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Homeostatic responses in the gut of *Porcellio scaber* (Isopoda: Oniscidea) optimize litter degradation

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Abstract In order to examine the influence of differences in food conditions on gut characteristics in *Porcellio scaber*, pH-manipulated and microbially inoculated leaf litter from three different tree species were offered. Microbial activity was clearly influenced by the pH levels of the leaves. Analyses of the pH levels in the gut indicated the ability of *P. scaber* to buffer the pH value in the intestinal tract to about 5.5–6.0 in the anterior hindgut, and to about 6.0–6.5 in the posterior hindgut. The pH levels of the gut sections remained in this range, within a range of food pH from 4.0 to 7.5, no matter what kind of leaves the animals were fed. Homeostatic responses to changes in food pH guarantee optimized digestion of leaf litter. However, when the pH level of the litter dropped below 3.5, *P. scaber* was not able to maintain the pH conditions in the gut. Furthermore, microorganisms colonizing the litter biased the pH level in the anterior hindgut where digestive processes mainly take place. These results indicate a decline of litter quality with regard to the nutrition of terrestrial isopods, caused by acidification and consequently reduced microbial activity.

Key words Terrestrial isopods · Acidification · Gut pH · Digestive enzymes · Litter decomposition

Introduction

Terrestrial isopods play an important role in the decomposition of leaf litter (e.g. Biwer 1961a,b; Cameron and LaPoint 1978). The food of saprophagous animals mainly consists of cellulose and other polysaccharides that cannot be easily digested (Kozlovskaja and Striganova 1977; Szegi 1988). Consequently, these animals need cellulolytic enzymes. Cellulolytic gut symbionts have been demonstrated in xylophagous termites (Breznak 1975, 1982). By contrast, woodlice, as a model for saprophagous soil animals, are thought to possess neither symbionts nor endogenous enzymes to decompose cellulose (Jeuniaux 1956, in *Porcellio scaber*; Hassall and Jennings 1975, in *Philoscia muscorum*; Kozlovskaja and Striganova 1977, in *Hemilepistus cristatus*; Kukor and Martin 1986, in *Trachelipus rathkii*). Hence, terrestrial isopods are assumed to utilize ingested microbial cellulases for the enzymatic breakdown of cellulose. Uesbeck and Topp (1995) recently described decreasing consumption rates and digestibility in *Oniscus asellus* foraging on sterilized leaf litter. Zimmer and Topp (1997) observed a decrease in reproduction when *Porcellio scaber* fed on acidic litter with little microbial activity. In choice experiments, terrestrial isopods clearly prefer litter that is colonized by microorganisms rather than litter without or with little microbial activity (Gunnarsson 1987; Stöckli 1990). Hence, *P. scaber* showed olfactory orientation towards microbial metabolites of cellulose digestion (Zimmer et al. 1996).

The physiology of the digestive system in terrestrial isopods has been described in detail. Differences in the pH levels of different gut sections (Nicholls 1931) are important with respect to the activity of digestive enzymes produced by the animal, or of microbial enzymes ingested with the food.

The present study was carried out to test the capability of the common woodlouse, *P. scaber*, of maintaining gut homeostasis. The maintenance of constant environmental conditions inside the gut should be adaptive, when acid rain and soil acidification cause low pH levels of the leaf litter.

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Materials and methods

Individuals of *P. scaber* were taken from a mixed alder-poplar forest near Cologne (Germany) during spring. Litter from the

sampling site was characterized by pH levels of 7–8. The isopods were maintained on a natural substrate in large plastic dishes at long-day conditions (16 h L:8 h D) and 15 °C for 3 weeks. As a food source, the leaf litter of three different palearctic tree species, namely alder (*Alnus glutinosa*), birch (*Betula pendula*) and oak (*Quercus robur*), was offered. Furthermore, to alter food quality, leaf litter of all species was manipulated (cf. Zimmer and Topp 1997):

1. We simulated the influence of acid rain and altered leaf quality by pH manipulation of the litter in different solutions of sulphurous acid (pH 2.0, pH 3.5 and pH 5.0, respectively) for 7 days. Additionally, we used NaOH solutions (pH 8.0) to raise the pH levels of the leaves. These procedures resulted in leaf litter exhibiting pH values within the following ranges: pH 3.0–3.3, pH 4.1–4.5, pH 5.2–5.8, and pH 7.3–7.8, respectively.
2. pH-manipulated leaf litter was inoculated with microorganisms by placing the leaves in gauze bags of mesh size 700 µm beneath litter originating from the field. The inoculation was carried out for 21 days at 20 °C in constant darkness. After the inoculation, the pH levels of the leaves ranged from pH 3.6–3.7, pH 4.1–4.8, pH 5.7–6.0, and pH 7.0–8.0, respectively.

In each of the 12 experimental treatments, 15 adult isopods were kept separately for 14 days in small petri dishes of diameter 4.5 cm. After 7 days, faecal pellets were removed. This time interval was chosen to ensure that the entire gut content had been replaced by the food offered. After a further 7 days, freshly deposited faeces were collected within 24 h of removing the litter from the petri dishes to prevent the pH levels of the faeces from being changed by contact with the litter. These faecal pellets were used to examine the pH level of the faeces.

The gut of adult *P. scaber* can be divided into three parts. Two pairs of lobes or caeca of the hepatopancreas as described by Hassall and Jennings (1975) fill up the body cavity along the alimentary canal. In the hindgut, beginning at the connection of the lobes to the alimentary canal and ending at the anus, an anterior and a posterior part can be distinguished. The anterior part is characterized by "typhlosole canals" (Hassall and Jennings 1975). The posterior part of the hindgut is a straightened tube consisting of different parts that can be subdivided morphologically. In this study, the entire posterior hindgut was considered as the sphincter region.

For the preparation of the gut, the specimens were frozen at −20 °C. To examine pH values, the gut was removed from the dissected specimens and divided into the caeca of the hepatopancreas, the typhlosole region of the hindgut and the posterior (i.e. sphincter) region of the hindgut. While the samples of the hepatopancreas included the surrounding cells, the gut tissue was removed in the case of anterior and posterior hindgut and the samples only included the gut content and the surrounding gut cuticle.

For pH measurements, aliquots of the leaves (10–20 mg), the gut sections (3–5 mg), and the faeces (5–10 mg) were each homogenized in 750 µl of 3 M KCl. After 30 min, the pH of the supernatant solution was determined. Some isopods ($n = 10$) were examined as described above immediately after their capture, in order to get information on the gut characteristics under natural conditions. Additionally, the influence of microorganisms colonizing the leaf litter was estimated by comparing these isopods to specimens that had fed on sterilized litter (60 °C, 48 h) for 7 days.

Cellulose is one of the most important food sources of saprophagous animals (cf. Kozlovskaja and Striganova 1977). Consequently, we compared the pH levels of the gut of *P. scaber* with the pH optima of cellulolytic enzymes of the leaf litter. Cellulase activity of pH-manipulated litter was determined according to Skambracks (1996). By this means, we obtained optimal activity of cellulases at pH levels of 5.5–6.0 (cf. Fig. 2). These values coincide with data given for cellulases of the gut fluids of *O. asellus* (Hartenstein 1964).

Since the data obtained from the different treatments were not normally distributed, data are represented as median ± median absolute deviation (M ± MAD) and statistical analyses were carried out using non-parametric tests. Gut sections of isopods fed on different leaf litter qualities were compared with the Kruskal-Wallis *H* test. Subsequently, significant differences were localized using the Mann-Whitney *U* test.

Results

When freshly collected specimens of *P. scaber* fed on the basic litter found in the field (pH = 7.5 ± 0.2, $n = 10$), the pH level of the examined sections of the gut were 6.2 ± 0.2 (caeca of the hepatopancreas), pH 6.0 ± 0.1 (anterior region of the hindgut, i.e. typhlosole region) and pH 6.5 ± 0.2 in the posterior region of the hindgut (i.e. sphincter region). After feeding on sterilized litter (pH 7.5 ± 0.4), the pH level in the typhlosole increased slightly but significantly (6.2 ± 0.1; $P < 0.05$) compared to freshly collected individuals. By contrast, the pH levels of the hepatopancreas (6.3 ± 0.1) and the sphincter region (6.5 ± 0.2) did not differ from those of freshly collected animals.

Experimental manipulation of the pH values of the litter resulted in a similar gut pH compare to freshly collected individuals ($P > 0.1$). The measured values ranged from pH 5.5 to pH 6.0 in the typhlosole, and from pH 6.0 to pH 6.5 in the hepatopancreas and the sphincter region.

The pH values of the litter treated at pH 8 did not differ from those of freshly collected poplar leaves. Hence, the pH levels of the gut fluids of isopods feeding on the former litter did not differ significantly from that of freshly collected animals.

When the pH values of the leaves varied between 4 and 6, the pH levels of either of the gut sections remained within the above-mentioned range of pH 5.5–6.0 or pH 6.0–6.5, respectively, no matter what kind of leaves the isopods had fed on. When the pH values of the litter declined below 3.5–4.0, the pH levels of the typhlosole region and the hepatopancreas dropped slightly, but significantly ($P < 0.05$). However, this was not observed in the sphincter region, where the pH remained constant even if the pH of the litter was about 3.5. In any case, the extracts of the typhlosole region were significantly more acidic than those of the hepatopancreas and the sphincter region ($P < 0.001$).

Statistical analyses of the presented data uncovered significant correlations between the pH levels of the distinct sections of the gut and the pH values of the leaves ($P < 0.01$ in each case). However, small regression coefficients of 0.03–0.08 indicated that large changes in the pH values of the litter (ranging from pH 3.5 to pH 8.0) resulted in only slight changes of the gut environment.

The pH values of the faecal pellets collected from 12 treatments ranged from pH 5.5 to pH 6.5 (Fig. 1) and were highly correlated with the pH level of the sphincter region ($P < 0.001$). Faeces that had contact with the pH-manipulated leaves exhibited altered pH levels that appeared to be influenced by the litter (data not presented), and differed significantly from faeces that did not have contact with the litter.

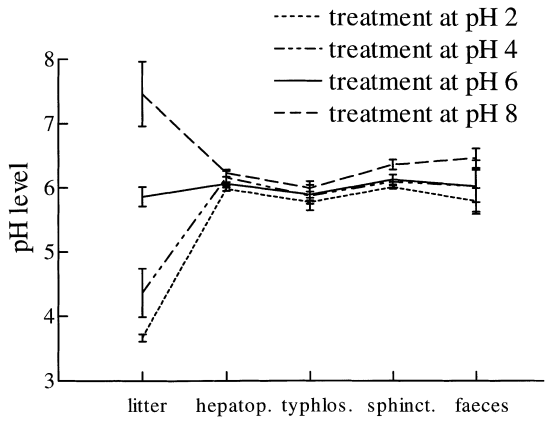

Fig. 1 pH levels of the food, the observed sections of the gut and the faeces of *Porcellio scaber* after having fed on manipulated leaf litter of three different tree species. *Alnus glutinosa*, *Betula pendula*, *Quercus robur*. The bars represent the ranges of pH levels of three different varieties after the same pH treatment ($n = 3 \times 15$) (*hepatop.*: hepatopancreas, *typhlos.*: typhlosole, *sphinct.*: sphincter)

Discussion

The morphology, histology and histochemistry of the intestinal tract of terrestrial isopods have been comprehensively described by several authors (e.g. Hassall and Jennings 1975; Lane 1988; Hames and Hopkin 1989). The pH level in the gut of *O. asellus* ranges from 6.4 to 6.8 (Hartenstein 1964). However, the author did not ascertain how pH differed between distinct sections of the gut. In our studies, the pH of distinct parts of the gut differed significantly. The pH levels of the hindgut of *P. scaber* ranged from 5.5 to 6.0 in the anterior part, while in the posterior part values of pH 6.0–6.5 were observed.

Our results clearly indicate that specimens of *P. scaber* buffer a wide range of pH values of the food source, maintaining homeostasis in different regions of the gut. The observed pH values remained fairly constant when the pH level of the food changed (Fig. 1). Slight effects on the pH levels in the hepatopancreas and the anterior hindgut could only be observed in the case of food with very low pH values (pH < 3.5–4.0). By contrast, the sphincter region showed reduced pH levels when the pH of the litter was about 6 or lower. Homeostatic responses concerning the pH level of the gut lumen are important with respect to the activity of endogenous and microbial enzymes. These enzymes are mainly active in the anterior hindgut (cf. Hassall and Jennings 1975; Hames and Hopkin 1989). Hence, changes in the pH level of the sphincter region might be relatively insignificant with respect to digestive processes.

A decrease in microbial activity on the ingested food (sterilized litter) resulted in altered pH conditions in the typhlosole, suggesting regulating functions of ingested microorganisms with respect to the pH level of the anterior hindgut. Correspondingly, isopods feeding on untreated litter from the field differed significantly in their gut pH from individuals that fed on manipulated litter with similar pH values. Generally, litter that has been pH-manipulated and microbially inoculated in the described way exhibits reduced microbial activity compared with litter in the field (cf. Skambracks 1996). Moreover, little microbial activity (Zimmer and Topp 1997) and low microbial counts (M. Zimmer, unpublished data) were found on strongly acidic leaf litter. Under these conditions, reduced reproductive success and increased mortality were observed in *P. scaber* (Zimmer and Topp 1997). These observations may be correlated with the lack of ability to buffer the pH levels of gut fluids in isopods fed on strongly acidic litter (pH < 4).

Ingested microorganisms, or at least their enzymatic activities, are supposed to be promoted during their stay in the hindgut by advantageous conditions (e.g. Hassall et al. 1987; Štrus et al. 1995). Cellulolytic breakdown of the food mainly takes place in the anterior hindgut (Hassall and Jennings 1975). These findings are in agreement with our results. The typhlosole supported optimal pH conditions for cellulases (Fig. 2), since it was characterized by the lowest pH level (pH 5.5–6.0, cf. Fig. 1), while the pH values in the hepatopancreas and the sphincter region remained at 6.0–6.5 (Fig. 1). Cellulolytic bacteria prefer environments with pH values of 6–8, while fungal cellulases are more active when the pH level drops below 6 (Szegi 1988). The overall activity of microbial cellulases on the leaf litter we used in our studies reached its maximum at pH 5–6 (cf. Fig. 2). Hartenstein (1964) found maximal activity of cellulases from the hepatopancreas and the hindgut of *O. asellus* at about pH 5. These values are close to the pH level of 5.5–6.0 we obtained from the typhlosole (Fig. 1). As illustrated in Fig. 2, the activity of microbial cellulases diminished rapidly when the pH values exceeded 6, as has been observed in the hepatopancreas and the

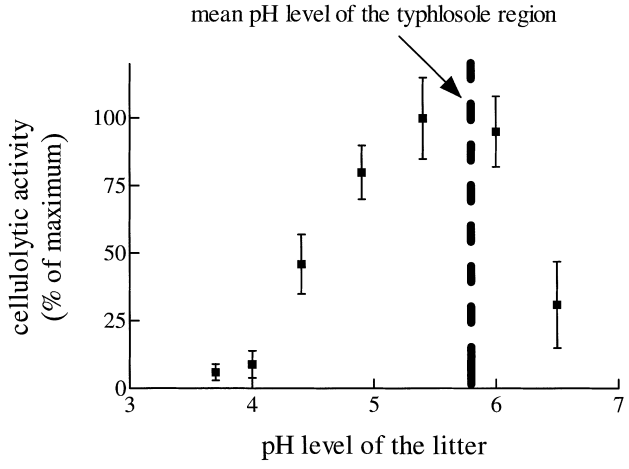

Fig. 2 pH optimum of the cellulolytic activity of leaf litter. The data show median ± MAD of the combined activities of different leaf species ($n = 3 \times 15$). The dotted line marks the mean pH level of the typhlosole region of *P. scaber* that coincides with the optimal enzymatic activity

sphincter region, respectively. The pH levels of these sections coincide with the pH optima of endogenous digestive enzymes that are mainly secreted by the hepatopancreas (Clifford and Witkus 1971; Storch and Štrus 1989). Similar optima of digestive carbohydrases have been observed in the genus *Porcellio* (Newcomer 1956) and in *O. asellus* (Beck and Friebe 1981). Alikhan (1968) isolated a maltase from the gut of *P. laevis* that was characterized by an optimum of activity at about pH 6. Overall, the observed pH homeostasis guarantees optimal conditions for digestive enzymes in different gut sections of *P. scaber*.

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