ISSN 1062-3590, Biology Bulletin, 2024, Vol. 51, No. 5, pp. 1438–1445. © Pleiades Publishing, Inc., 2024.

PHYSIOLOGY OF ANIMALS AND HUMANS

Dynamics of Food Passing through the Digestive Tract in the Nocturnal Rodent *Meriones crassus* as a Response to the Rhythm of Feeding Activity

E. I. Naumova[a], *, G. K. Zharova[a], and T. Yu. Chistova[a]

[a] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia*

**e-mail: einaumova@gmail.com*

Received March 26, 2024; revised April 6, 2024; accepted April 7, 2024

Abstract—The dependence of the food passing through the digestive tract (DT) on feeding activity in the nocturnal rodent *Meriones crassus* was studied. Two groups of gerbils were housed in the laboratory under artificial lighting at a photoperiod of 12 : 12 L : D for 36 h. The groups were named "day" (L : D = 20 : 16) and "night" (L : D = 12 : 24) according to the predominance light or dark time during the experiment. The mean retention time of markers in the stomach in these groups was 20 and 30 h, respectively. The kinetics of the contents changed depending on the specific time of consumption of the markers and the saturation level of the rodents with food. During the daylight hours, sequential evacuation of markers entering the DT begins from the stomach after two hours, and at night, during a period of higher feeding activity, part of the food located in the fornix of the stomach and in the cecum stays here for a longer time. In the "day" group, by the end of the experiment, 84% of the markers were removed from the DT, and in the "night" group, about 55%.

Keywords: rodents, digestive tract, multiple marking of food, kinetics of dietary fiber, feeding activity

DOI: 10.1134/S1062359024608206

INTRODUCTION

For several decades, the rate of movement of food through the digestive tract (DT) of mammals, as an important component of digestive physiology, has received considerable attention. The results obtained, including research methods, have been repeatedly summarized and analyzed (Warner, 1981; Stevens and Hume, 1998; Hume et al., 1993; Sakaguchi, 2003; Clauss et al., 2007, 2013; Müller et al., 2013). Many factors influencing the exposure time of feed in the digestive tract are considered, such as body size of the animal, DT morphology, type of fermentation, level of intake, and the quality of feed, as well as methodological shortcomings that reduce the reliability of the results.

The study of the kinetics of the solid fraction of food in several species of voles and gerbils using the method of multiple marking of food (Naumova and Kucheruk, 1996; Zharova et al., 2002, 2010; Naumova et al., 2007, 2024) made it possible to clarify important nuances of the circulation of the content in herbivorous rodents with small body sizes that are not recorded using a single food marking. An important feature from a functional point of view was discovered, which is the uneven progression of content, namely, a violation of the sequence of output of markers entering the DT at certain intervals. This violation is due to the specific nature of the collection and absorption of food plants by small phytophages. Size restrictions exclude the possibility of small rodents consuming a sufficient amount of poor plant food at a time. Regardless of the type of feeding activity, rodents often refill their stomach with food, which is associated with rapid evacuation of the contents and its rapid further passage through the DT. This situation is characterized by large fluctuations in the level of stomach filling, on which the localization of the marked feed and, accordingly, the dynamics of its passage through the DT depend. This is one of the reasons for the high variability in the kinetics of the DT content, demonstrated by the example of the diurnal gerbil (Naumova et al., 2024).

To clarify the dependence of the nature of the movement of food along the DT on the rhythm of feeding activity, we performed multiple marking of the food of the nocturnal rodent *Meriones crassus* in different phases of feeding activity. Previously, we studied several species of rodents (voles and gerbils) with round-the-clock feeding activity and *M. crassus*, rodents that consume food mainly at night (Khokhlova et al., 2005). Although some publications have noted the dependence of the speed of feed passage on the level of its consumption (Pei et al., 2001; Clauss et al., 2007), the single-marking method does not allow taking into account the rhythm of filling the stomach and cecum with food or the order in which food is removed from them.

MATERIALS AND METHODS

Sundevalla gerbil, *Meriones crassus,* is a nocturnal seed-eating rodent the main food of which is seeds and leaves of desert plants as a source of moisture. We conducted two series of experiments on food marking for gerbils during the day and at night. The animals were fed freshly harvested grass *Salsola tetrandra* that was beginning to bear fruit. Each experimental group consisted of six adult captive-born nonbreeding gerbils of both sexes. Animals were kept in cages individually under artificial light with an equal ratio of light and dark time of day (L : D = 12 : 12). The work was carried out in the laboratory of Ben-Gurion University.

To detail the paths of movement of solid fractions of food, we used five- and seven-fold marking of food with inert markers. Thin plastic film of different colors was used as an inert marker of the fiber fraction of the feed. The method is described in detail in previous publications (Naumova and Kucheruk, 1996; Zharova et al., 2002, 2010; Naumova et al., 2007, 2024). Each portion of the marker consisted of small pieces of film mixed with bait, the total amount of which in the DT and excrement was calculated at the end of the experiment and taken as 100%. The time of eating and the duration of exposure of markers in each experimental group of gerbils is given in Table 1. At the end of the experiments, the quantitative distribution of markers in the DT in sacrificed rodents was analyzed. The DT and separately the stomach and cecum were weighed on an electronic scale with an accuracy of 0.001.

The mean retention time (*MRT*) in the DT was calculated using the formula (Warner, 1981)

$$MRT = \sum_{i=1}^{n} m_i t_i \Big/ \sum_{i=1}^{n} m_i ,$$

where m_i is the number of marker particles in excrement excreted over time t_i after eating markers and n is the number of defecation during the complete removal of the marker. *MRT* were calculated separately for each marker. The mean retention time of food in the stomach and cecum was calculated similarly. At the same time, for m_i the number of marker fragments passing through a given organ (i.e., detected in the DT behind the stomach or cecum and excrement) was taken. As t_i the time of exposure of the marker in the stomach or cecum was taken.

Table 1. Schedule of markers arriving in the DT

Group of gerbils	No. of the marker	Marker eating time	Duration of marker exposure in the DT, h
1 (daytime)	1	12:00	36
	2	18:00	30
	3	24:00	24
	4	9:00	15
	5	16:00	8
	6	18:00	6
	7	22:00	2
2 (night)	1	20:00	36
	2	8:00	24
	3	20:00	12
	4	2:00	6
	5	5:00	3

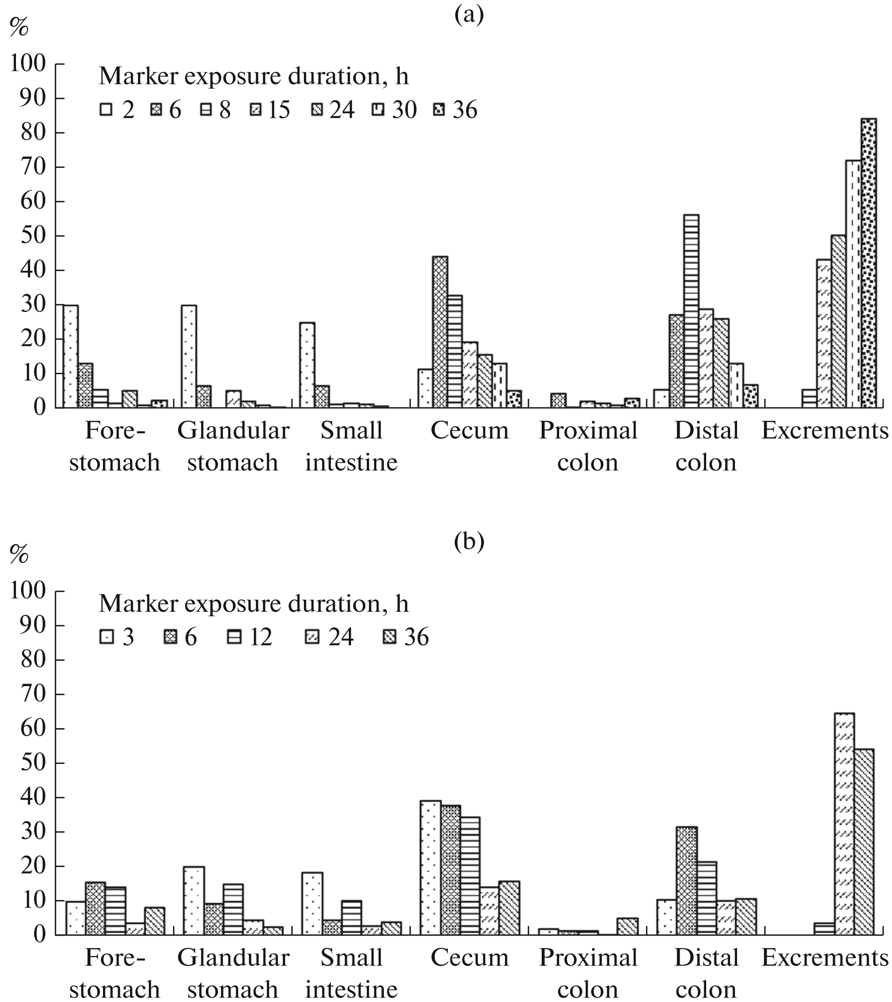

Fig. 1. The content of markers (%) with different exposure durations in each organ of the DT according to the results of dissection in the (a) day and (b) night groups of gerbils.

Generalized diagrams of the kinetics of contents in the DT for individual organs and in excrement were constructed based on data on the distribution of markers at the end of the experiment in sacrificed animals. The obtained data were processed using nonparametric statistical methods (Statistica 12).

RESULTS AND DISCUSSION

The duration of the experiments was 36 h, during which the rodents received marked bait at different phases of activity and at different times of the day. The experimental groups differed in the total duration of the light and dark periods and the start time of the experiments. We designated the group in which the first marker was given to the animals at 12:00 and the ratio of light and dark time was 20 : 16 as "daytime." We designated the group in which the start of the experiment was at 20:00 and in which the ratio of light and dark time in the experiment was 12 : 24 as "night."

Total Marker Delay Time in DT

The duration of the experiments did not allow us to determine the full time of food retention in the DT of Sundevalla gerbil. In the first experiment (day group), which covered two periods of daylight and one night, after 36 h, more than 80% of the markers that entered the DT at noon had left the DT (Fig. 1). In the second experiment (night), which covered two periods of night activity and one daytime, after 36 h, 55% of the markers eaten by gerbils in the evening at 20:00 was evacuated from the DT, and the marker which had lain

Table 2. Average retention time of markers in the stomach and intestines

Group	MRT, h		
	total	stomach	cecum
Daytime	19 (15–26)	20 (16–30)	20 (16–29)
Night	–	30 (27–35)	28 (23–35)

in the DT for 24 h and had been eaten at 8:00 in the morning, was removed by 65%.

The first particles of markers (about 5%) were recorded in the excrement of the day group of gerbils after eight hours of exposure (from 16:00), and in the night group the first fragments of markers (less than 5%) were noted after 12 h of exposure in the DT (from 20:00). The average retention times of markers in the stomach and intestines differed markedly in the two groups of gerbils (Table 2).

Movement of Marked Feed through Separate Organs of the DT in the Day Group

Marked food that entered the stomach of gerbils at noon exited the stomach and reached the cecum relatively quickly. Two hours after consuming the bait, about 40% of the marker had been evacuated from the stomach of day-group gerbils (Fig. 1a), and the remaining portion was distributed approximately equally between the forestomach and the glandular part. At this time, about 10% of the marker has already reached the cecum. Further marking showed the sequential passage of markers injected into the DT and their retention in the cecum, with most of the remaining marker particles retained in the forestomach. At the longest exposures (24, 30, and 36 h), trace amounts of markers still remained in the stomach. After 6–8 h, up to 45% of markers had accumulated in the cecum, and after eight hours their active excretion began in the excrement. After 24 h, more than 15% of the markers still remained in the cecum, and after 36 h, about 5%. In the proximal part of the colon, markers were almost not retained. In the distal colon, the highest concentration of markers was observed after an 8-h exposure (more than 50%).

Movement of Marked Feed through Separate Organs of the DT in the Night Group

The gerbils received the first marker at 20:00, and had was in the DT for more than a day. After 36 h of exposure about 10% of the first marker still remained in the stomach, but subsequent markers that arrived did not remain in the stomach for long (Fig. 1b). After three hours, 30% of the marker remained here, and about 40% was concentrated in the cecum. Markers with exposure in the DT of 6 and 12 h (entered into the stomach in the dark) advanced to the cecum in approximately the same way as the first marker. In the stomach their content was about 20%, and about 40% settled in the cecum. In the proximal part of the colon, the markers did not remain, and in the distal part the maximum concentration (30%) was reached by the marker with a six-hour exposure. After 24 and 36 h of exposure, about 25% of all markers were retained in the stomach and cecum.

Quantitative Ratio of Markers in Separate Organs of the DT Depending on the Time of Their Entry into the Stomach

Calculation of the quantitative ratio of markers that entered the stomach at different time and that remained in the DT until the end of the experiments made it possible to register two possibility for food distribution (Fig. 2) depending on the duration of exposure and the time of arrival of the markers in the DT. At the same time, clear differences emerged between the nature of the localization of marked portions of food eaten at different time of the day. In both experimental groups, the rapid entry of part of the markers into the cecum (after 2–3 h) and the long retention (for the entire experimental period) of part of the marked food in the forestomach should be noted. An important difference between the groups was the duration of retention of food consumed at different stages of feeding activity.

A comparative analysis of the quantitative content of markers in the stomach, cecum, and excrement revealed significant differences in the movement of markers along the DT depending on the time of entry into the stomach (Table 3). Markers that arrived at the DT in the morning and afternoon, with exposure durations of about 13 and 36 h, were cleared from the stomach and cecum much faster than markers with the same exposure duration that arrived in the DT in the evening. The content of markers that entered the DT

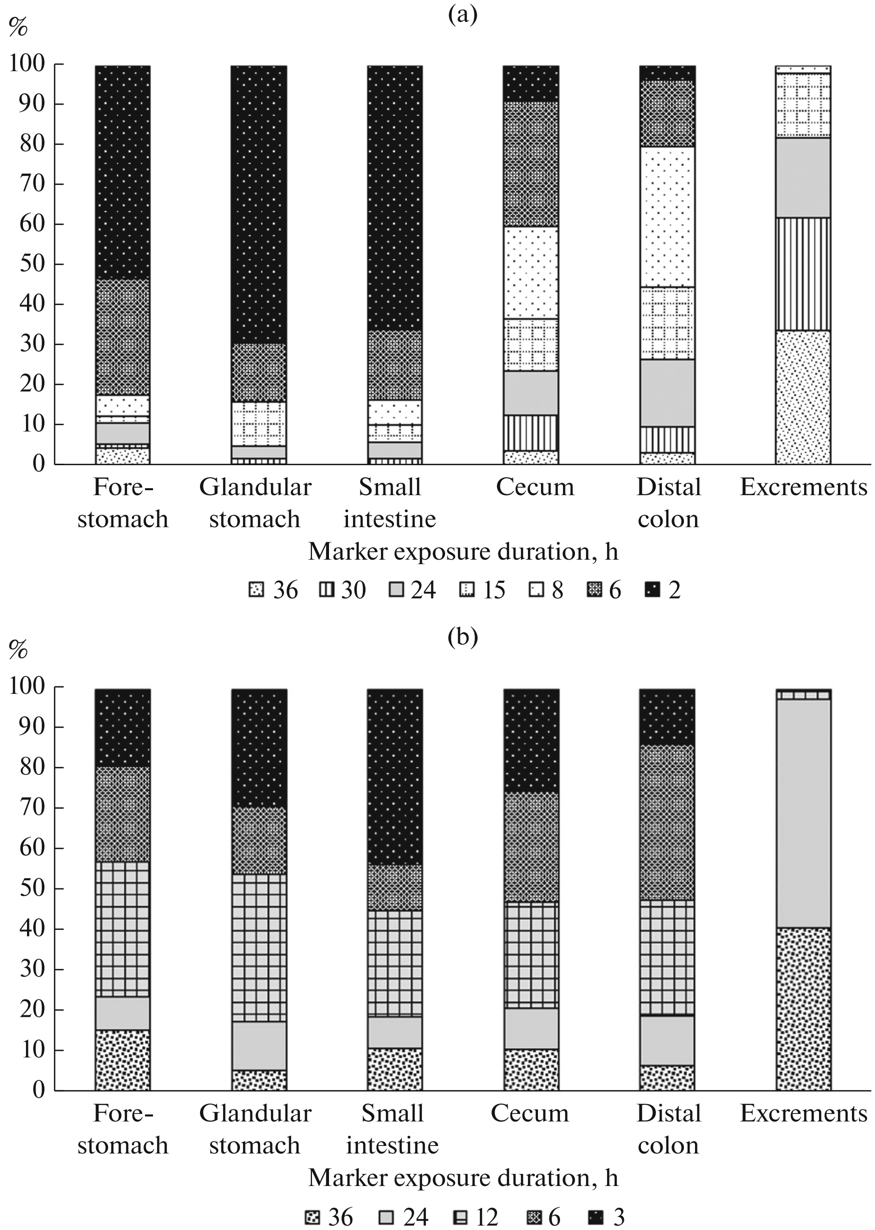

Fig. 2. Quantitative ratio of markers (%) in different sections of the DT at the end of the experiment in the day (a) and night (b) groups of gerbils.

during daylight hours, with exposure durations of 13 and 36 h, in excrement was significantly higher than those that entered the DT during the dark. In other cases, the differences were not significant, but when analyzing the advancement of markers with the shortest exposure in the DT (2–3 h), it was found that more markers entering the stomach in the evening (at 22:00), at the beginning of feeding activity, stagnated in the stomach than markers entering the DT at the end of feeding activity (at 5:00). In the cecum, the opposite situation is observed: markers that entered the DT at the beginning of feeding activity accumulate there to a greater extent than those that entered the DT at the end of feeding activity (Fig. 3).

Relationship between the Kinetics of Marker Passage, Feeding Activity, and Filling of the DT with Food

A study of the kinetics of the contents of a fat sand rat (a rodent with round-the-clock activity) provides examples of the dependence of food promotion on the degree of filling of the DT (Naumova et al., 2024). These differences were associated with frequent irregular intake of fresh food into the stomach during the

Table 3. Comparative analysis of the dependence of the quantitative content of markers on the time of their arrival in the DT

Location of the marker	Duration ofmarker exposure, h	Marker arrival time		Quantitative marker content, %		*p*
Stomach	2–3	22:00	5:00	59.27	29.99	0.083
	6	18:00	2:00	19.26	24.55	0.635
	12–15	9:00	20:00	6.22	29.05	0.0001*
	24	8:00	24:00	8.33	6.65	0.642
	36	12:00	20:00	2.41	10.68	0.007*
Cecum	2–3	22:00	5:00	11.11	39.11	0.061
	6	18:00	2:00	43.59	37.89	0.434
	12–15	9:00	20:00	18.99	34.51	0.017*
	24	8:00	24:00	13.95	15.48	0.517
	36	12:00	20:00	4.81	15.89	0.028*
Excrement	12–15	9:00	20:00	42.94	3.70	0.001*
	24	8:00	24:00	64.54	49.97	0.07
	36	12:00	20:00	83.48	54.00	0.035*

* The differences are statistically significant.

day. A similar situation has been analyzed in sufficient detail using the example of the great gerbil (Naumova and Kucheruk, 1996). A newly incoming dose of food enters the fornix of the stomach and displaces the contents already present there to the periphery (towards the greater curvature). During active feeding, the food that has already occupied the fornix does not have time to move into the small intestine, and part of it gets stuck in the fornix area for a long time. In a full stomach, new food is located in the pyloric part of the stomach.

In Sundevalla gerbil, this feature is also manifested, but against the background of a noticeable general decrease in feeding activity during daylight hours, which consists of a reduction in the number and duration of episodes of eating food (Khokhlova et al., 2005). Consequently, in Sundevalla gerbil, food marking during the daytime was carried out against the background of weak filling of the stomach. Therefore, the rate of advancement of separate markers that have lain in the DT for similar periods of time, but are absorbed by animals during the day or night, differ significantly. For example, markers with similar exposure (2–3 h), but that entered the stomach at different times, were excreted from it with different intensities. The marker eaten at the beginning of feeding activity (22:00) was removed from the stomach by 40%, and the marker that arrived at the end of feeding activity was removed by 70%. The marker with an exposure of 36 h, which entered the stomach at noon (day group), was practically eliminated from the DT, and the marker that entered the stomach at the beginning of feeding activity at 20:00 was still noticeable in small quantities in all sections of the DT. In the cecum, the opposite situation was observed: at the beginning of feeding activity, only 10% of the marker was located here, and at the end of feeding activity, about 40% of the marker was concentrated in it.

The paths and speed of advancement of different markers in Sundevalla gerbil, which consumes food with different intensities during the day and at night, are determined by the nature of filling the DT with food. When marking is carried out during daylight hours, when feeding activity is reduced, the marker enters the weakly filled stomach and is quickly evacuated from it. In cases of night marking during active feeding, the next marker enters the stomach, which is already largely filled with food. Therefore, a signifi-

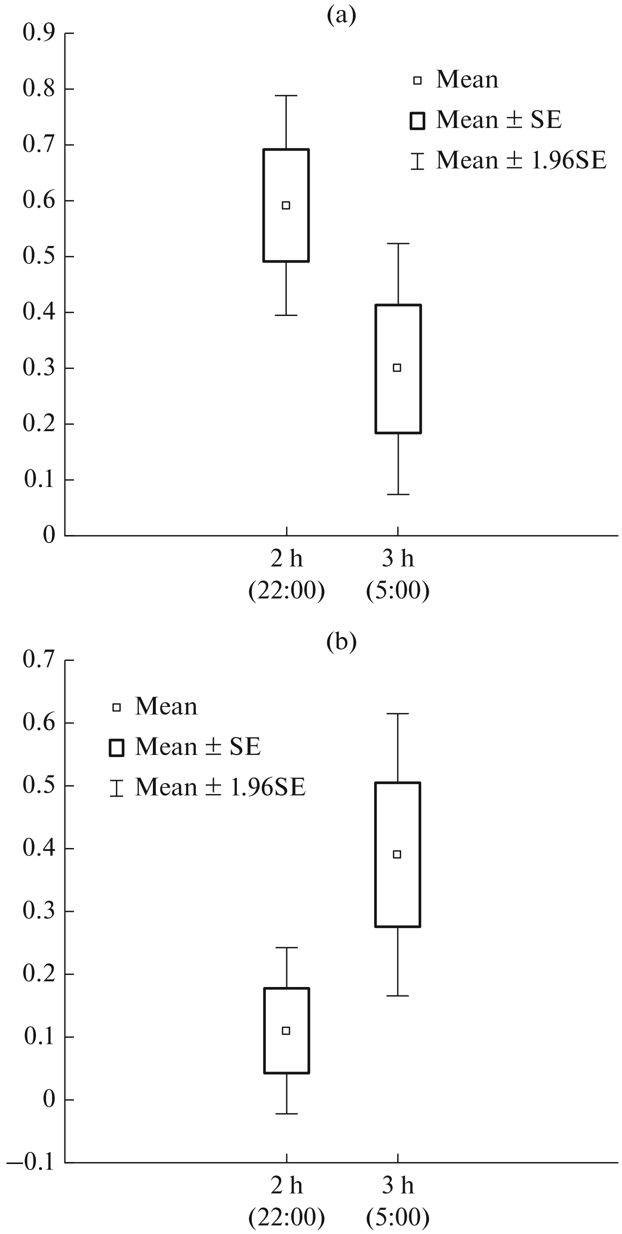

Fig. 3. Relative content of markers (a) in the stomach and (b) in the cecum, received in the DT at different time of the day.

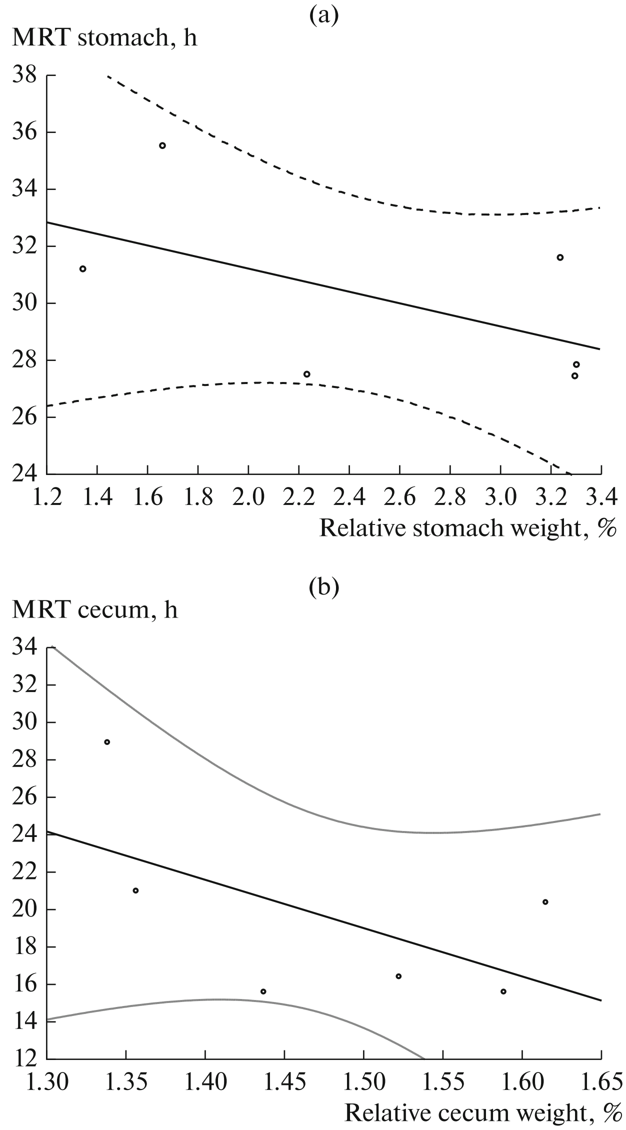

Fig. 4. Correlation between (a) the relative stomach mass and the duration of retention of food in it, $r = -0.5591$, and (b) the relative mass of the cecum and the duration of retention of food in it, $r = -0.5906$.

cant part of the marked feed is quickly evacuated into the small intestine, and the fornix of the stomach remains occupied for a long time with the feed that arrived earlier. The noted features of content progression are consistent with the filling of the stomach and cecum at different stages of feed consumption. In the daytime group, feeding activity took up noticeably less time than in the night group, which was reflected in the relative weight of the DT, 7.02 and 11.48% of the body weight, respectively. A tendency towards a correlation between the relative mass of organs and the average retention time of markers in them was observed in most of the cases considered. This dependence was especially noticeable when comparing the time of food retention and the weight of the stomach in the dark and the weight of the cecum in the daytime (Fig. 4).

Thus, multiple marking of food in rodents allows us to visualize the variability in the pathways of digesta movement into the DT, depending on the regimen of feeding activity. In addition to other factors already studied that influence the content kinetics of the DT, such as feed quality and feed intake, feeding activity may compromise the reliability of the results obtained. This effect can be especially significant in animals with a shift in feeding activity to light or dark times of the day. The noted dependence should be taken into account when studying the kinetics of contents in rodents.

ACKNOWLEDGMENTS

The authors are grateful to Israeli colleagues A. Degen, I.S. Khokhlova, B.R. Krasnov, and M. Kam, for providing the material and organizing the work.

FUNDING

This work was supported by ongoing institutional funding. No additional grants to carry out or direct thisparticular research were obtained.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All studies on experimental animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy, Washington, D.C., 1996), which is the basis for the document "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes."

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Clauss, M., Schwarm, A., Ortmann, S., Streich, W.J., and Hummel, J., A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores, *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.*, 2007, vol. 148, no. 2, pp. 249–265.
https://doi.org/10.1016/j.cbpa.2007.05.024

Clauss, M., Steuer, P., Müller, D.W.H., Codron, D., and Hummel, J., Herbivory and body size: allometries of diet quality and gastrointestinal physiology, and implications for herbivore ecology and dinosaur gigantism, *PLoS One*, 2013, vol. 8, no. 10, p. e68714.
https://doi.org/10.1371/journal.pone.0068714

Hume, I.D., Morgan, K.R., and Kenagy, G.J., Digesta retention and digestive performance in sciurid and microtine rodents: effects of hindgut morphology and body size, *Physiol. Zool.*, 1993, vol. 66, no. 3, pp. 396–411.
https://doi.org/10.1086/physzool.66.3.30163700

Khokhlova, I.S., Krasnov, B.R., Kuznetsov, V., Sartor, C.E., Zan, M., Salek, L., Ghazaryan, L., Kam, M., and Degen, A.A., Dietary intake and time budget in two desert rodents: a diurnal herbivore, *Psammomys obesus*, and a nocturnal granivore, *Meriones crassus, Mammalia*, 2005, vol. 69, no. 1, pp. 57–67.
https://doi.org/10.1515/mamm.2005.005

Müller, D.W.H., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J., and Clauss, M., Assessing the Jarman-Bell principle: scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores, *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 2013, vol. 164, pp. 129–140.

Naumova, E.I. and Kucheruk, V.V., An experimental study of the rate and dynamics of movement of various foods through the great gerbil digestive tract, *Biol. Bull.* (Moscow), 1996, vol. 23, no. 6, pp. 591–598.

Naumova, E.I., Zharova, G.K., and Chistova, T.Yu., Investigation of food movement through the digestive tract of voles by repeated injection of plastic markers, *Zool. Zh.*, 2007, no. 6, pp. 739–750.

Naumova, E.I., Zharova, G.K., and Chistova, T.Yu., Kinetics of dietary fibers in a fat sand rat: importance of the digestive tract isolating structures and food quality, *Biol. Bull.* (Moscow), 2024, vol. 51, no. 4.

Pei, Y.-X., Wang, D.-H., and Hume, I., Effects of dietary fibre on digesta passage, nutrient digestibility, and gastrointestinal tract morphology in the granivorous Mongolian gerbil (*Meriones unguiculatus*), *Physiol. Biochem. Zool.*, 2001, vol. 74, no. 5, pp. 742–749.
https://doi.org/10.1086/322928

Sakaguchi, E., Digestive strategies of small hindgut fermenters, *Anim. Sci. J.*, 2003, vol. 74, no. 5, pp. 327–337.
https://doi.org/10.1046/j.1344-3941.2003.00124.x

Stevens, C.E. and Hume, I.D., Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients, *Physiol. Rev.*, 1998, vol. 78, no. 2, pp. 393–427.
https://doi.org/10.1152/physrev.1998.78.2.393

Warner, A.C.I., Rate of passage of digesta through the gut of mammals and birds, *Nutr. Abstr. Rev., B*, 1981, vol. 51, pp. 789–820.

Zharova, G.K., Naumova, E.I., Chistova, T.Yu., Nesterova, N.G., and Podtyazhkin, O.I., Specific characteristics of food passage through the digestive tract in meadow voles, *Dokl. Biol. Sci.*, 2002, vol. 382, pp. 41–43.

Zharova, G.K., Chistova, T.Yu., and Naumova, E.I., Characteristics of digesta passage through the gastrointestinal tract of the tamarisk gerbil (*Meriones tamariscinus*), *Dokl. Biol. Sci.*, 2010, vol. 435, pp. 431–434.
https://doi.org/10.1134/S0012496610060177