# Nitrogen characteristics of ungulates faeces: effect of time of exposure and storage

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A b s t r a c t . An experiment was conducted to monitor the effect of the length of environmental exposure of faeces on the content of nitrogen and diaminopimelic acid. We used samples of the droppings of wild red deer and examined them for the content of N and DAPA upon exposure to field conditions for 0–7 days during the growing season and for 0–30 days in winter, and after a year of storage in dried and frozen state. In relation to nitrogen level, there were no differences between the samples of fresh droppings and those after different lengths of exposure to ambient conditions before analysis and no differences between fresh and stored samples. As to DAPA level, there were no differences between the samples of fresh droppings and those after exposure. Nitrogen and DAPA levels in the droppings were stable and can be measured in both fresh samples and samples that have been exposed to ambient conditions for one week in summer or one month in winter.

## Introduction

Wildlife management in a cultivated landscape focuses on seeking a balance between the numbers of animals present and their impact on the environment. Special attention is paid to meeting the food requirements of free ranging ungulates whilst attempting to minimise the impact of grazing on the vegetation. Monitoring of the quality of ungulates' food is important in this context. Evaluation of the capacity of food sources is as a rule based on the botanical composition of the food and the knowledge of the nutritive value of the various ingredients (A b e l l & G i l b e r t 1974, L e s l i e et al. 1984, M e r r i l l et al. 1995, R a y m o n d et al. 1996, H o m o l k a & H e r o l d o v á 2001). Such methods require determination of the diet composition (direct observation, analysis of the rumen content, faecal analysis or feeding experiments) and determination of nutritive value in the samples of the plants corresponding to the composition of the diet of the animals under study. As direct determination of diet quality often involves complications, indirect methods are frequently used, based on the correlation between the content of the selected substances (nitrogen, DAPA and others) in the faeces and the nutrient value parameters of the food the animal eats (fibre, crude protein, digestible organic matter). These relations have been demonstrated in a number of species of both free ranging and domestic ruminants (H o l l o w a y et al. 1981, L e s l i e & S t a r k e y 1985, P u t m a n & H e m m i n g s 1986, L e s l i e & S t a r k e y 1987, I r w i n et al. 1993, H o d g m a n et al. 1996).

Nitrogen content is the most frequently used food quality indicator (S i n c l a i r et al. 1982, L e s l i e & S t a r k e y 1985, B e i e r 1987, L e s l i e et al. 1989, H o d g m a n et al. 1996) even thought is not fully reliable (E i s f e l d 1983, H o b b s 1987).

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The use of faecal indicators involves problems, often including the difficulty of collecting a sufficient number of fresh samples of the droppings (due to low density of occurrence of the animals and rapid decomposition of the faeces). As a result, samples of different age are often used together. However, the length of exposure to field conditions may affect the chemical composition of the faeces because of the activity of insects and microbes and the influence of weather. The time of persistence of the droppings in the environment varies with weather conditions, including precipitations, and may range between several days and several months (D z i e c i o l o w s k i 1976, W i g l e y & J o h n s o n 1981, A u l a g & B a b i n s k a - W e r k a 1990, L e h m k u h l et al. 1994, M a s s e i et al. 1998).

This paper contains the results of a trial in which we studied the changes in the levels of nitrogen (N) and diaminopimelic acid (DAPA) in samples of droppings after exposure to effects of weather for different periods of time and after preservation by different methods.

## Material and Methods

In September and December 2000, we collected 5 fresh (< 6 hours after defecation) faecal groups of red deer each of 50 pellets. Samples were collected in an area, where the diet composition of ungulates is representative for the Czech Republic (H o m o l k a 1996). We immediately divided the samples into seven (ser. No. 1 – 7) and five (ser. No. 8 – 12) sub-samples in summer and in winter respectively. Each subsample contained 5 pellet (enough for 2 g weight of dry matter needed for the laboratory analysis) and placed them on soil surface partially shaded by vegetation. After different intervals of time (Table 1), the samples were collected for further treatment. We tested for N and DAPA content of fresh pellets from one particular pellet group sample and then compare the values for N and DAPA derived from that same sample after exposure for some days in the field, or after freezing or drying.

After exposure to ambient conditions, the samples were dried at 60 °C in a ventilated drier and were analysed. Sub-Sample no. 5 was slowly dried for 7 days in the laboratory (21 °C) after collection and then the drying process was completed in the drier and the sample was analysed. Sample no. 6 was first dried in the drier and then kept under laboratory conditions and sample no. 7 while fresh, was frozen and stored at –21 °C. Experiments with the summer samples no 5, 6 and 7 was made in laboratory conditions

**Table 1.** Characteristics of faeces group samples (five pellets per each group) used to evaluation of changes in nitrogen and DAPA volume (mean ± SD).

| No. | Season | Exposure (day) | Stocking (day) | Position | N (g/100g) | Dapa (g/kg) |
|-----|--------|----------------|----------------|----------|------------|-------------|
| 1 | summer | 0 | 0 | - | 2.62 ± 0.25 | 2.90 ± 0.52 |
| 2 | summer | 1 | 0 | open air | 2.59 ± 0.25 | - |
| 3 | summer | 3 | 0 | open air | 2.64 ± 0.27 | 3.70 ± 1.32 |
| 4 | summer | 7 | 0 | open air | 2.65 ± 0.27 | 2.60 ± 0.42 |
| 5 | summer | 7 | 0 | laboratory | 2.68 ± 0.28 | 3.13 ± 0.92 |
| 6 | summer | 0 | 380 | dried | 2.78 ± 0.24 | 4.22 ± 1.39 |
| 7 | summer | 0 | 380 | frozen | 2.69 ± 0.27 | - |
| 8 | winter | 0 | 0 | - | 1.72 ± 0.10 | 3.73 ± 0.66 |
| 9 | winter | 3 | 0 | open air | 1.68 ± 0.15 | 4.88 ± 0.46 |
| 10 | winter | 10 | 0 | open air | 1.70 ± 0.12 | 4.30 ± 0.56 |
| 11 | winter | 20 | 0 | open air | 1.69 ± 0.10 | 4.10 ± 0.62 |
| 12 | winter | 30 | 0 | open air | 1.71 ± 0.16 | 4.18 ± 1.03 |

independently of weather. It also was why the same procedures were not done with the winter samples to save any financial means. In summer the daytime temperature was around 15 °C during the trial. During the first 3 days the samples had a fresh appearance; later their surface began drying and changing colour.

In winter the samples were placed on the ground thinly covered with snow. The daytime temperatures were 0–5 °C, the night temperatures were < 0. During the first 10 days the samples maintained a fresh appearance. On the 20–30th day their surface began drying and cracks occurred on it.

The dried samples were crushed (1 mm mesh). Nitrogen content was determined by the standard Kjeldahl method, DAPA content was determined after hydrolysis at 145 °C for 4 hours in HCl (c = 6 mol/l) on the automatic amino acid analyser AAA 400 (INGOS Prague).

Volume of nitrogen and DAPA in samples were normally-distributed (1-sample Kolmogorov-Smirnov test; P > 0,05) thus 1-way ANOVA and t-tests were used to determine whether N and DAPA means would change with increased time of exposure.

**Results**

Nitrogen content (g/100 g dry matter) in the fresh samples ranged between 2.25 and 3.05 g in the growing season and between 1.51 and 1.95 g in winter. Exposure of the samples to ambient conditions before collection for analysis (0–7 days in the growing season, 0–30 days in winter) had no effect on nitrogen level in the growing season ($F_{4,20} = 0.27$; P = 0.95) and in winter ($F_{4,20} = 0.11$; P = 0.97) (Fig 1,2). Nor did one year of storage of samples preserved by drying or freezing appear to have any effect on nitrogen ($F_{2,12} = 0.19$; P = 0.83). Nitrogen content in the samples put in the drier when fresh did not differ from that in the samples left to dry slowly in the laboratory (t = -1.02; df = 8; P = 0.34).

DAPA content (g/kg of dry matter) in the fresh samples ranged between 1.91 and 6.86 g in the growing season and between 2.69 and 5.61 g in winter. Environmental exposure of the samples before collection for analysis had no impact on DAPA level ($F_{4,25} = 1.68$; P = 0.19 in the growing season and $F_{4,25} = 1.81$; P = 0.16 in winter) (Figs 1,2). DAPA content in the samples put in the drier when fresh did not differ from that in the samples left to dry slowly in the laboratory (t = -1.78; df = 8; P = 0.11). In the preserved samples, DAPA level could not be evaluated.



y = 0.0068x + 2.6299
$R^2$ = 0.0058
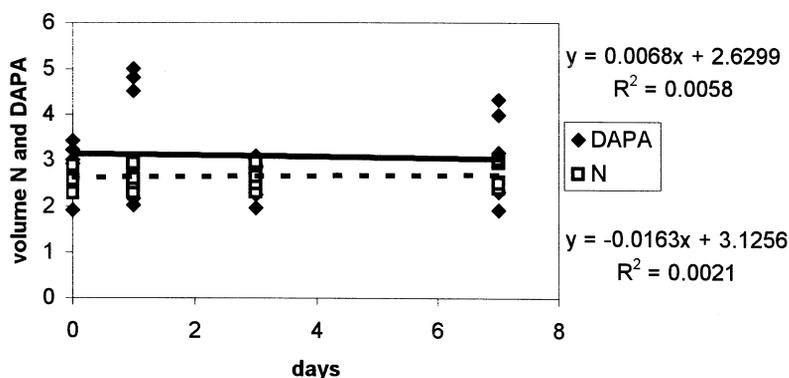
y = -0.0163x + 3.1256
$R^2$ = 0.0021

**Fig 1.** Dynamics of nitrogen (g/100g; dashed line) and diaminopimelic acid (g/kg; continuous line) volume in summer deer faeces in relationship to time of exposure.
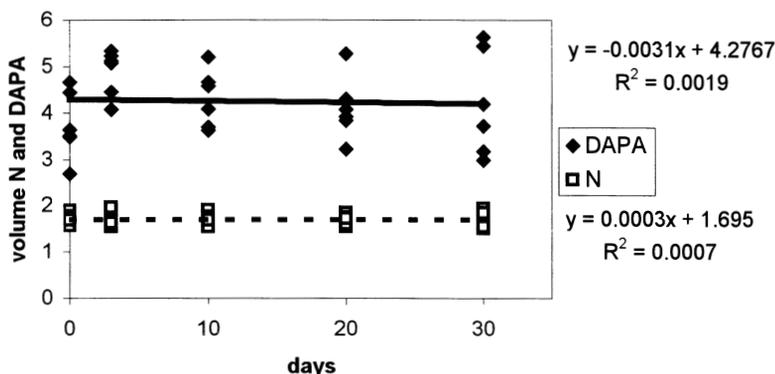
$$y = -0.0031x + 4.2767$$
$$R^2 = 0.0019$$

$$y = 0.0003x + 1.695$$
$$R^2 = 0.0007$$

**Fig 2.** Dynamics of nitrogen (g/100g; dashed line) and diaminopimelic acid (g/kg; continuous line) volume in winter deer faeces in relationship to time of exposure.


## Discussion

Samples were kept in shady place with short loan during the environmental exposure. Neither in winter nor in summer did insects attack them. In summer, older samples can be distinguished from the fresher ones by appearance (dried surface, cracks). In winter the faeces continue looking fresh for about 2 weeks, but droppings old 21 days or more can already be clearly distinguished from fresh samples. The time of sample exposure used in the trial (7 days in summer, 30 days in winter) exceeded the period for which the samples maintain fresh appearance.

Nitrogen levels in the analysed samples of red deer faeces showed a comparatively narrow variance both in the growing season and in winter (SD = 5.9–10.3 % of the average) and even did not change after environmental exposure following storage for a year. Nor did the method of drying of the samples exert any influence on nitrogen level. The stability of nitrogen level, as found in our trial, corresponds to the results reported by J e n k s  et al. (1990), who observed no differences between samples of white-tailed deer droppings stored in the laboratory for 12 days and samples exposed to environmental conditions for as long as 24 hours.

DAPA level varied more significantly than did oxygen level in both fresh and older samples (SD = 10.2–40.0 % of average). Neither in summer nor in winter did DAPA levels show any significant change when the samples were exposed to field conditions.

Both DAPA and nitrogen belong among the most frequently used quality indicators of the food of free-ranging ruminants. Correlations between DAPA and N levels in faeces and the content of available energy in the food have been demonstrated on a number of occasions (H o d g m a n  et al. 1996, K u c e r a  1997). Our results proved the stability of N and DAPA contents in red deer faeces when the samples were exposed to field conditions before collection and when they were stored. The content of these indicators was affected neither by the freshness of the samples, nor by the speed of their drying and time of their storage.

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