysis of Faeces

There are some specific aspects in small rodent studies which should be considered. Likely, the most problematic point is the small size of these mammals.

For diet studies, the most usual type of sample is faeces. Faeces can be used also for middle-size animals [5][15]. However, the usual body size of rodents ranged from 7 to 35 grams, thus a piece of faeces is too small not only for a classic chemical analysis but even for the NIRS analysis (**Figure 1**). Theoretically, more faeces can be collected in one place for NIRS analysis of one single mixed sample. Dozens of fresh faeces from one place should be used for one sample; unfortunately, these are very difficult to find in larger quantities. In addition, most rodent species live socially and more individuals share burrows and living space [16]. Therefore, one sample would be composed of faeces of several individuals. This means that the results of the NIRS analysis cannot be linked to a specific individual of a specific age, sex, size, etc., sometimes not even to a specific species. Demographic studies of wild rodents based on such faecal analysis are therefore practically impossible. However, analyses of rodent faeces can be a useful tool in laboratory conditions, where a sample can be reliably assigned to a specific individual (e.g., for food preference tests [17]).

Figure 1. Rodent faeces. Their collection is limited by defecation rate of the individual species. It also depends on food. While on a grain diet and water, herbivorous voles (*Microtus* sp.) produce about 1 g of faeces a day, the granivorous *Apodemus* sp. produce 1 g of faeces within 4 to 5 days.

2.2. Analysis of Stomach Contents

A more promising type of rodent sampling involves stomachs; some stomachs contain sufficient chyme to perform NIRS analysis. For the analysis of nutrients from stomach content, NIRS is probably the only possible analysing method, as stomachs are only rarely sufficiently large for traditional chemical analysis. Stomach NIRS analysis has been successfully used in ruminants [18] and dugongs [19].

For stomach contents analysis, material obtained from research into the spread of zoonoses in small mammal populations was used. Whole stomachs were taken from snap-trapped animals. Each individual was determined by species, sex, and age, measured, and weighed. Stomachs were dried in a kiln for 4 hours in 50–60 °C. Rodent tissue was then removed, and the remaining stomach contents were preserved in a standard freezer at a temperature of −20 °C. The following processing is described in Section 3.2. Important steps of the processing are shown in **Figure 2**.

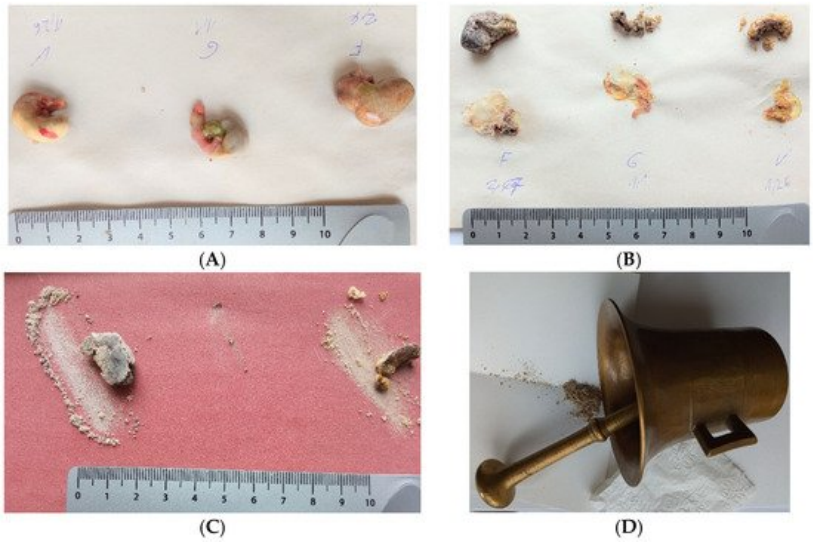

Figure 2. Steps of stomach content samples processing. Fresh samples show the difference between the light cardiac and dark pyloric part (**A**). Nitrogen NIRS analysis however showed similar results for both parts of the stomach. Samples are marked with letters (identification of a specific individual); the number indicates the weight of a fresh sample. After drying, the rodent tissue was removed, and the stomach content (chyme) was prepared (**B**). It can be seen in the same figure that the samples contained different amounts of water; the residual dry matter in the middle sample (letter G) is at the limit of processability. An example of processing samples by abrasion with sandpaper is in (**C**), grinding in a mortar in (**D**). Most samples were processed by abrasion because it is significantly faster.

2.3. Calibration

The NIRS analysis of stomach content has a specific requirement, building a calibration curve that is usually based on samples analysed both by NIRS and by a classic chemical analysis. Results of traditional analysis and of NIRS spectrums are then incorporated into a NIRS calibration model. However, a vast majority of stomachs are too small for a chemical analysis and cannot be used for building a NIRS calibration model. The size of a stomach is mainly affected by the species, the size of an animal, and the food consumed. Stomachs of herbivorous species tend to be smaller, and their chyme contains significantly lower amounts of dry matter than in granivorous species [20][21]. These differences have been caused both by the type of food consumed and the way it is ingested [22].

Another issue is the variability of rodent food. To make the calibration curve as reliable as possible, a wide range of types of food samples should be included, preferably an entire spectrum. However, this is almost impossible with omnivorous rodents. They consume both plant and animal food in very variable proportions, sometimes also rather unexpected substances (for example, ash or clay) [23]. NIRS calibrations generally less accurately predict the chemical composition of compound materials, compared to raw materials [24]. In varied diets, most compounds can occur in more types of materials and chemical bonds (for example nitrogen in vertebrate muscles, seeds, vegetation tissues, etc.) [25]. Despite an accurate calibration curve based on many different types of diet, some samples deviate extremely from the calibration model. These circumstances limit the possibility of creating a precise calibration and make NIRS results for omnivores weaker than results for food specialists [26][27]. The NIRS results should be interpreted regarding some specific features of species and natural conditions. One of them is the spatial behaviour of rodent species; the home range of voles is usually much smaller than areas used by mice [28]. Unlike voles, mice can be caught in a different environment than the one in which they feed. Another feature is that the grass and herbal diet in the stomachs of herbivores (voles) is more susceptible to damage in hot weather than the seed food in the stomachs of mice; this can lead to inaccuracies in some samples.

3. What Has Already Been Found?

3.1. NIRS Analysis of Rodent Dietary Preferences

The first case study tested the possibility of using NIRS to determine the number of cereals and acorns in the stomach content. The subject of the study was the food preferences of *Apodemus flavicollis*, depending on the yield of acorns and the availability of supplementary food in a pheasantry. The applicability of the method for this type of work was verified on 94 samples. The results confirmed that *A. flavicollis* prefer cereals over the common available food (e.g., acorns). In the “seed year” (a year with a high harvest of acorns), there was a higher proportion of acorns in the diet of *A. flavicollis* [29].

To the best of current knowledge, the analysis of rodent faeces using NIRS has been used only once, to predict the proportion of two types of cereals (wheat and barley) in the diet of common rodents occurring in the arable landscape of Moravia. The calibration equation was developed by measuring samples obtained by mixing two types of faeces in various known ratios. Each type of faeces was collected in a laboratory experiment from a wood mouse (*Apodemus sylvaticus*) fed either barley or wheat. The percentage diet preferences were determined by comparing faeces from preference tests with a calibration equation. The calibration was shown to be sufficiently accurate also for the pygmy field mouse (*A. uralensis*) and black-striped mouse (*A. agrarius*) (R^2 = 0.99, p < 0.001; SECV = 1.78). However, estimates for the common voles’ faeces varied substantially. The reason is probably taxonomic and/or physiological differences in the process of digestion [22]. Hence, a special calibration equation had to be developed for the common vole. Results based on the NIRS method showed that all *Apodemus* species preferred wheat over barley, while the common vole showed no preference for any crop [17].

3.2. Rodent Stomach Sample Preparation for the NIRS Analysis

During the above-mentioned research, it was found that, for samples from stomach contents, it would be useful to verify the influence of chyme composition in different stomach parts as well as the methodology of sample preparation on the results. There were two main reasons; first, rodents chew different foods differently and the food can then progress through the stomach at different speeds [30]. Second, homogenization of whole samples by grinding is much more time consuming than abrasion of a sample part with sandpaper and subsequent analysis of the ground part. Therefore, it had to be verified that none of these reasons affected the outcome of the NIRS analysis. It was found that there are no differences in the nitrogen content between the pyloric and cardiac chyme or between the sample prepared by abrasion and grinding [31].

After verifying the methodology, the nitrogen content was selected as an ideal indicator of food quality for further study. Samples from forest and field habitats were available. Because they differ both in potential food sources and, to some extent, in the species spectrum of rodents, both groups of habitats were examined separately.

3.3. NIRS Analysis of Rodents Food Quality in Forest Habitats

The NIRS method was used to compare the food quality (i.e., nitrogen content) of four species of the most numerous forest rodents in the Czech Republic (*Apodemus flavicollis, A. sylvaticus, Clethrionomys glareolus,* and *Microtus arvalis*) [32]. Both *Apodemus* species showed higher variability and higher mean food quality compared to *M. arvalis* [32]). Results for *C. glareolus* were intermediate for both variability and mean value. Differences in diet composition (or ratio of nitrogenous substance) in different biotopes was demonstrated only for the *C. glareolus*. Moreover, the *C. glareolus* diet varied within the same biotope over different years. *C. glareolus* was the only species to display differences in diet between males and females, with males having higher nitrogen concentrations. In addition, for *A. sylvaticus*, changes in food quality during the year were intensively monitored [33]. The highest quality as well as variability in food quality was found outside the growing season (i.e., through the winter).

Analysis by the NIRS method highlighted differences in food quality between species with different foraging ecology. For example, omnivorous mouse species have eaten food with higher and more variable content of nitrogenous substances than the herbivorous common vole. Additionally, food quality changes throughout the year (at least in *A. sylvaticus*).

3.4. NIRS Analysis of Rodents Food Quality in Arable Landscape

Three articles dealt with the study of food quality by the NIRS method of the most numerous rodents in Central European agroecosystems. In the first article, changes in the quality of food were observed in two populations of *Microtus arvalis* (the most numerous rodents in this habitat). A higher proportion of nitrogenous substances was found in breeding females at the highest population densities [34]. The second article compared the food quality of *A. sylvaticus* and *M. arvalis* in extensively and intensively agriculturally used localities. The effect of population density, crop, and season on the food quality was found in both species [35]. The third article sought to verify whether the knowledge found in previous works on food of the genus *Apodemus* can be applied to *A. uralensis*; it is the dominant species in some areas together with the *M. arvalis*. *A. uralensis*' food was shown to have, on average, a lower proportion of nitrogenous substances and higher variability; the food quality was influenced by the season. In *M. arvalis*, the most important factor was the overall abundance of rodents [36]. A strong correlation was found between food quality and population density in the common vole and wood mouse [35][36]. This finding clearly confirms that food quality is related to rodent population dynamics. In arable ecosystems, the amount of nitrogen in consumed food differs in various crops in relation to their nitrogen content [35]. In forest and arable habitats, reproduction and season of year were also reflected in changes in food quality [34][33] in contrast to sex, body size, and age.

4. Conclusions

The NIRS method has been used only rarely to study small mammals; this is a shame, especially in connection with the invention of microsensors that have great potential. NIR spectroscopy can help find answers to questions that are difficult to answer by other methods because of time, technology, and financial requirements; these are issues of both basic and applied research. Basic research could use NIRS for monitoring physiological variables (e.g., type and quality of food, occurrence of pathogens, environmental pollution, etc.). The applied area could use the acquired knowledge for population monitoring (e.g., frequency of pathogens or environmental pollution), and subsequently to manage rodent populations more precisely (detection and prediction of conditions under which damage occurs facilitates better timing of interventions to minimize the damage). The main limitation of using the NIRS method is likely the high demand on technological equipment and expertise in a number of different branches, such as experimental design, spectrum removal, calibration, and the interpretation of results (i.e., the need to create a team of people of very different specializations).

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