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The Method of Quantitative Determining of the Food Composition of Rodents [1])

[With 3 Tables]

The method was developed for the quantitative determining of plant components of the rodent stomach contents. Small fragments of vegetative parts and seeds of several plant species were mixed in known weight proportions and diluted using 0.04 ml of water for 1 mg of the mixture. After thoroughful mixing the area occupied by all plant fragments seen in the microscopic field was determined using the eye-piece grid dividing the field into 144 squares. From each mixture 10 slides were made and on each slide the surface of fragments was determined in 10 microscopic fields. The area occupied by all plant species was considered 100% and the per cent of area occupied by every component was calculated. In all cases the difference between calculated and the actual weight was not significant. The results indicate that this method can be used for quantitative analysis of rodent stomach contents.

I. INTRODUCTION

The food composition of rodents is usually determined from the analysis of stomach contents. In this group of Mammals the stomach, besides its usual digestive functions, serves as a temporary food storage. Consequently, food stays in stomach for the longest time and is least changed. Full analysis of stomach contents should consider both the quality of consumed food and the quantity of each food component. Determining the species composition of food can be done relatively easily using histological methods. For this purpose a set of microscopic slides is made from different parts of all plants occuring in the studied area.

Systematical identity of plant epidermis fragments from rodent stomach is being determined by comparison with previously prepared test slides (H o l i š o v a, 1959, 1960, 1965; H o l i š o v a *et al.* 1962; W i l-

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[1]) This study was carried out under the Rodent Project of the International Biological Programme in Poland.

l i a m s, 1955, 1959, 1962). The key classification of the epidermis fragments of many forest and meadow plants is now being prepared in Poland in connection with the International Biological Programme; it should help considerably in this type of analysis. However, the problem of quantitative estimation of food components is still far to be solved.

Table 1.

Pooled results of analysing 10 samples of some mixtures of plant vegetative parts.

Galeobdolon luteum Huds.			Asperula odorata L.			Viola silvestris Rchb.			Aegopodium podagraria L.		
1	2	3	1	2	3	1	2	3	1	2	3
40	41.4	1.24	40	38.6	1.25						
20	20.9	1.24	60	59.0	0.47						
40	38.5	1.67	40	40.9	0.70				40	41.8	0.81
60	59.7	0.90	20	20.1	0.66	20	20.0	1.34			
15	15.5	1.74	40	39.1	1.44	25	25.3	0.75			
60	60.0	0.42	50	49.3	0.50	8	8.5	0.49			
20	21.7	4.75	10	12.0	8.53	50	49.4	5.56	40	37.2	5.21

1 — Actual weight in mg., 2 — Computed weight in mg., 3 — χ^2.

Most of the studies were usually limited to determining the "frequency index" i.e. frequency of occurence of separate food components in the series of stomachs from specimens of the studied species. Calculating the frequency index as well as some attempts to determine the volume of different components in the stomach contents (M c K e e v e r, 1964)

Table 2.

Pooled results of analysing 10 samples

Galeobdolon luteum Huds.			Asperula odorata L.			Viola silvestris Rchb.		
1	2	3	1	2	3	1	2	3
40	40.3	0.64	60	63.0	3.99	20	19.4	0.54
						10	9.7	0.40

1 — Actual weight in mg., 2 — Computed weight in mg., 3 — χ^2.

seemed not satisfactory. They did not allow to establish quantitative relationship between the individual components of food eaten.

Determining the quantity of food eaten by rodents is very important in studying processes involved in the production of living matter. Consequently, this information is of considerable practical significance. For this reason the attempt was made to develop a method for quantitative determination of food eaten by these mammals.

II. THE METHOD

The material of this study consisted of vegetative parts of four green plant species: *Galeobdolon luteum* H u d s., *Asperula odorata* L., *Viola silvestris* R c h b., and *Aegopodium podagraria* L. and seeds of three species of trees: *Quercus robur* L., *Acer platanoides* L., *Corylus avellana* L. Both green plants and seeds were differentially stained with the method of C a s t l e (1956) as modified by G i l l (1957) using brilant green, basic fuchsin, gentiana violette, aurantia and methylene blue. Staining was used to help the identification of species in microscopic examination. Stained material was dried and thoroughfully ground into parts corresponding to the size of plant fragments found in rodent stomachs. From the material processed in this way the mixtures were prepared containing 2, 3, 4 or five components in different proportions. The mixtures were diluted with 0.04 ml of water for 1 mg of stained plants. After thoroughful mixing a drop of suspension to be studied was placed on the microscopic slide and covered with the cover glass. In the eye-piece of microscope the grid with squares 0.5 × 0.5 mm. was placed. Using 200 × magnification the grid was dividing the microscopic field into 144 squares. The grid was employed to determine the surface occupied by fragments of different plants in the microscopic field. The surface of individual plant fragments was estimated to the nearest half of the square. In this way ten microscopic fields were studied on each slide. From the preliminary experiments it was concluded that from each

of vegetative parts of plants and seeds.

Quercus robur L.			Acer platanoides L.			Corylus avellana L.		
1	2	3	1	2	3	1	2	3
120	119.8	0.31	10	10.1	0.38			
40	39.7	0.69						
			40	38.6	1.43	40	38 9	1.31
40	39.6	1.85	50	50.7	0.95	20	20.1	1.01

mixture 10 slides should be made and analyzed. Thus the surface of components of every mixture was determined in 100 microscopic fields. This number proved sufficient for obtaining precise results. Above experiments were carried out by two authors, each doing 5 samples. The area occupied by each species of studied plants was summed up in 10 microscopic fields for every mixture. Then, knowing the total weight of whole mixture, the weight of every component was computed. The calcu-

lations were done separately for each of 10 samples. Experimentally computed weight of components was compared with known weight composition of the mixture using χ^2 test.

III. DISCUSSION

The results of this study suggest that microscopically measured proportions of area occupied by different food components can be used to determine the quantitative composition of plant food of rodents. The comparison of experimentally computed weight of components with their known actual weight revealed no significant differences. This was true for samples made from 2, 3 or 4 components mixed in even or uneven proportions (Table 1). Similarly for the mixtures composed of tree seeds fragments or vegetative parts of plants together with seeds the computed and known values did not differ significantly. It was found experimentally that it is sufficient to analyse 10 samples from each mixture (Tab. 2). Although, in individual samples the differences between computed and actual weight of components may be considerable, the mean weight computed from 10 samples was very close to the actual value (Table 3).

Table 3.

Results of analysing 10 samples of the mixture of vegetative parts of 4 plant species.

Samples No.	Viola silvestris Rchb.			Corylus avellana L.			Quercus robur L.			Acer platanoides L.		
	1	2	3	1	2	3	1	2	3	1	2	3
1	39	10.0	9.5	81	20.0	19.8	160	40.0	39.2	210	50.0	51.4
2	43	10.0	10.2	95	20.0	22.5	165	40.0	39.1	204	50.0	48.3
3	45	10.0	10.0	94	20.0	21.0	165	40.0	36.8	234	50.0	52.2
4	51	10.0	10.6	100	20.0	20.8	189	40.0	39.3	238	50.0	49.5
5	44	10.0	8.9	90	20.0	18.2	223	40.0	45.0	237	50.0	47.9
6	84	10.0	9.3	154	20.0	17.1	380	40.0	42.2	465	50.0	51.6
7	24	10.0	9.0	53	20.0	20.7	88	40.0	34.4	142	50.0	55.5
8	51	10.0	9.0	115	20.0	20.2	230	40.0	40.5	287	50.0	50.5
9	45	10.0	9.7	94	20.0	20.3	183	40.0	39.5	233	50.0	50.3
10	43	10.0	10.4	83	20.0	20.2	163	40.0	39.6	204	50.0	49.6
$\bar{x}$		10.0	9.7		20.0	20.1		40.0	39.6		50.0	50.7

1 — Surface in squares, 2 — Actual weight in mg, 3 — Computed weight in mg.

Consequently, it appears that the method described can be succesfully used for the quantitative analysis of stomach contents composition in rodents. The stomach contents to be studied should be processed in the same way as the mixtures considered above. Food contents should be air dried in the thermostat and precisely weighed. Then they should be diluted with water and thoroughly mixed. The area occupied by each component of the studied stomach contents should be determined using

the microscope with grid. The per cent of area occupied by different parts of each plant species can be calculated, considering the total area of all identified fragments as 100%. Assuming that the surface proportions between individual components correspond to their weight proportions and knowing the weight of total stomach contens the weight of separate components can be computed.

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METODA ILOŚCIOWEGO OKREŚLANIA SKŁADU POŻYWIENIA GRYZONI

Streszczenie

Opracowano metodę ilościowego oznaczania składników roślinnych treści żołądkowej gryzoni. Sporządzano mieszaniny wysuszonych drobnych fragmentów części wegetatywnych i nasion kilku gatunków roślin w znanych proporcjach wagowych

i rozcieńczano je dodając na mg mieszanki 0.04 ml wody. Po dokładnym wymie-
szaniu określano pod mikroskopem powierzchnię zajmowaną przez wszystkie frag-
menty roślinne, znajdujące się w polu widzenia mikroskopu. W tym celu w okula-
rze umieszczano siatkę dzielącą pole widzenia na 144 kwadraty. Z każdej miesza-
niny sporządzano 10 preparatów, na których określano powierzchnię szczątków
w 10 polach widzenia. Powierzchnię zajmowaną przez wszystkie gatunki w każdej
próbie przyjmowano za 100⁰/₀ i obliczano procentowy udział w niej wszystkich
składników mieszaniny. We wszystkich wypadkach otrzymane różnice pomiędzy cię-
żarami wyliczonymi a faktycznymi były nieistotne statystycznie. Doświadczenia te
wykazały, że metodę tę można będzie wykorzystać do ilościowej analizy treści po-
karmowej gryzoni.