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GENERAL BIOLOGY

Nitrogen-Fixing Microorganisms in the Hare Gastrointestinal Tract

T. A. Kuznetsova[a], M. V. Vechersky[a], M. V. Golichenkov[b], N. V. Kostina[b], M. M. Umarov[b], and E. I. Naumova[a]

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The key specific feature of phytophagous animals is feeding on cellulose-containing food with a low protein content. A full-scale use of this food as a source of energy and nutrients is possible due to microbial endosymbionts. They not only destroy polysaccharides that are hard to digest, but also enrich food mass with bacterial protein as a result of nitrogen fixation. In animals with postgastric fermentation (for instance, lagomorphs and many rodents), the accumulated biomass of symbionts cannot be completely lysed and assimilated in the large intestine. Therefore, in some mammals, including lagomorphs, a specific mechanism of consumption of such a microbial biomass–obligate autocoprophagy– has formed [1]. One of original assumptions on the physiological role of coprophagy, mainly studied on domestic rabbits, was that bacterial cellulases produced in the blind gut are thus transported to the stomach and small intestine, where they are otherwise absent [2]. Subsequently, it was shown that caecotrophs (specially produced excreta of soft consistence representing a rapid evacuate from the blind gut consumed by lagomorphs) contain a great amount of protein of bacterial origin: up to 30% in the rabbit (*Oryctolagus cuniculus*) [3], 40% in the polar hare (*Lepus timidus*) [4], and 47% in the northern pika (*Ochotona hyperborea*) [5]. According to some estimates, the daily intake of additional protein through caecotrophy in polar hares is 23–56 g [2]. The use of molecular biological methods also demonstrated the presence of nitrogen fixers in caecotrophs of the northern pika (*Ochotona hyperborea*), a representative of lagomorphs [6]. However, the functional importance of the microorganisms found has not been clarified, because, at the moment, there are practically no data on the level of nitrogenous activity in the digestive tract of lagomorphs.

The subject of this study was the activity of nitrogen fixation in the gastrointestinal tract of hares (*Lepus europaeus*) in different periods of their feeding activity.

MATERIAL AND METHODS

The objects of the study were samples of the gastrointestinal tract of 18 hares obtained in Belgorod oblast (10 individuals) and in the Republic of Kalmykia (8 individuals). The hares were obtained in different periods of feeding activity—in the daytime, in the "resting" period (12 individuals), and in the twilight during "foraging" (6 individuals). Samples were taken from the vault and body of the stomach, small intestine, blind gut, and colon (separately proximal and distal parts).

The activity of nitrogen fixation in the samples of the gastrointestinal tract (GIT) was determined using the acetylene method according to the standard protocol [7] with the use of a Kristall 2000 gas chromatograph (Russia) with a flash-ionization detector.

Statistical analysis of the data obtained was performed using the Statistica 8.0 for Windows software.

RESULTS

Nitrogen fixation was detected in all regions of the hare GIT studied (Figs. 1 and 2). Minimum values of diazotrophic activity were obtained for the contents of small intestine; maximum, for the large intestine. In addition, the diurnal dynamics of this process was detected. The diazotrophic activity in all regions of GIT studied except for the distal part of the colon was significantly higher in the "resting" period than in the "foraging" period ($p < 0.05$). The activity of nitrogen fixation also significantly differed in different regions of GIT of the hare during the "resting" period

[a] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 119071 Russia;*
[b] *Department of Soil Science, Moscow State University, Moscow, 119991 Russia*
e-mail: tashka_u@mail.ru

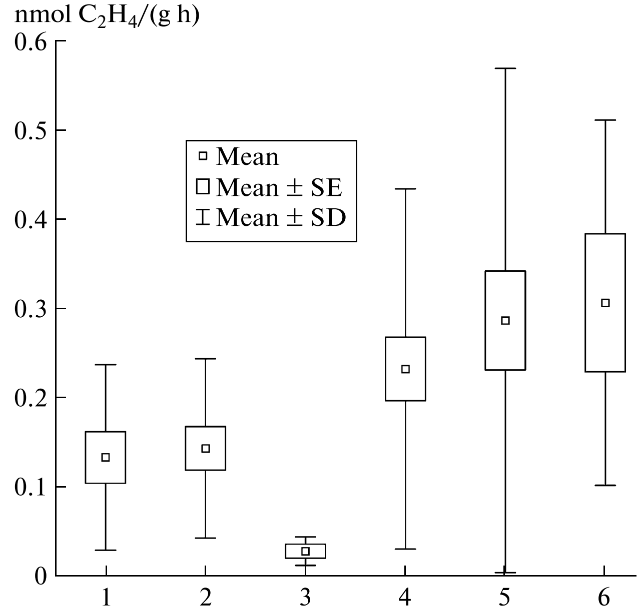

Fig. 1. Activity of nitrogen fixation in different dividions of the hare GIT in the “resting” period. Here and in Fig. 2: (1) stomach vault, (2) stomach body, (3) small intestine, (4) blind gut, (5) proximal part of colon, (6) distal part of colon.

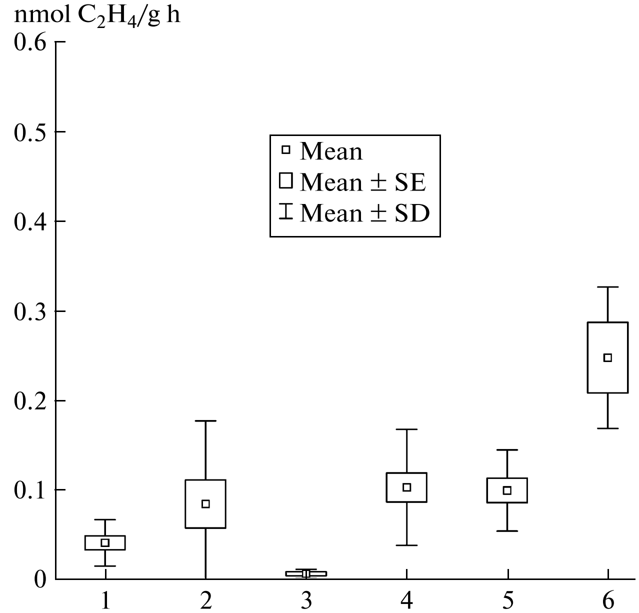

Fig. 2. Activity of nitrogen fixation in different divisions of the hare GIT in the “foraging” period.

(Kruskall–Wallis test, $H_{(5, N = 99)} = 21.78$, $p = 0.0006$) and was higher in the large intestine (Fig. 1). During “foraging,” no significant differences in the levels of nitrogen fixation in the blind gut or different parts of the colon were found ($p = 0.3764$). The vault and body of the stomach did not differ with respect to this index either ($p = 0.9167$).

In the “foraging” period, diazotrophic activity was also higher in the large intestine; however, in this case, the minimum values were recorded in the distal part of the colon (Fig. 2), ($p = 0.0155$). Although no significant differences in the levels of nitrogen fixation between the vault and body of the stomach were found ($p = 0.0966$), some increase in the diazotrophic activity in the vault contents was still observed.

DISCUSSION

It is common belief that, in monogastric mammals with a glandular stomach, the microbial transformation of food components in this organ is impossible [8]. However, the food that has arrived in the stomach is not immediately impregnated by the acidic gastric juice. During some time, its digestion is exercised under the impact of saliva enzymes, as well as of enzymes brought with caecotrophs that remain in the stomach for a long time [1, 19]. A considerable concentration of bacteria in caecotrophs (up to 10^9 cells/g) indicates that microbial enzymatic processes may take place in the stomach.

The increased values of nitrogen fixation in the large intestine are related to the formation of caecotrophs here. The consumption of the evacuate from the blind gut enriched with bacterial mass occurs in hares in the “resting period” [4, 9, 10]. This explains a considerable increase in the activity of nitrogen fixation in different regions of GIT of the hare in the “resting period” and confirms that precisely caecotrophs affect this process. Soft caecotrophs of the hare after consumption rather rapidly mix with the stomach contents [10]; therefore, no differences in the diazotrophic activity in the vault and body of the stomach have been found.

However, the consumed caecotrophs are retained in the stomach vault for a long period [1, 10], remaining there for some time after the “resting” period. The time of food passage from the proximal region of the colon to the distal one exceeds 2 h [10]. This possibly explains an increase in the diazotrophic activity in the distal part of the hare colon in the “foraging” period, when fresh food has already filled GIT.

The high level of nitrogenase activity in GIT indicates a large contribution of nitrogen-fixing microorganisms to digestion in hares and confirms the importance of coprophagy as a nitrogen source in logomorphs.

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