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RESEARCH ARTICLE

Effect of the abrasive properties of sedges on the intestinal absorptive surface and resting metabolic rate of root voles

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ABSTRACT

Recent studies on grasses and sedges suggest that the induction of a mechanism reducing digestibility of plant tissues in response to herbivore damage may drive rodent population cycles. This defence mechanism seems to rely on the abrasive properties of ingested plants. However, the underlying mechanism has not been demonstrated in small wild herbivores. Therefore, we carried out an experiment in which we determined the joint effect of abrasive sedge components on the histological structure of small intestine as well as resting metabolic rate (RMR) of the root vole (*Microtus oeconomus*). Histological examination revealed that voles fed with a sedge-dominated diet had shorter villi composed from narrower enterocytes in duodenum, jejunum and ileum. Reduction in the height of villi decreased along the small intestine. Activity of the mucus secretion increased along the small intestine and was significantly higher in the ileum. The intestinal abrasion exceeded the compensatory capabilities of voles, which responded to a sedge-dominated diet by a reduction of body mass and a concomitant decrease in whole body RMR. These results explain the inverse association between body mass and the probability of winter survival observed in voles inhabiting homogenous sedge wetlands.

KEY WORDS: Body mass, Plant defensive mechanism, Sedges, Silicon, Small intestine

INTRODUCTION

Grasses and sedges dominate many terrestrial ecosystems and are the food base for numerous cyclical populations of small herbivores. This plant food contains a high concentration of silicon (Hodson et al., 2005), which together with fibre (Vincent, 1982; Grzelak et al., 2011), increases its abrasive properties (Montagne et al., 2003; Massey and Hartley, 2006). The abrasive properties of this plant food have been proposed as an anti-herbivore defence mechanism (Massey et al., 2008). Our previous studies (Wieczorek et al., 2014) showed that changes in the silicon concentration in fibrous tussock sedge (*Carex appropinquata* Schumacher 1801) were induced by a high density of cyclical population of the root voles (*Microtus oeconomus* Pallas 1776) at the end of the previous summer. We also found that smaller (lighter) voles were characterised by lower mortality during early winter (Wieczorek et al., 2014; Zub et al., 2014), which might be correlated with the low quality of their food base. However, the underlying proximate mechanism promoting smaller individuals is not known.

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Low body mass of voles feeding on a highly silicated and fibrous diet (Massey et al., 2008) may reflect the need to reduce absolute energy requirements (Ergon et al., 2004), to bring them into line with reduced digestive efficiency (Massey et al., 2006). This efficiency may be compromised because of a reduction of the intestinal surface and its function of nutrient absorption, stemming from the mechanical abrasion of the apex of villi where the widest mature enterocytes, which produce digestive enzymes, are located (Montagne et al., 2003; Abbas et al., 1989; Barker et al., 2008). However, as far as we know the above putative mechanism has not yet been demonstrated in the context of the effect of dietary silicon and fibre on the intestinal histology of wild rodents. Several laboratory studies have demonstrated that passage of the abrasive food components through the digestive tract elicits protective mucus secretion by the mucus cells (Allen et al., 1982; Enss et al., 1994; Lentle and Janssen, 2011) and contributes to enterocyte shedding, thereby shortening the height of villi (Pluske et al., 1997). The food being digested undergoes significant physical changes that increase abrasive properties along the small intestine during its intestinal transit (Lewis and Southern, 2001). If the abrasive diet exerts the same effect on rodent herbivores, one can expect reduction of their absorptive surface, increasing along the small intestine and followed by faster turnover of enterocytes (Jin et al., 1994), leading to a compensatory increase in the size of the herbivores' intestine (Hammond and Wunder, 1991; Lee and Houston, 1993; Young Owl and Batzli, 1998; Pei et al., 2001).

Predictions related to the association between the effect of abrasive diet and basal or resting metabolic rate (BMR or RMR) are not straightforward (Cruz-Neto and Bozinovic, 2004). On the one hand, BMR is positively genetically correlated with the ability to cope with a low-quality diet (Sadowska et al., 2009). Indeed, many studies report a positive correlation between BMR and the intestinal mass/size (Konarzewski and Diamond, 1995; Książek et al., 2004; Książek et al., 2009; Naya et al., 2013), which may reflect metabolic costs of the intestinal hypertrophy triggered by a hard-to-digest diet. On the other hand, rodents fed a poor-quality diet reduced their BMR or RMR and body mass or size (Muñoz-Garcia and Williams, 2005; Perissinotti et al., 2009). This suggests that, at least at the phenotypic level, individuals faced with abrasive diet components primarily balance energy budgets by minimisation of energy expenditure.

Here, for the first time, we quantify the joint effect of abrasive components of plant diet on intestinal histological structure as well as RMR. We chose the root vole (*M. oeconomus*) as a model herbivore, because they feed primarily on sedges (Tast, 1966) that contain high fibre (Grzelak et al., 2011) and silicon content (Hodson et al., 2005). We carried out a laboratory experiment, in which we fed voles with a sedge-dominated diet for 27 days. We predicted that this will result in: (1) a decrease of the digestive efficiency and compensatory increase of food consumption; (2) abrasion of the intestinal mucosa due to removal of the mature enterocytes, decrease

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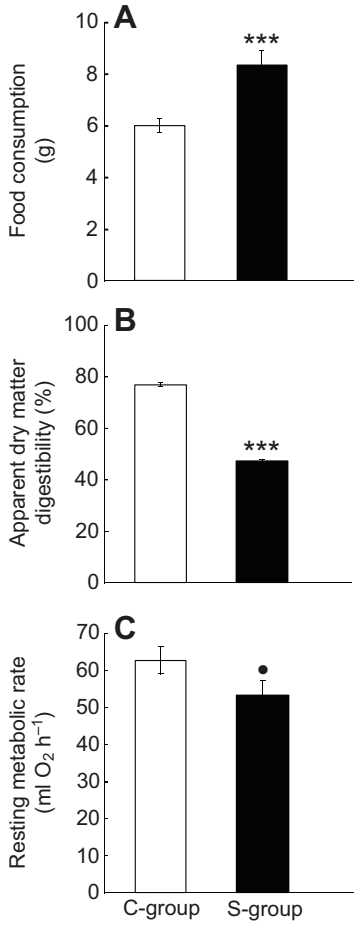

Fig. 1. Food consumption, digestibility and metabolism measurements in the root vole (*Microtus oeconomus*). (A) Food consumption (g), (B) the apparent dry matter digestibility (DMD) and (C) whole body resting metabolic rate (ml O_2 h^{-1}) after the feeding trial of root voles from the control (C-) group and sedge-fed (S-) group (means ± s.e.). n.s., not significant, *P<0.05, ***P<0.001.

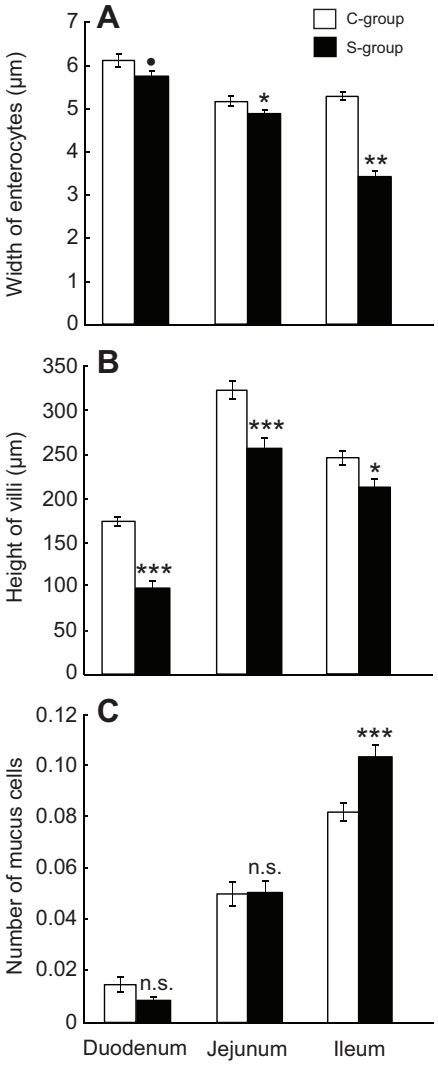

Fig. 2. Histological measurements of the digestive system of root voles fed a diet rich in sedge. (A) Width of enterocytes (µm), (B) height of villi (µm) and (C) number of the mucus cells per height of villus in duodenum, jejunum and ileum of root voles from the control (C-) group and sedge-fed (S-) group (means ± s.e.). n.s., not significant, *P<0.05, **P<0.01, ***P<0.001.

of the villi height and increase of mucus secretion, which increase along the small intestine; (3) an increase of RMR due to the incurred metabolic costs of rebuilding the intestinal surface or, alternatively, a decrease in RMR and body mass to save energy.

RESULTS

Voles fed with the sedge-dominated diet (S-group) had 39% higher food consumption ($F_{1,27}$=14.37, P=0.001; Fig. 1A) but lower apparent dry matter digestibility (DMD) than individuals from the control group (C-group) ($F_{1,27}$=667.90, P<0.001; Fig. 1B).

In the duodenum, jejunum and ileum enterocytes of voles from the S-group were narrower than voles from the C-group (Table 1; Fig. 2A, Fig.3). In both the duodenum and jejunum, the width of the S-group enterocytes was reduced by 6% and in the ileum by 8%. In all three segments of the small intestine voles from the S-group had lower villi than those from the C-group (Table 1; Fig. 2B, Fig. 4). In the duodenum the height of villi were reduced by 44%, in jejunum by 20% and in ileum by 13%. Ileum (but not duodenum nor jejunum) of voles from the S-group had higher number of mucus cells (Table 1; Fig. 2C, Fig. 3).

Before the feeding trial, the body mass of voles did not differ between the diet groups ($F_{1,27}$=0.24, P=0.63). Repeated-measures ANOVA showed that during the course of the feeding trial voles from the S-group significantly decreased their body mass (Fig. 5),

Table 1. ANOVA results for the effect of diet type on width of enterocytes, height of villi and number of mucus cells in root voles

	Duodenum			Jejunum			Ileum		
	N	*F*	*P*	*N*	*F*	*P*	*N*	*F*	*P*
Width of enterocytes (µm)	14	$F_{1,12}$=4.91	**0.05**	26	$F_{1,24}$=4.54	**0.04**	26	$F_{1,24}$=11.01	**0.003**
Height of villi (µm)	13	$F_{1,11}$=42.63	**<0.001**	22	$F_{1,20}$=17.06	**<0.001**	24	$F_{1,22}$=7.58	**0.01**
Number of mucus cells	13	$F_{1,11}$=2.46	0.14	22	$F_{1,20}$=0.03	0.86	24	$F_{1,22}$=15.46	**0.001**

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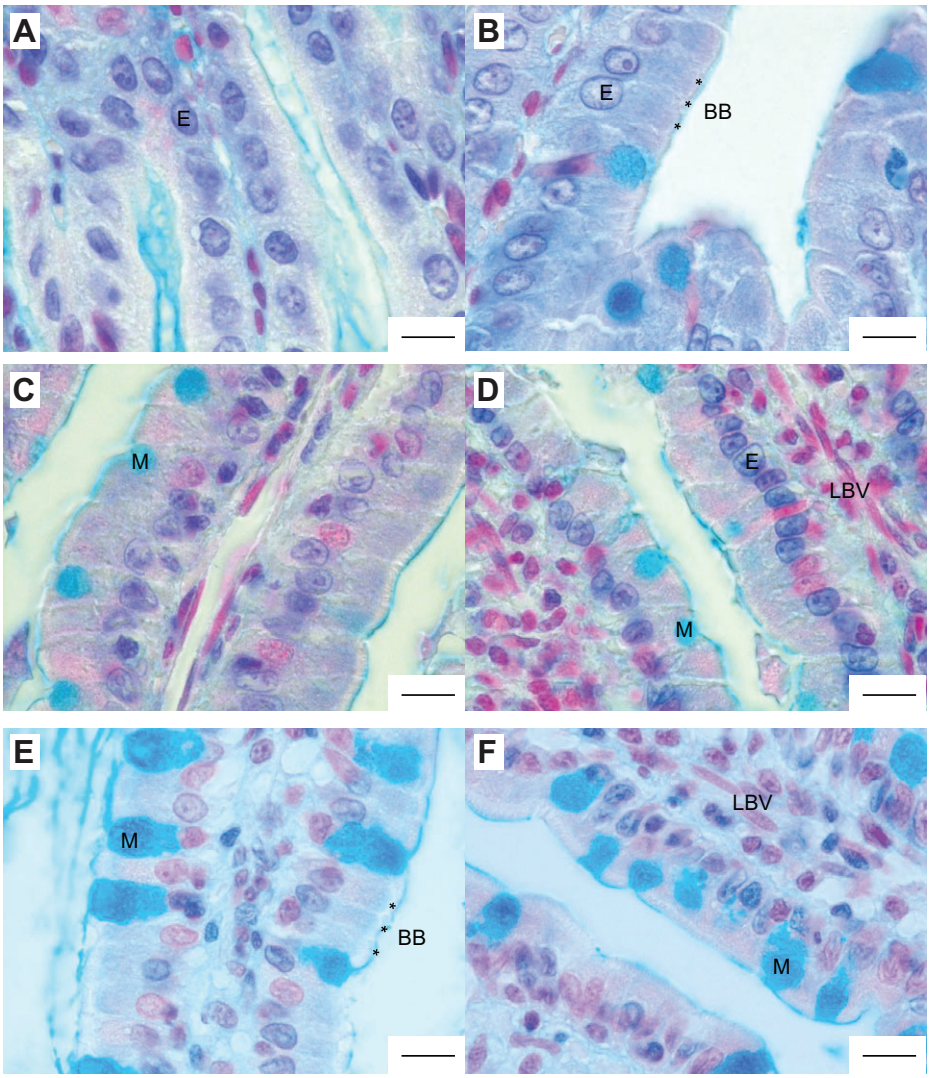

Fig. 3. Longitudinal sections through digestive system of root voles. Villi with enterocytes and mucus cells in duodenum (A,B), jejunum (C,D) and ileum (E,F). Comparison between C-group (A,C,E) and S-group (B,D,F). BB, brush border with microvilli (asterisks); E, nucleus of enterocyte; LBV, lymph and blood vessels; M, mucus in the mucus cell. Scale bar: 10 µm.

as indicated by a significant effect of diet ($F_{1,26}$=10.8, P=0.003) and a significant time×diet interaction ($F_{10,260}$=4.9, P=0.001).

Whole-body RMR measured before the feeding trial did not differ between the diet groups (for C-group: 79.04±3.24 ml O_2 h^{-1}; for S-group: 82.59±4.72 ml O_2 h^{-1}; $F_{1,26}$=0.20, P=0.65; mean ± s.e.). In both experimental groups, RMR decreased during the course of the feeding trial [RMR measured after the experiment in the C-group: 64.28±3.53 ml O_2 h^{-1}, paired t-test=4.76, P=0.0004; S-group: 53.60±3.61 ml O_2 h^{-1}, t (paired)=4.63, P=0.0005]. Whole-body RMR measured after the experiment was lower in the S-group ($F_{1,26}$=4.02, P=0.05, Fig. 1C), which was partly due to a slight reduction of body-mass-corrected RMR ($F_{1,25}$=3.93, P=0.06; body mass was not significant as a covariate, $F_{1,25}$=0.40, P=0.53).

DISCUSSION

Feeding of voles with the sedge-dominated diet for 1 month resulted in a significant abrasion of mature enterocytes located at the apex of villi, which reduced the nutrient absorptive surface (Montagne et al., 2003). An increased secretion of mucus in the ileum of the S-group was probably due to increasing abrasiveness of the digesta during its transit along the small intestine. As is well known, once the digesta enters the duodenum, it is broken down by a series of enzymatic secretions that convert nutrients into forms more easily absorbed (Lewis and Southern, 2001). Therefore, the digesta transported along the intestine becomes increasingly less fluid and upon reaching ileum it contains increasingly condensed solid indigestible particles. Our results strongly suggest that the digesta of voles from the S-group contains high concentrations of silicon and fibres, which initiates protective mucous secretion in the ileum. However, the abrasive effect of the digesta on the height of villi was most clear-cut in the duodenum, where little protective mucous secretion (most likely interfering with nutrient absorption) was observable.

It should be noted that the experimental diet we used contained only 62% sedge, which was enough to cause abrasion to the voles' intestine. Under natural conditions the root voles feed almost exclusively on sedges (Tast, 1966), and therefore they probably suffer from even more intense mucosal shedding than that reported here. In our recent study, we found that high densities of the root vole population inhabiting homogenous sedge wetland induces an increase of the silicon concentration in sedges at the end of summer (Wieczorek et al., 2014), which, in turn, is followed by an increased mortality of larger (heavier) individual voles during early winter (Wieczorek et al., 2014; Zub et al., 2014). Thus, our laboratory experiment provides a mechanistic explanation to the field observations by showing that a sedge-dominated diet can reduce the intestinal absorptive surface. The same mechanism may also be relevant to Massey et al. (Massey et al., 2008) who found that highly silicated grasses reduced the body mass of overwintering voles.

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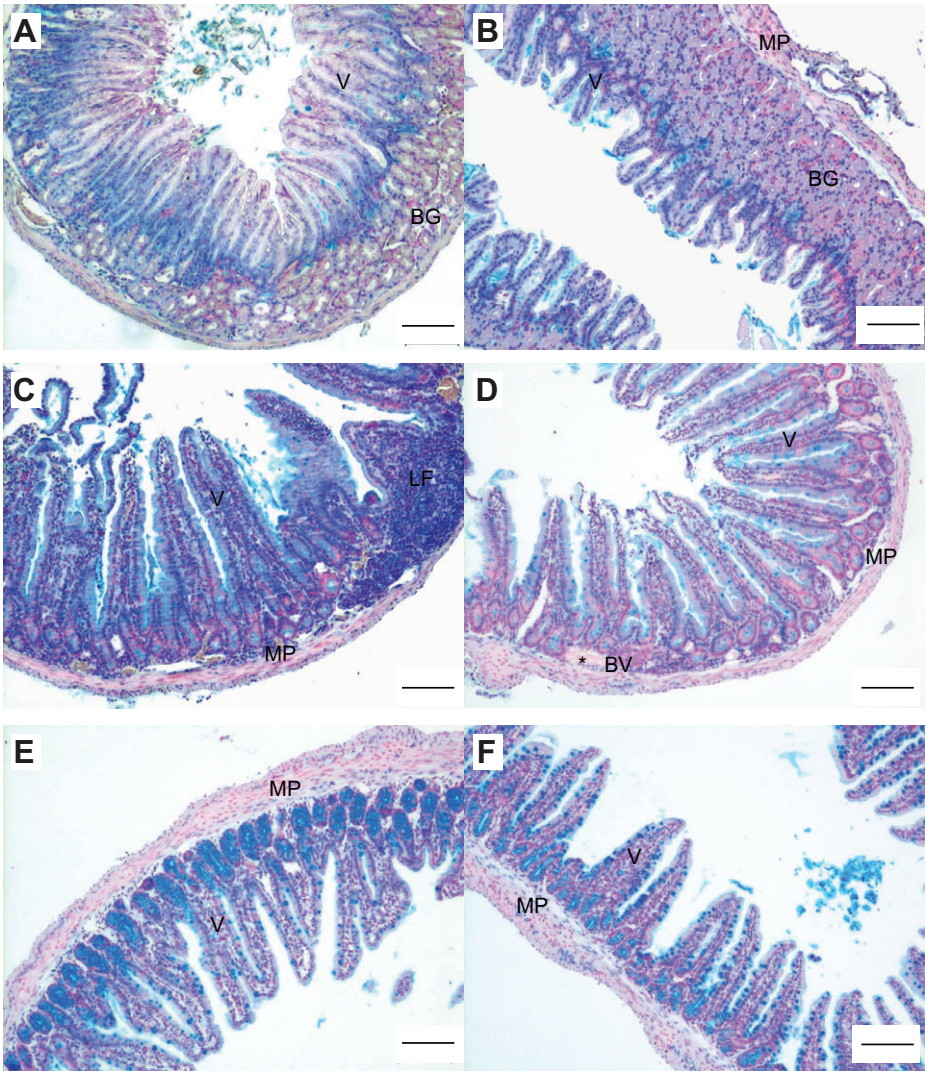

Fig. 4. Cross-sections through digestive system of root voles. Duodenum (A,B), jejunum (C,D) and ileum (E,F). Comparison between C-group (A,C,E) and S-group (B,D,F). BG, Brunner's glands in submucosa; BV (asterisk), blood vessel; LF, intestinal lymph follicle; MP, muscularis propria; V, villi covered with epithelium in mucosa. Scale bar: 100 µm.

These findings are also supported by the lower DMD and higher food consumption of voles from the S-group. Several experimental studies on voles also reported that animals increased their consumption to compensate for the loss of nutrients and energy deficiency from hard-to-digest food (Hammond and Wunder, 1991; Young Owl and Batzli, 1998). In our case, however, voles were not able to compensate for the intestinal abrasion and responded by a reduction of body mass.

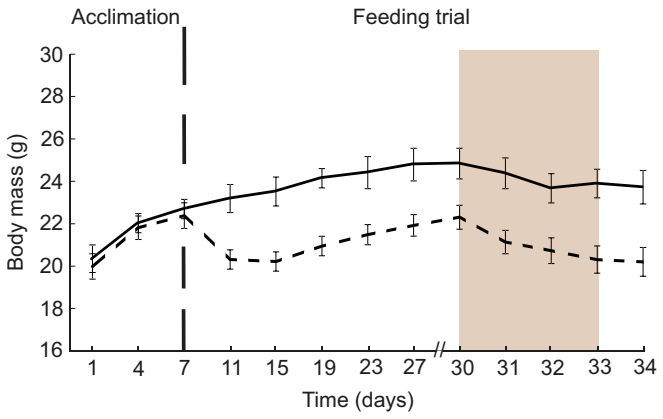

Fig. 5. Body mass of root voles measured throughout the acclimation period and feeding trial. C-group, solid line; S-group, dashed line (values are means ± s.e.). The shaded area indicates the period for which measurements for the food consumption and digestibility trial were taken.

Reduction of the RMR resulted mainly from the reduction of body mass, rather than mass-specific metabolic rate, because the latter was not statistically significant. In any case, we did not find any indication of metabolic costs incurred by rebuilding of the intestinal surface. It is also important to note that our experiment does not allow for a clear-cut discrimination between the reduction of body mass and RMR as manifestations of the adaptive response to poor quality diet, versus the non-adaptive loss of body mass merely due to energy deficiency. Nevertheless, our results are consistent with theoretical considerations predicting that voles should limit their energy expenditure through reduction in body mass to increase their survival when access to the digestible nutrients and energy is limited (Ergon et al., 2004). Phenotypic reduction of RMR in response to a poor-quality diet reported herein is in opposition to the positive genetic correlation between metabolism and the ability to cope with a low-quality food found in the bank voles (Sadowska et al., 2009). However, within-individual phenotypic responses may not reflect genetic correlations, which, by definition, are analysed on the between-individual level (Lynch and Walsh, 1998).

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In summary, we provide evidence that the rate of intestinal abrasion may exceed the compensatory abilities of voles. When they have no alternative in the selection of food components voles seem to be forced to reduce their energy expenditure to increase the probability of overwinter survival (Wieczorek et al., 2014).

MATERIALS AND METHODS

Animal collection and acclimation

We live-trapped root voles (*M. oeconomus*) in September 2011 in the Białowieża Glade, NE Poland (~52°42′ N, 23°52′ E). Captured animals were transported to the nearby laboratory of the Mammal Research Institute, Polish Academy of Sciences, where they were sexed and weighed. To standardise the experiment, we used voles weighing less than 30 g, i.e. born at the end of summer or early autumn (Gliwicz, 1996). The criteria were met by 29 voles of both sexes, which were placed in separate cages and provided with food and water *ad libitum*. They were fed with maintenance fodder containing 17.54 kJ g^{-1} of energy, 1.04% silicon, 21.16% reducing sugars and 54.62% di- and polysaccharides in dry matter (for detailed diet composition, see below). Photoperiod (9 h:15 h, light:dark) and temperature (20°C) were adjusted to the environmental conditions at that time of year. Voles were acclimated to these laboratory conditions for 7 days. All experimental protocols were approved by the Local Ethical Committees in Białystok (43/2010 and 38/2011).

Analysis of diet component and chemical composition

During the feeding trial, the control group of voles (C-group) was fed with the maintenance fodder (Labofeed KB, Morawscy, Kcynia, Poland), while the other group of animals (S-group) received a modified fodder with 62.16% of the grinded mixture of leaves and rhizomes of fibrous tussock sedges (*Carex appropinquata*). Components of both diets are listed in supplementary material Table S1.

Before chemical analysis, samples of fodders were dried at 55°C for 48 h. The total energy content was measured in a Berthelot-type bomb calorimeter (IKA, Germany). Total mineral content was quantified as the ash mass left after combustion. The total fat concentration was determined by the Soxhlet extraction in petroleum ether for 10–12 h. The total protein concentration was determined by near infrared (NIR) spectroscopy with reflectance measurements in the wavelength range of 1100–2500 nm (Feed & Forage Analyser, FOSS, USA). The concentration of reducing sugars was determined by the Somogyi–Nelson method (Somogoyi, 1952) using 80% ethanol alcohol, Somogoyi–Nelson reagent at a wavelength of 540 nm (Beckman Coulter, USA). The concentration of the remaining saccharides (di- and polysaccharides) was determined by subtracting the summed concentrations of reducing sugars, ash, protein and fat from 100%. Silicon concentration was determined by the atomic absorption spectrometry method, using 70% nitric acid, 30% hydrogen peroxide and 10% NaOH solutions (Haysom and Ostatek-Boczynski, 2006) with the absorbance measured at 251.6 nm (Avanta PM atomic absorption spectrometer, GBC Scientific Equipment, Australia). All results were converted to percentage dry matter (supplementary material Table S2).

Feeding trial and experimental design

Following acclimation, we randomly assigned voles into one of the two dietary groups: 14 voles to the control group (C-group) and 15 voles to the group fed with sedges (S-group). The C-group was fed with the maintenance fodder. The S-group received a modified fodder containing 62.16% (dry matter) of the fibrous tussock sedges (*C. appropinquata*). Highly fibrous sedges (Grzelak et al., 2011) contain silica in the form of phytoliths of 10–45 µm size (Ollendorf, 1992), deposited within cells and incorporated in their walls. Leaves and rhizomes from aboveground tussocks of sedges were collected in November 2010 from the enclosures inhabited by root voles (Wieczorek et al., 2014). As voles do not feed on decaying litter, we collected leaves and rhizomes physically connected with the tussocks, composed of dried tissues. Sedge samples were cleaned under running water, dried at 80°C, ground and mixed with the maintenance fodder. Thus prepared diet contained 18.78 kJ g^{-1} energy, 1.87% silicon, 10.03% reducing sugars and 63.98% di- and polysaccharides in dry matter. Both fodders were in the form of 1.2-cm-diameter pellets. The feeding trial lasted for 27 days (Fig. 5). Measurements of RMR were made at the beginning and end of the feeding trial. Food consumption and digestibility was measured from 30th to 33rd day of the experiment. After the second RMR measurement, voles were killed for histological examination of their small intestine.

Food consumption and digestibility

We estimated food consumption and digestibility during a 4 day trial. We placed each animal in a wire-bottomed metabolic cage, which enabled separation of the provided food from faeces and orts. However, the metabolic cage exposes the animals to grid floors and a lack of nesting materials that may lead to the rapid heat loss (Tarland, 2007). Therefore, the first 2 days were an introductory period, and we based our estimates on the measurements taken during the next 2 days of the trial. Daily food consumption was calculated by subtracting the mass of uneaten food from the mass of food provided (weighed to the nearest 0.01 g) and presented as an average from 2 days. Food provided was in the form of dry pellets. Faeces and food orts were collected daily and dried at 55°C. As the mean retention time of low-quality diet in small rodents shows substantial variation (Young Owl and Batzli, 1998; Hammond, 1989; Hume et al., 1993), we averaged the apparent dry matter digestibility (DMD) for summed 2 day food consumption calculated according to the following equation:

$$\mathrm{DMD} = \frac{\left(\text{mass of given food} - \text{mass of uneaten food}\right) - \text{mass of faeces}}{\text{mass of given food} - \text{mass of uneaten food}} \times 100\% . \quad (1)$$

Resting metabolic rate (RMR)

Measurements of RMR were carried out during the day, in the 2–4 h trials at 30°C, a temperature within the thermoneutral zone (Wang and Wang, 2000). Prior to the trials, voles had unlimited access to food and water to avoid the possibility that longer fasting would compromise the animals' welfare.

We used a computer-controlled positive pressure, open-circuit respirometry system. Outside atmospheric air was pushed through a column of Drierite (W. A. Hammond Drierite Co.) to remove water vapour, and forced through a copper coil submerged along with the metabolic chamber in a water bath to equalise and control the temperature. The airstream was then divided into reference and measurement streams, each fed to a separate mass-flow controller (Sierra Instruments, Monterey, CA, USA or ERG-1000, Warsaw, Poland). The measurement stream was forced through the metabolic chamber (volume ~800 cm^3) at a mean rate of 400 ml min^{-1}. The air streams were then directed to a computer-controlled Sable Systems TR-1 setup (Las Vegas, NV, USA). The analysed gas stream was re-dried (Drierite), subsampled at 120 ml min^{-1} with a subsampler, and then passed through the sensor of an FC-10b oxygen analyser (Sable Systems). Digital signals from the analyser were stored using Winwedge 3.0 software (Taltech, Philadelphia, PA, USA) and subsequently analysed with Sable Systems' Datacan V software.

For each individual (weighed to the nearest 0.1 g prior to the trial), we selected the three lowest read-outs of 4 min length that did not change more than 0.01% of the oxygen consumption. In the final analyses, we used values characterised by absolute lowest RMR (regardless of s.d.). Oxygen consumption rate was calculated according to Hill (Hill, 1972, equation 5), assuming RQ=0.8 (Koteja, 1996).

Body mass measurements

To control for the condition of the voles, we repeatedly measured their body mass (to the nearest 0.1 g) at the beginning, in the middle and at the end of acclimation period. During the feeding trial, we measured body mass every 4 days. The only exception was the consumption and digestibility trial, when we weighed voles daily (Fig. 5).

Histological analyses

After the feeding trial, voles were killed by intramuscular injection of a lethal (1 ml) dose of ketamine hydrochloride (Bioketan, Vetoquinol, Biowet, Poland). All animals were killed between 8:30 h and 11:30 h. To minimise the damage incurred by evacuation of foodstuffs from the intestine, voles

were given no food after the completion of the second RMR measurement. The small intestine was dissected out from the gastrointestinal track and divided into duodenum, jejunum and ileum. From each segment, we cut out the 1-cm-long samples, rinsed out their luminal content with the 1×phosphate buffered saline (PBS) and fixed them in 6% buffered neutral formalin for 2 weeks. Then, samples were dehydrated with a graded series of ethanol alcohol (75, 80, 90, 96, 99%), cleared in ST Ultra (Leica, Germany) and embedded in Paraplast®PlusTM (Leica). We cut the 4-µm-thick serial sections with a motorised rotary microtome Hyrax M 55 (Zeiss, Germany), stained them with Alcian Blue (Carl Roth, Germany) and Nuclear Red (Carl Roth), cleared in ST Ultra (Leica) and mounted on CV Ultra (Leica). We chose sets of five cross-sections from each sample and digitised them under a light microscope BX 51 (Olympus, Japan) equipped with a digital camera and dotSlide (Olympus) software (version 2.1). We examined the width of enterocytes, the height of villi and the activity of mucus secretion by mucus cells separately for each intestinal segment. In the duodenum, we measured the height of villi only in the regions with the Brunner's glands in submucosa.

The width of enterocytes (Abbas et al., 1989), as well as their maturity (Barker et al., 2008), increase from the bottom to the apex of the villi. Therefore, we used this parameter as an indicator of the enterocytes' enzymatic activity and ability to absorb nutrients. We arbitrarily divided each villus into three equal parts and measured enterocytes only in the middle one. Plasmalemma was not visible in the enterocytes, therefore their width was measured as the distance between the nuclear membranes of neighbouring enterocyte nuclei. We used sets of a minimum of two cells, measuring ~120 enterocytes per individual.

The height of villi was measured as the distance from the apex to the base of a single villus. In the case of two unevenly located crypts of the villus, we measured the height to a crypt with higher located opening. The measured villi had to be cut perpendicularly to the intervillous area and completely preserved. These criteria were met by a variable number of villi (from 5 to 40 per individual). Villi with detached tops were excluded from the analysis, although they were still suitable for measuring the width of enterocytes in their middle parts. As a result, there were a different number of samples used for analysing the width of enterocytes and the height of villi (Table 1). The activity of mucus secretion was measured as the number of mucus cells per villous height. The mucus cells were recognised on the basis of the visible mucus-filled apical regions of cytoplasm.

Statistical analyses

We log-transformed body mass, RMR, width of enterocytes and height of villi, and arcsine-transformed DMD to correct the right-skewed distributions. We used a stepwise approach and retained only significant variables and interactions in the final models.

To test whether voles maintained their body mass during the experiment, we used the repeated-measures ANOVA with the diet type as the between-subject factor, time course as the within-subject factor and the respective interaction terms. To test the effect of the diet type on body mass before the feeding trial, we constructed a one-way ANOVA model with the diet as a fixed factor. To analyse the effect of food consumption, digestibility, intestinal histology and RMR (measured before and after the feeding trial) among voles, we constructed ANCOVA models with diet type and sex as fixed factors, body mass as a covariate and the respective interaction terms. Sex and body mass of voles were never significant and were therefore not retained in the final models. For the second measurement of RMR, we built an additional model with food consumption as a covariate, to analyse its potential effect on the results. Consumption was not significant, and was thus eliminated from the final model. The stepwise approach did not allow us to correct the second RMR for the effect of body mass, thus we separately presented a model with body mass as a covariate. To compare RMR of voles before and after the feeding trials, we used a *t*-test for matched pairs. All statistical analyses were undertaken using the stats package in R software (R Development Core Team, 2012).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.W., P.S., M.K. conceived the experiment. M.W., P.S, J.L. performed the laboratory work (trapping and handling of voles in the laboratory, measuring their body mass, RMR and digestibility). M.W. performed euthanasia. A.M.L. conceived the histological analyses, prepared histological slides, coordinates histological measurements. M.W. performed histological measurements. M.W., M.K. performed the statistical analyses. M.W., M.K. prepared the manuscript. M.K. coordinated the entire experiment. All authors discussed and commented on the manuscript.

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Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.117168/-/DC1

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