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Choice of Indicator in the Investigation of the Passage of Foodstuffs through the Alimentary Tract of Rodents

Dobór wskaźnika w badaniu przechodzenia treści w przewodzie pokarmowym gryzoni

[With 3 Tables & 4 Figs.]

I. INTRODUCTION

The rate of passage of foodstuffs through the alimentary tract of animals has been examined by different authors by means of different and gradually improving methods. Research workers directed their efforts at finding the most natural indicator which would be easy to identify in excrement. The result of this search is the stained food method worked out by the Mangold school (Lenkeit & Habeck, 1930; Mangold, 1950) and improved by Balch (1950) and Castle (1956).

Honigmann (1936), in emphasising the advantages of this method, criticises the non-physiological method of sudden successive changes in diet used by Weiske in 1878 and the method applied by Browne, who in 1922 used red millet, white and black oats, all of which are difficult to identify in excrement, and also stained bread, which loses colour under the action of digestive juices. Honigmann (1936) also criticises the way in which experimental food was marked by Kaupp & Ivey (1923), who used soot, which adheres to the mucous membranes of the intestines and only superficially stains excrement, and also a mixture of flour with methylene blue, and maize grain steeped in a gentian violet solution, causing disturbances to the function of the alimentary tract of the animals.

In research on the rate of passage of foodstuffs through the alimentary tract Taylor (1935) used the spores of *Lycopodium*, and Benedict in 1936 (cited after Gill, 1960) pieces of rubber from the inner tube of a car wheel, which as foreign bodies could not of course give a true picture of the passage of foodstuffs.

The attempts made at using the X-ray method to trace the rate of passage of foodstuffs have not as yet given satisfactory results on account of the injurious effect of the contrast food and X-rays on the animals' organism (Spiridonova, 1949; Velitshko, 1956).

For the reasons discussed above the only proper method of examining the rate of passage of foodstuffs through the alimentary tract of animals would seem to be that of the stained food method, the best staining agents being basic fuchsin and brilliant green. This method should be used in accordance with the instructions given by Lenkeit & Habeck (1930), who wrote that the animals should be given the same kind of food throughout the experiment, and that the stained part of this food should form the indicator.

In the light of these data the continued use by certain contemporary authors (Koźniewski, 1961; Horszczaruk, 1962) of pieces of rubber as an indicator is unjustified.

It is also difficult to understand why, when animals are fed on a mixed experimental food, part of one of the components only of this mixture is used as an indicator (Castle, 1956; Gill, 1957, 1959), and even more so, in examination of the rate of passage of a certain food, the use as indicator of a different kind of food stained for this purpose (Honigmann, 1936; Sławiński, Sławoń & Bednarz, 1962; Sławiński, Bednarz & Sławoń, 1962; Piekarz, 1963).

A serious defect in the majority of studies so far made on the rate of passage of foodstuffs through the alimentary tract (Lenkeit & Habeck, 1930; Lenkeit, 1931; Balch, 1950; Castle & Castle, 1956; Castle, 1956; Velitshko, 1956; Gill, 1957, 1959, 1960; Gill & Bieguszewski, 1960; Koźniewski, 1961, Horszczaruk, 1962; Piekarz, 1963) is the small number of animals used for the experiments, making it impossible to draw conclusions in relation to the species investigated.

On account of the existance of the above methodical errors we decided to trace the rate of excretion of the indicator, that is, of the stained part of one of the components of the food mixture, and compare it with the rate of excretion of the same indicator, that is, the stained part of a uniform food of experimental animals (Kostelecka-Myrcha & Myrcha, 1964). We also decided to investigate the rate of excretion of the index formed by stained food differing from the experimental food, and find out whether this indicator reveals the rate of passage of the kind of food examined.

II. MATERIAL AND METHODS

The experiments were made on experimentally reared animals composed of 60 individuals of *Microtus agrestis* Linnaeus, 1761 (30 ♂♂ and 30 ♀♀), and 9 individuals of *Clethrionomys glareolus* Schreber, 1780 (5 ♂♂ and 4 ♀♀). All the animals were sexually mature.

Test food stained with basic fuchsin by the Castle method (1956), modified by Gill (1957), was used in the experiments. The conditions and way of carrying out the experiments, and also the method of counting the stained remains of food in the excrement, have already been described by us in our previous paper (Koste-

Table 1.

Description of excretion of the stained remains of the vegetative parts of plants in *Microtus agrestis* fed on wheat and green plants.

Sex	Animal no.	Excretion times /hours and minutes/				R
		5%	50%	90%	100%	
MALES	1	0.10	1.56	4.38	18.00	2.26
	2	0.07	1.19	3.50	16.00	1.44
	3	0.29	2.30	4.55	15.00	2.49
	4	0.36	2.09	3.58	19.00	2.34
	5	0.10	1.28	4.24	19.00	2.00
	6	1.05	1.55	3.26	17.00	2.14
	7	0.28	1.43	4.36	15.00	2.12
	8	0.09	1.53	5.43	18.00	2.33
	9	0.14	2.18	5.40	16.00	2.40
	10	1.05	2.02	5.16	15.00	2.39
	11	0.09	1.47	6.00	20.00	2.28
	12	0.07	1.15	5.46	18.00	2.08
	13	0.24	2.34	11.27	21.00	4.04
	14	0.07	1.16	5.44	19.00	2.19
	15	0.07	1.13	3.48	16.00	1.36
Avg.		0.22 ± 0.11	1.49 ± 0.15	5.17 ± 1.03	17.28 ± 1.03	2.26 ± 0.18
FEMALES	16	2.05	3.36	7.13	18.00	4.04
	17	0.14	2.14	5.38	18.00	2.42
	18	0.06	1.04	6.06	17.00	2.02
	19	0.17	1.45	4.40	15.00	2.20
	20	0.24	1.56	4.07	22.00	2.36
	21	0.13	2.54	5.41	16.00	3.00
	22	0.57	1.57	5.45	19.00	3.01
	23	0.12	1.40	4.39	16.00	1.57
	24	1.08	2.24	5.25	16.00	3.00
	25	0.51	2.32	4.45	19.00	3.12
	26	1.08	2.35	5.41	17.00	3.06
	27	0.08	1.39	6.35	18.00	2.29
	28	1.17	2.36	4.26	14.00	3.03
	29	1.03	1.55	7.26	16.00	3.20
	30	0.50	2.27	5.37	14.00	3.04
Avg.		0.43 ± 0.19	2.13 ± 0.20	5.35 ± 0.32	17.00 ± 1.06	2.52 ± 0.17
Avg. /♂♂ + ♀♀/		0.32 ± 0.11	2.01 ± 0.13	5.26 ± 0.33	17.14 ± 0.42	2.39 ± 0.13
Coefficient of variation		27.68	9.45	20.16	0.23	6.97

lecka-Myrcha & Myrcha, 1964). Both in the preceding and in the present study the experiments, the results of which were intended to serve as a point of reference for all statistical comparisons, were made using series each consisting of 30 animals. This number was established on the basis of the size of the division of confidence based on variable *t*. For statistical comparisons (*t*-Student test for difference of mean values for two independent groups) the times of excretion of 5%, 50%, 90% and 100% of the indicator were taken, and also the *R* values (Castle, 1956) which formed the mean time of retention of the foodstuff in the alimentary tract of the animals. Coefficients of variation were also calculated for all these values. Curves according to Balch (1950) were drawn on the basis of the percentages of data obtained for each hour of the experiments.

III. RESULTS

1. Rate of excretion of the indicator formed by the stained part of one of the components of the mixed food.

The experiment was made on 30 individuals of *M. agrestis* (15 ♂♂ and 15 ♀♀) which were fed on wheat and the green parts of plants. The stained vegetative parts of the plants were used as the indicator. It was found that the animals on an average ate 4.850 g of grain and 15.450 g of greenstuffs. Mean times of excretion of 5%, 50%, 90% and 100% of the test food and *R* values (Table 1) were compared with analogical data obtained for *M. agrestis* fed on greenstuffs only (K o s t e l e c k a - M y r-

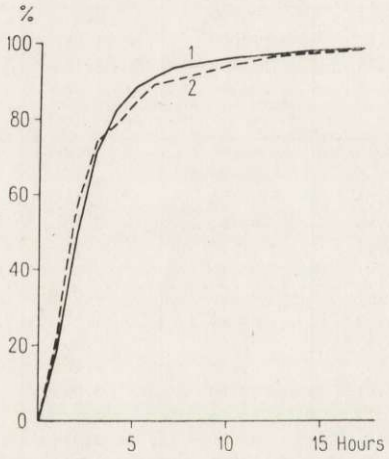

Fig. 1. Average course taken by the excretion of the stained remains of the vegetive parts of plants in *Microtus agrestis*, fed on green plants and wheat (1), and in animals fed on a green diet only (2).

Table 2.

Description of excretion of the stained remains of wheat in *Clethrionomys glareolus* fed on wheat and the vegetative parts of plants.

Sex	Animal no.	Excretion time /hours and minutes/				R
		5%	50%	90%	100%	
MALES	1	11.12	21.48	29.15	41.00	20.54
	2	2.23	14.56	23.18	39.00	14.48
	3	1.22	7.02	22.45	41.00	9.49
	4	1.08	15.38	30.50	40.00	14.18
	5	2.02	5.10	21.20	44.00	8.12
FEMALES	6	1.21	11.38	29.00	39.00	12.47
	7	2.10	10.49	23.26	44.00	11.30
	8	0.29	3.25	27.33	41.00	9.16
	9	1.09	5.54	16.26	39.00	8.35
Avg. /♂♂ + ♀♀/		2.35 ± 2.31	10.42 ± 4.36	24.52 ± 3.36	40.54 ± 1.09	12.14 ± 3.07
Coefficient of variation		33.20	178.71	47.13	1.87	70.52

cha & Myrcha, 1964). Non-significant differences were obtained for all the values compared, and excretion of the indicator takes an almost identical course in all the hours of both experiments (Fig. 1). This is evidence that the wheat did not affect the rate of excretion of greenstuffs. This fact suggests that both components of the food mixture pass through the alimentary tract at a rate independent of each other. In order to obtain confirmation of this assumption an additional experiment was made using 9 individuals of *C. glareolus* (5 ♂♂ and 4 ♀♀) fed on wheat and greenstuffs. The indicator in this experiment was formed by the stained wheat grains. During the experiment the animals ate on an average 4.600 g of wheat and 3.050 g of the vegetative parts of plants per 24 hours.

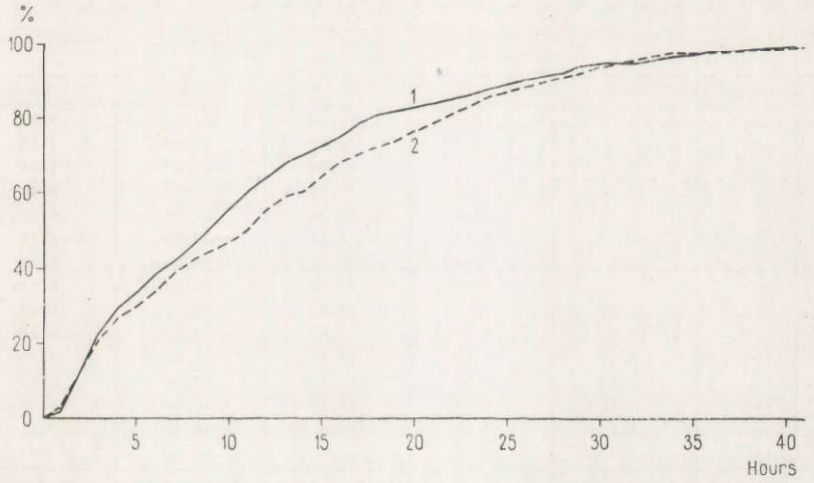

Fig. 2. Average course of excretion of the stained remains of wheat in *Clethrionomys glareolus*, fed on wheat and the green parts of plants, and fed on wheat only.

1 — Wheat — indicator wheat, n = 30 (after Kostelecka-Myrcha & Myrcha, 1964). 2 — Wheat + green plants — indicator green plants.

The mean times of excretion obtained for 5%, 50%, 90% and 100% of the stained remains of food and also the average R values (Table 2) were compared with the corresponding data for *C. glareolus* fed on wheat only (Kostelecka-Myrcha & Myrcha, 1964). The comparisons made show that in this case also both components of the food mixture pass through the alimentary tract at a rate independent of each other (differences statistically non-significant for all the values compared). The curves of excretion of the stained remains of wheat grain take very similar courses in both cases (Fig. 2).

2. Vegetative parts of plants as an indicator of the passage of wheat grains.

The experiment was made using 30 individuals of *M. agrestis* (15 ♂♂ and 15 ♀♀) which were fed on wheat grains. The test food in this experiment was formed by the stained green parts of plants. Average times of excretion of 5%, 50%, 90% and 100% of the indicator and *R* values (Table 3) were compared with analogical values obtained for *M. agrestis* fed on wheat, the indicator of passage of which were the stained wheat grains, and for those fed on the vegetative parts of plants, the indicator

Table 3.

Description of excretion of the stained remains of green plants in *Microtus agrestis* fed on wheat.

Sex	Animal no.	Excretion times /hours and minutes/				R
		5%	50%	90%	100%	
MALES	1	1.21	4.36	10.36	27.00	5.25
	2	2.04	5.53	18.50	37.00	7.46
	3	1.15	3.43	10.04	24.00	5.01
	4	1.14	7.48	17.35	39.00	8.38
	5	1.33	3.46	13.36	28.00	6.22
	6	0.38	3.10	9.17	39.00	4.11
	7	0.21	2.58	15.23	36.00	6.09
	8	1.10	8.00	17.38	28.00	8.11
	9	0.24	5.11	15.43	36.00	6.43
	10	0.07	2.39	10.43	23.00	4.20
	11	2.17	5.18	11.39	21.00	6.26
	12	2.11	4.55	15.13	29.00	6.42
	13	1.16	7.21	25.41	42.00	10.11
	14	1.25	7.05	13.32	34.00	7.32
	15	1.38	7.58	20.09	41.00	10.11
Avg.		1.16 ± 0.21	5.21 ± 1.04	15.03 ± 2.28	32.16 ± 3.50	6.55 ± 1.02
FEMALES	16	0.36	2.46	7.06	27.00	3.42
	17	0.29	9.00	19.26	35.00	9.38
	18	0.33	3.38	9.45	31.00	4.44
	19	0.25	3.41	15.58	37.00	6.36
	20	0.42	9.12	24.40	40.00	11.51
	21	1.02	3.10	14.14	38.00	5.52
	22	1.07	2.12	7.42	23.00	3.29
	23	1.45	3.31	11.46	27.00	4.51
	24	3.03	3.35	8.40	27.00	4.24
	25	1.09	6.07	25.12	39.00	9.58
	26	0.23	5.25	19.29	32.00	7.46
	27	1.17	4.37	18.15	28.00	7.02
	28	1.38	7.22	14.45	23.00	7.49
	29	2.11	4.59	12.51	21.00	6.55
	30	0.31	4.36	11.06	23.00	5.35
Avg.		1.07 ± 0.26	4.55 ± 1.26	14.44 ± 3.10	30.04 ± 3.34	6.41 ± 1.20
Avg. /♂♂ + ♀♀/		1.11 ± 0.16	5.08 ± 0.52	14.53 ± 1.53	31.10 ± 2.29	6.48 ± 0.48
Coefficient of variation		24.76	46.57	104.70	1.71	38.49

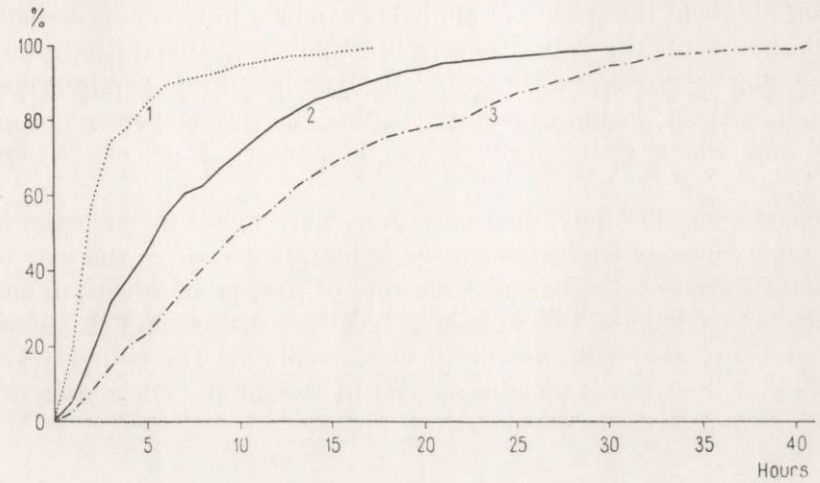

Fig. 3. Average course of excretion of the indicator in *Microtus agrestis* in three experiments differing as to the experimental or indicator foods.

1 — Green plants — indicator green plants. 2 — Wheat — indicator green plants. 3 — Wheat — indicator wheat. (Data for 1 and 3 after K o s t e l e c k a - M y r c h a & M y r c h a, 1964).

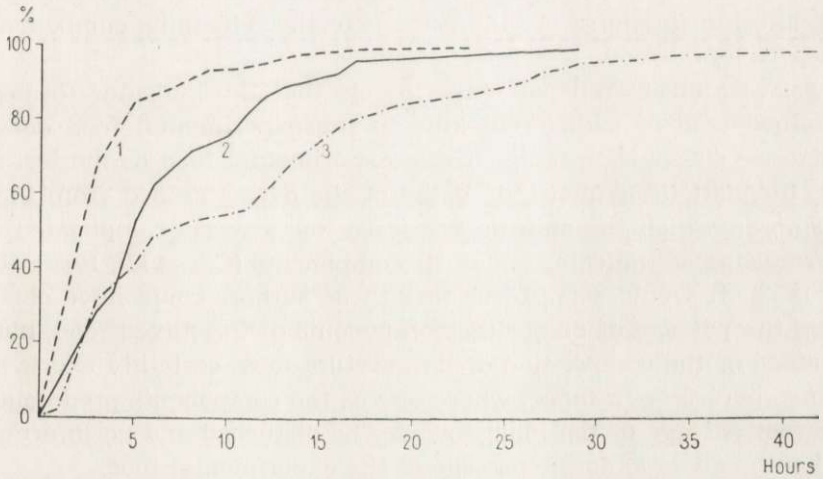

Fig. 4. Course taken by the excretion of the stained remains of the vegetative parts of plants in three individuals of *Microtus agrestis* fed on wheat.

1 — Female, no. 22, 2 — Male, no. 12, 3 — Male, no. 13.

of passage of which were the stained green parts of plants. As was the case previously, data for comparisons were taken from our preceding paper (K o s t e l e c k a - M y r c h a & M y r c h a, 1964). The comparisons made show that the stained greenstuffs used as an indicator of passage of wheat do not characterise either the passage of grains or the vegetative

parts of plants in the species of animals examined (differences statistically significant for all the values compared). The curve illustrating excretion of the indicator during each hour of this experiment takes an intermediate course in relation to the curves illustrating the rate of passage of greenstuffs and wheat grain through the alimentary tract of *M. agrestis* (Fig. 3).

Very considerable individual variations were found in this experiment (very high values of coefficients of variation). In certain of the individuals the stained greenstuffs indicated the rate of passage of wheat, in another the rate of passage of the vegetative parts of plants, and in yet others they were excreted with an intermediate rapidity. The course taken by excretion of the stained remains of food in several animals chosen at random from this series is illustrated by Fig. 4.

IV. DISCUSSION

The results of the above experiments confirm our assumption that the indicator formed by the stained part of one of the components of a food mixture indicates only the rate of passage of this one kind of food, without giving any information as to the passage of the remaining components of the experimental food.

It was also clear from our experiments that the indicator formed by the stained food of a different kind to the experimental food does not characterise either the passage of the experimental food or the test food.

It is difficult to estimate the value of the data obtained from the experiments in which the animals were fed on a very complicated food mixture, using as indicator one of its components (Castle, 1956; Gill, 1957; 1959). It would seem to us that these authors could have obtained data on the passage of one of the components of the mixed food, but the percentage of the composition of the mixture most certainly exerts some influence here. For instance, where one of the components predominates, the rate of passage of the indicator may be disturbed and no information will then be given as to the passage of the experimental food.

An extreme case of disturbance of the excretion of the indicator may occur in experiments in which the test food did not occur at all in the experimental food (Sławiński, Sławoń & Bednarz, 1962; Sławiński, Bednarz & Sławoń, 1962; Piekarz, 1963).

A sufficiently accurate picture of the passage of food can only be obtained when the stained part of this food forms the indicator. When the passage of mixed food is investigated part of all the components should be stained with different staining agents and the rate of passage of each component traced separately.

The use of a suitably large number of individuals for the description of the course taken by passage of foodstuffs through the alimentary tract in the species of animals investigated is of no less importance. In our experiments the necessary and adequate number of animals proved to be 30 individuals. The results of experiments carried out by a large number of authors on very small numbers of animals are only of importance as pointers (Balch, 1950; Castle, 1956; Castle & Castle, 1956; Velitshko, 1956; Gill, 1957; 1959; 1960; Gill & Bieguszewski, 1960; Kōźniewski, 1961; Horszczaruk, 1962; Piekarz, 1963). In the light of our data it is impossible to make use of such results when comparing the rate of passage of foodstuffs through the alimentary tract of different species of animals (Gill, 1957), and even more so to make a statistical analysis of them and to reach conclusions as to the existence of sex differences on this basis (Gill & Bieguszewski, 1960). As is now known, on account of the existence of considerable individual variations, the differences obtained may be entirely fortuitous.

V. SUMMARY

Investigation was made of the rate of excretion of the vegetative parts of plants in 30 individuals of *M. agrestis* fed on wheat and greenstuffs, the rate of passage of wheat through the alimentary tract of 9 individuals of *C. glareolus* fed on greenstuffs and wheat, and the rate of excretion of the stained parts of green plants in 30 individuals of *M. agrestis* fed on wheat grain.

The way in which the experiments were carried out has already been described by us in our previous paper (Kostelecka-Myrcha & Myrcha, 1964).

The results obtained make it possible for us to state that:

1. The stained vegetative parts of plants, used as an indicator of the passage of foodstuffs through the alimentary tract of *M. agrestis* fed on wheat and green plants, provides information only as to the rate of passage of green food. Stained wheat used as an indicator of the passage of foodstuffs in the case of *C. glareolus*, fed on wheat grain and the vegetative parts of plants, reveals only the rate of passage of the grain. Therefore if the indicator consists of the stained part of one of the components of a mixed food, we obtain data as to the passage of only one of the components of the mixed food.

2. The stained green parts of plants used as an indicator of the passage of wheat in *M. agrestis* do not describe the rate of passage of grain and are not excreted with the rapidity proper to greenstuffs. Therefore the use as indicator of a stained food different from the experimental food gives no information as to the passage of foodstuffs in the animals examined.

3. In order to obtain a sufficiently exact picture of the rate of passage of food through the alimentary tract of the species investigated, experiments must be carried out on a suitably large (established statistically) series of individuals.

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STRESZCZENIE

Zbadano tempo wydalania wegetatywnych części roślin u 30 osobników *M. agrestis* karmionych pszenicą i roślinami zielonymi, szybkość transportu pszenicy u 9 *C. glareolus* karmionych zielonymi częściami roślin i pszenicą oraz szybkość wydalania barwionych roślin zielonych u 30 *M. agrestis* karmionych nasionami pszenicy.

W eksperymentach zastosowano metodę opracowaną przez Castle (1956) i zmodyfikowaną przez Gilla (1957). Sposób prowadzenia doświadczeń został już przez nas opisany w poprzedniej pracy (Kostelecka-Myrcha & Myrcha, 1964).

Otrzymane wyniki pozwalają nam stwierdzić, że:

1. Barwione wegetatywne części roślin, użyte jako wskaźnik transportu treści w przewodzie pokarmowym *M. agrestis* karmionych pszenicą i roślinami zielonymi, charakteryzują tylko szybkość przechodzenia pokarmu zielonego (Tabela 1, Ryc. 1). Barwiona pszenica, będąca wskaźnikiem transportu treści pokarmowej u *C. glareolus* karmionych nasionami pszenicy i wegetatywnymi częściami roślin, wskazuje tylko szybkość przechodzenia ziarna (Tabela 2, Ryc. 2). Wobec tego, jeżeli wskaźnikiem jest zabarwiona część jednego ze składników pożywienia mieszanego, otrzymujemy dane o transporcie tylko tego jednego składnika mieszanki pokarmowej.

2. Barwione zielone części roślin, użyte jako wskaźnik transportu pszenicy u *M. agrestis*, nie charakteryzują szybkości przechodzenia nasion i nie są też wydalane z szybkością właściwą dla roślin zielonych (Tabela 3, Ryc. 3). U niektórych osobników szybkość wydalania wskaźnika jest zgodna z szybkością przechodzenia wegetatywnych części roślin, u innych z tempem wydalania nasion, a u jeszcze innnych przedstawia wartości pośrednie (Ryc. 4). Wobec tego, stosowanie jako wskaźnika barwionego innego rodzaju pożywienia niż pokarm doświadczalny nie mówi nic o transporcie treści pokarmowej u badanych zwierząt.

3. Aby otrzymać dostatecznie dokładny obraz szybkości przechodzenia pokarmu przez przewód pokarmowy badanego gatunku, eksperymenty należy przeprowadzać na odpowiednio dużych (określonych statystycznie) seriach osobników.