

## DYNAMICS OF NITROGENOUS SUBSTANCES CONTENT IN THE DIET OF THE WOOD MOUSE (*APODEMUS SYLVATICUS*)

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### Abstract

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The representation of nitrogenous substances in the stomach content of *Apodemus sylvaticus* was determined using the NIRS (near-infrared spectroscopy) method. Out of the total of 247 examined stomachs, 66 were male and 181 female. Sampling of study material was conducted in 2003–2010 at three isolated forest sites in South Moravia with different habitat conditions and different type of management (old semi-natural forest with dominance of English Oak (*Quercus robur*); production forest with dominant Sessile Oak (*Quercus petraea*) and Black Locust (*Robinia pseudoacacia*); pheasantry with variable mixture of forest plots). The determined nitrogenous substances content ranged from 9.5–64.4% of dry matter. With respect to nitrogenous substances content, the habitat, site and sex factors were assessed as statistically insignificant. On the other hand, the factor of a given year ( $\chi^2 = 31.14$ ;  $p < 0.000$ ) and that of sexual activity ( $\chi^2 = 7.86$ ;  $p = 0.005$ ) showed significant differences. In relation to season, both the average nitrogenous substances content in diet and the standard deviation oscillated. The highest average nitrogenous substances content was determined in winter months, when the most significant dispersion in values was determined as well. In years following mast years (2004 and 2007) high values of standard deviations in dietary nitrogenous compounds content were determined.

*Apodemus sylvaticus*, wood mouse, diet quality, near-infrared spectroscopy, nitrogenous substances

The wood mouse (*Apodemus sylvaticus*) is one of the most common rodent species in Central Europe, where it occurs in a wide spectrum of habitats ranging from agrocenoses to ecotones and open forested areas to continuous forest stands of various ages and composition (FLOWERDEW and GARDNER, 1978; GURNELL, 1985; SUCHOMEL and HEROLDOVÁ, 2004). The species has been subject to intensive study, particularly under research projects studying the structure and character of small mammal communities (ZEJDA, 1973; 1981, 1991; PELIKÁN, 1989; SUCHOMEL *et al.*, 2007), while some studies focus on its population dynamics (PELIKÁN, 1964, 1976) or trophic relations (HOLIŠOVÁ, 1960; HEROLDOVÁ, 1994). Dietary studies, however, are not numerous and are based on demanding microscopic analyses

of individual diet components (fruits, green parts of plants, invertebrates, fungi, etc.) and cannot evaluate their quality (nutritional value). Latest analytical methods (here NIRS – Near Infrared Reflectance Spectroscopy) represents a prompt low-cost analytical technique that can support conventional laborious methods and is a highly promising new method for diet quality analysis in free-living species. One of the most commonly used indicators is content of nitrogenous substances (LESLIE *et al.*, 2008). A direct analysis and quality assessment of digested food (energy, nitrogenous substances, fibre) from the stomach content of wild small terrestrial mammals has probably been the focus of a single study focusing on the common vole (*Microtus arvalis*) in Brittany (BUTET, 1996). This issue in the wood mouse has not been subject to research so far.

The presented paper therefore focuses on issues related to nitrogenous substances content in this species' diet. The aim is to evaluate its dynamics in the course of the year as well as over the years, changes with respect to the given biotope and site and relation to the sex and sexual activity of the studied individuals.

## MATERIAL AND METHODS

### Study area

Samples for this study were collected between 2003 and 2010 from three model lowland forest biotopes in southern Moravia (Czech Republic): Lednice – Horní les (48° 48.5' N, 016° 47.08' E), a semi-natural floodplain forest; Hájek – Vranovice (48° 57.4' N, 016° 35.62' E), a typical commercial oak forest; and the Rumunská pheasantry near the village of Blučina (49° 02.41' N, 016° 42.8' E), which encompasses a variety of biotopes and stands. All sites were divided into different forest biotopes (spruce, ash, sessile or English oak forest in different age) or non-forest biotopes situated on the edge of forest (i.e. meadows, clear-cut areas, field edges, wetlands).

### Small mammal sampling

*Apodemus sylvaticus* were captured using snap traps laid in lines. Nineteen lines were laid in total (five in Horní les and Hájek, and nine in the pheasantry), each with 20 traps set two metres apart, and always positioned in a different type of biotope. Wicks fried in flour and smeared with peanut butter were used as bait. Traps were exposed for three nights and they were checked each morning following exposure. Sampling was repeated every 2 months five times a year (with the exception of December/January).

### Examination of captured mammals

Captured individuals were determined to species, measured (body length; in mm) and weighed (in g; if necessary, other important species determination characteristics were also recorded). The rodents were dissected in order to determine the sex and sexual activity of the specimen, reproductive state being specified as immature or adult. Adult males were identified through enlarged testes and seminal vesicles, adult females were those with embryos or placental scars on the uterus, and animals that had not yet entered into reproduction were designated as immature (PELIKÁN, 1959). Adult males with sexual glands in regression were registered as in the regression stage. The stomachs were removed and dehydrated by freezing and subsequent drying in a kiln.

### NIRS analysis of nitrogenous substances content in digested food

The stomach wall was removed from the frozen and dried sample in order to provide a sample of pure content. One of sample's sides was then

levelled through abrasion such that a) the area treated was larger area than the detection window of the NIRS equipment, and b) the sample was sufficiently thick that it could not be penetrated by a light beam. Samples that met the above criteria were measured in spinning cups using a near infrared spectrophotometer FOSS NIR System 6500 in the 1 100–2 500 nm wavelengths range. This produced a spectrum representing the complete physical and chemical composition of the sample. The NIR calibration model of nitrogen content was developed by chemometric and statistical methods and partial least square regression was used according to Janová *et al.* (in preparation). In short, this calibration model utilises 34 stomach samples of known nitrogen value measured using the Kjeldahl method (AOAC, 1980). The calibration model obtained had a simple coefficient of determination 0.841 ( $R^2$ ) and a standard error of validation (SEV) of 43.5. After Gross validation, the simple coefficient of determination was 0.843 and the 154 SECV 44.1. This model was used to estimate the nitrogen content of all stomach samples collected. Verification of the calibration model was done by EffiChem software designed for validation calibration curves. For this, 20 independent samples not included into calibration model were used. Comparison between the conventional Kjeldahl and NIRS method provided statistical identical results, so the NIRS method was usable.

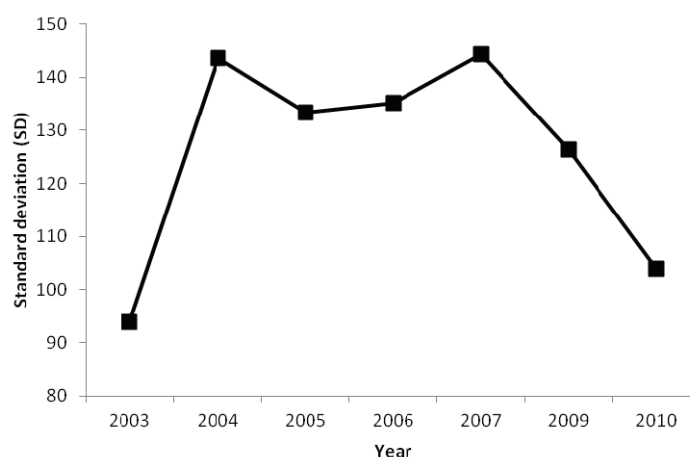
### Statistical analysis

All calculations have been done in programmes Microsoft Excel 2007 or Statistica 8.0 (StatSoft 2007), using the generalised linear model (multivariate analysis) to test for statistical significance of individual factors.

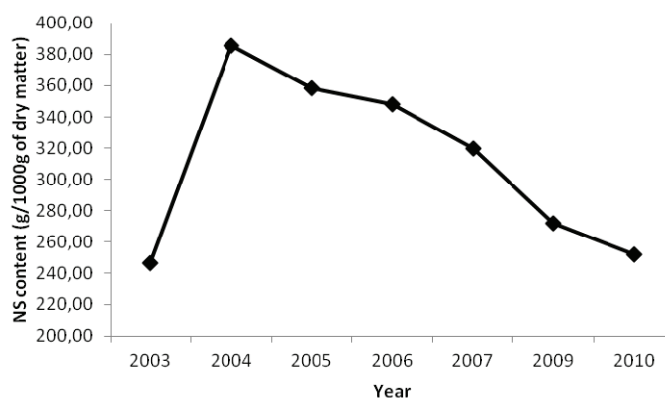
## RESULTS

Nitrogenous substances content was measured in stomach contents of 247 individuals, representing 66 males and 181 females. The determined nitrogenous substances content ranged from 9.5–64.4% of dry matter. Thus obtained data were consequently processed statistically. It was not possible to establish a statistically significant dependence on the biotope, site or sex factors. This means that no differences were determined in the above cases with respect to content of nitrogenous substances in the wood mouse diet.

The year factor differed significantly ( $\chi^2 = 31.14$ ;  $p < 0.000$ ). It may be inferred that due to different food supply in individual years the nutrient content in wood mouse diet changes as well. The highest dietary nitrogenous substances content values do not correlate with mast years or with the consequent periods of wood mouse population peaks. However, the years immediately following mast years (2004 and 2007) showed increases in standard deviations and dietary nitrogenous substances content variability respectively (Fig. 1).



1: Dynamics of standard deviations of nitrogenous substances average content in the *Apodemus sylvaticus* stomach content in the monitored period



2: Development of nitrogenous substances average content in the *Apodemus sylvaticus* stomach content in the monitored period

I: Average nitrogenous substances content in the diet of individual categories of *Apodemus sylvaticus* (AD – adult males; MC+ – females which have given birth; MC0 – females which have not given birth; R – males in the regression stage; SAD – subadult males)

Category	Average nitrogenous substances content (%)
AD	32.44
MC+	30.70
MC0	36.10
R	28.10
SAD	36.34

The average nitrogenous substances content in wood mouse diet decreased slightly in time (Fig. 2).

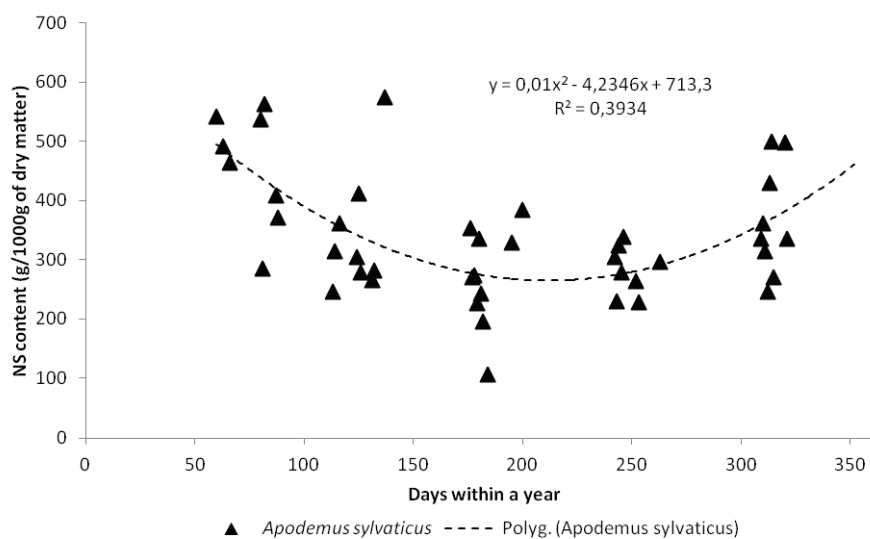
Sexual activity proved to be another conclusively significant factor ( $\chi^2 = 7.86$ ;  $p = 0.005$ ). Higher nitrogenous substances content in a diet was determined in sexually inactive individuals of both sexes and was always slightly higher in males (Tab. I). The lowest nitrogenous substances content was determined in the diet of males in regression stage (on average 28.1% of dry matter).

The dynamics of dietary nitrogenous substances content in *Apodemus sylvaticus* in the course of the

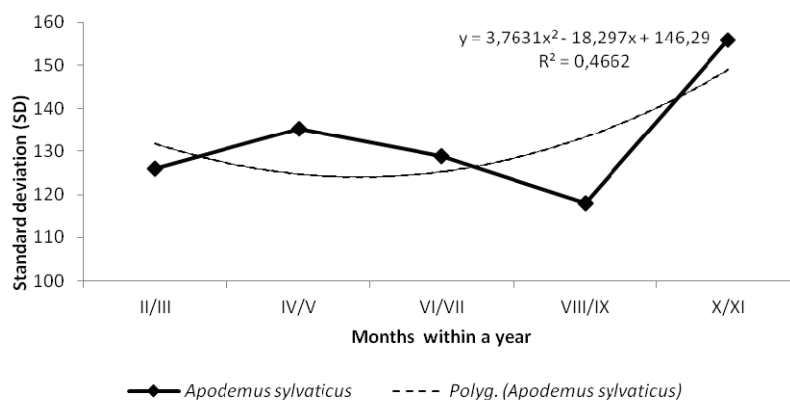
year may be best assessed from a graph displaying the polynomial trend line (see Fig. 3). Based on this it may be observed that higher relative nitrogenous substances content in the wood mouse diet can be detected in the period from late autumn to early spring than during the vegetation season. Similarly, the variability of nitrogenous substances in diet is higher outside the vegetation season, as a comparison of standard deviations in individual parts of the year reveals (see Fig. 4).

## DISCUSSION

The highest dietary nitrogenous substances content values do not correlate with mast years (which took place in 2003 and 2006, see SUCHOMEL, 2008) or with the consequent periods of population peaks in forest rodents. Similarly, BUTET (1996) did not find a significant relation between the dietary nitrogenous substances content and population dynamics of the studied *Microtus arvalis* populations in Brittany. An explanation of the cyclic population peak phenomenon must therefore remain open for the time being. Although a number of hypotheses about a direct relation



3: Dynamics of nitrogenous substance content in the *Apodemus sylvaticus* diet in the course of the year



4: Variability of nitrogenous substances content in the *Apodemus sylvaticus* diet in the course of the year

between population cycles and a group of chemical substances in diet did not prove true (OKSANEN and OKSANEN, 1981; ANDERSSON and JONASSON, 1986; JONASSON *et al.*, 1986; LAINE and HENTTONEN, 1987; OKSANEN *et al.*, 1987), the influence of other than the tested antinutritional components may not be ruled out. It will probably involve a complex synergy of a number of factors, such as predation, diet quantity and quality, weather and self-regulatory mechanisms within populations (HANSSON, 1979; BATZLI, 1985; BUTET and DELETTRE, 2011).

With respect to dietary nitrogenous substances content, a statistically significant difference between individual biotopes was not determined. Yet, most analyses comparing the diet of *Apodemus sylvaticus* in various biotopes state that the proportion of animal-based diet is significantly higher in coniferous stands (HOLIŠOVÁ, 1960; MONTGOMERY and MONTGOMERY, 1990; KHAMMES and AULAGNIER, 2007).

Higher nitrogenous substances content was determined in the diet of subadult individuals.

Most authors agree that this animal category feeds on animal-based diet in a relatively lowest extent (HOLIŠOVÁ, 1960; ZEMÁNEK, 1972; HEROLDOVÁ, 1994; KHAMMES and AULAGNIER, 2007), possibly substitutes it with fungi (HOLIŠOVÁ, 1960). But it is clear this category has higher needs of nutrients because of their continuous growth. It is possible they are able to find wider sources of nitrogen-rich vegetable and fungi food thanks to their higher mobility and agility (FLOWERDEW and GARNER, 1978; GURNELL, 1985). A relatively low ratio of nitrogenous substances in the diet of adult females is also surprising, as their nutrient consumption due to pregnancy and lactation is expected to be the highest (FLOWERDEW and GARNER, 1978). However, when assessing the volume of ingested nutrient, both its absolute quantity and the efficiency of its utilization, i.e. physiological absorption efficiency, prove to be key factors (MYRCHA, 1965). One of the methods comparing absorption efficiency is a relative or absolute comparison of the gastrointestinal tracts, particularly of the

weight and length of their individual parts or the absorption surfaces (SCHIECK and MILLAR, 1985; BORKOWSKA, 1995). It is a general truth that the bigger the relative size, the higher the efficiency. With regard to rodents, most studies agree on seasonal dynamics and differences in the size or weight of gastrointestinal tracts heavily in favour of females, by up to several tens percent (HOLIŠOVÁ, 1960; MYRCHA, 1965; BORKOWSKA, 1995). In this case the actually absorbed amount of nutrients is higher in females, which explains their higher demand (offspring investment) even upon lower nitrogenous substances content in digested food (GORMAN and AKBAR, 1993).

The dynamics of nitrogenous substances content in the diet of small terrestrial mammals in the course of the year is best assessed from a graph displaying a polynomial trend line (see Fig. 3). It may be concluded that in late autumn as well as in early spring, the relative nitrogenous substances content in rodent diet is higher than during the summer. In the summer months the content of less concentrated food richer in fibre is probably higher in rodent diet, which corresponds with diet analyses from the Czech-Moravian Highlands (HOLIŠOVÁ, 1960). BUTET (1996), who studied nitrogenous substances content in the diet of *Microtus arvalis* in Brittany, drew an identical conclusion. In contrast, the situation in the environment of the Swedish tundra seems to be the exact opposite, with the maximum dietary nitrogenous substances content falling on the vegetation season (HANSSON, 1972). In the conditions of the Czech Republic, the variability of nitrogenous substances content

in diet decreases in summer months as well. If the results obtained in the Czech-Moravian Highlands (HOLIŠOVÁ, 1960) could be generalized for South Moravia as well, then the higher dietary nitrogenous substances content outside the vegetation season is caused by consumption of seeds and fruits, as the maximum consumption of animal-based diet focuses on spring and early summer (from March through to June).

## CONCLUSION

Nitrogenous substances content in the diet of *Apodemus sylvaticus* was analysed using the NIRS method. Stomach contents of 66 males and 181 females, i.e. a total of 247 individuals, were analyzed. It was confirmed that population cycles and population cycle peaks in small terrestrial mammals are not directly related to increased nitrogenous substances content in digested food. Nevertheless, in years with population peaks, i.e. in years immediately following mast years, a slight increase in the variability of nitrogenous substances content in digested food was detected. A generally higher concentration of nitrogenous substances in the diet of subadult individuals was determined. In the course of the year, increased concentrations of dietary nitrogenous substances connected with higher variability of samples were monitored outside the vegetation season from late autumn to early spring. On the other hand, the summer months showed an opposite trend, with a lower variability in diet combined with lower concentration of nitrogenous substances.

## SUMMARY

The wood mouse (*Apodemus sylvaticus*) is one of the most common rodent species in Central Europe. The presented paper therefore focuses on issues related to nitrogenous substances content in this species' diet. The aim is to evaluate its dynamics in the course of the year as well as over the years, changes with respect to the given biotope and site and relation to the sex and sexual activity of the studied individuals. Nitrogenous substances content in the diet of *Apodemus sylvaticus* was analysed using the NIRS (near-infrared spectroscopy) method. Sampling of study material was conducted in 2003–2010 at three isolated forest sites in South Moravia with different habitat conditions and different type of management (old semi-natural forest with dominance of English Oak (*Quercus robur*); production forest with dominant Sessile Oak (*Quercus petraea*) and Black Locust (*Robinia pseudoacacia*); pheasantry with variable mixture of forest plots). *Apodemus sylvaticus* were captured using snap traps laid in lines. Captured individuals were determined to species, measured, weighed and dissected in order to determine the sex and sexual activity of the specimen, reproductive state being specified as immature or adult. Out of the total of 247 examined stomachs, 66 were male and 181 female. The stomachs were removed and dehydrated by freezing and subsequent drying in a kiln. The determined nitrogenous substances content ranged from 9.5–64.4% of dry matter. With respect to nitrogenous substances content, the habitat, site and sex factors were assessed as statistically insignificant. On the other hand, the factor of a given year ( $\chi^2 = 31.14$ ;  $p < 0.000$ ) and that of sexual activity ( $\chi^2 = 7.86$ ;  $p = 0.005$ ) showed significant differences. A generally higher concentration of nitrogenous substances in the diet of subadult individuals was determined. In relation to season, both the average nitrogenous substances content in diet and the standard deviation oscillated. In years following mast years (2004 and 2007) were determined higher values of standard deviations in dietary nitrogenous compounds content. Within a year the highest average nitrogenous substances content was determined in winter months, when the most significant dispersion in values was determined as well. On the other hand, the summer months showed an opposite trend, with a lower variability in diet combined with lower concentration of nitrogenous substances.



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