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Food Consumption by Microtus agrestis and the Unsuitability of Faecal Analysis for the Determination of Food Preference

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Phillipson, J., Sarrazin-Comans M. & Stomatopoulos C., 1983: Food consumption by *Microtus agrestis* and the unsuitability of faecal analysis for the determination of food preference. Acta theriol., 28, 26: 397—416 [With 6 Tables, 4 Figs. & Plate XIII]

In the laboratory adult *Microtus agrestis* (Linnaeus, 1761) consumed an average of 21.4 g wet wt. (=6.3 g dry wt.) of fresh grass indiv^{-1} d^{-1} with an annual mean digestibility of 52.8%. When four common species of food grass were on offer consumption in Autumn and Winter was directly proportional to availability. A preference for the more "succulent" species was exhibited in Spring and Summer. Over the year digestibility coefficients ranged between 33.6 and 67.8%, the highest values occurring in Spring and Summer. Faecal analysis suggested an order of food preference different to that determined by direct observation, the differences being attributable to the differential "desirability" and "digestibility" of the food grasses during the course of the year.

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INTRODUCTION

There are four main approaches to the quantitative study of the food habits of animals, (1) direct observation of feeding or food remains, (2) analysis of gut contents, (3) faecal analysis, and (4) food preference experiments. None of them is ideally suited to every type of animal and circumstance. In the case of the primarily grass feeding *Microtus agrestis* (Linnaeus, 1761) direct observation of food consumption under natural conditions is extremely difficult. Adoption of the exclosure approach of Chitty, Pimentel & Krebs (1968) does not permit separation of actual consumption from the amount of food removed but not eaten (Petrusewicz & Macfadyen, 1970). Gut-content analysis, favoured by Hansson (1970, 1971) and Evans (1973), has the disadvantage of a) killing the animal, and b) reflecting only the composition of the most recent meal (Ferns, 1976). Faecal analysis, used by Godfrey (1953) and Ferns (1976), raises problems of a) differential digestibility of different food items, and b) differential digestibility of the "same" food item at different seasons. Laboratory experiments on food preference are subject to the major criticism of non-natural conditions.

Irrespective of the approach employed, current evidence generally

supports the thesis that *M. agrestis* exercises some degree of selection in its choice of food items (Godfrey, 1953; Myllymäki, 1959; Chitty *et al.*, 1968; Hansson, 1970, 1971; Evans, 1973 and Ferns, 1976). What remains uncertain is whether the results obtained by different methods of diet determination truly reflect the rank order of food preference.

In this paper we combine the laboratory approach of food preference experiments with that of faecal analysis and explore two hypotheses, namely: (1) Consumption of different food items by *M. agrestis* is directly proportional to the availability of those foods. (2) Diet composition as determined by faecal analysis reflects the rank order of preference for the available food items.

MATERIAL & METHODS

Background Information

The field voles and food used in this study came from a limestone grassland (Rough Common — Nat. grid ref. SP 457 081) which forms part of the Wytham Estate of Oxford University. It is the same grassland used by Godfrey (1953) and Chitty *et al.* (1968) in their studies of feeding by *M. agrestis*.

At the time of the investigation the relative abundance of the main grass species comprising the grassland mosaic was *Brachypodium pinnatum* (L.) Beauv $>$ *Bromus erectus* Hunds. $\approx$ *Arrenatherum elatius* (L.) J. & C. Pressl $>$ *Dactylis glomerata* L. (see Gibson, 1976 & 1976a). Eleven other grass species were recorded but nowhere did they exceed 10 per cent of the local cover. Only the four most common species were used in the laboratory investigation of food preference.

Food Preference Experiments

Figure 1 shows in some detail the apparatus used in these studies. The main features to be noted are:

1. The provision of a central nest box with an opening into each of the four feeding arenas.
2. The four food-hoppers, each of which presents an equal area of access from its associated arena to the contained food supply.
3. The gridded floors of the feeding arenas, which allow food remains and faeces to fall into a collecting tray without being permanently moistened with urine.
4. The interlocking nature of the apparatus, which facilitated collection of food remains and faeces as well as cleaning at the end of each trial.

A total of 62 food preference trials were made between. June, 1979 and June, 1980. On each experimental occasion the following pre-trial procedure was adopted:

1. At least 1.2 kg of each of the four test grasses (*B. pinnatum*, *B. erectus*, *A. elatius* and *D. glomerata*) was collected fresh from the field and transported to the laboratory in labelled polythene bags.
2. In the laboratory the live (green) material was separated from the dead (brown), only the former being retained for the food preference trials.
3. The green material of each species was mixed separately.

4. The mixed material from each species was divided into individual lots of ca. 200 g fresh weight. These weights were recorded.

5. One 200 g lot was stored in a perforated metal container and kept as a control for subsequent determination of wet weight — dry weight relationships. The remaining lots were offered as food in the preference trials.

6. Each trial apparatus was allocated, at random, one 200 g lot of each of the four test grasses. The "arena" to which each grass species was allotted was also randomly chosen.

7. Taking each grass species in turn, the grass was used first to fill the appropriate food-hopper, surplus material being stored in the associated "spare-food" compartment.

8. The nest box was supplied with distinctively coloured, yellow, hay.

At this stage of assembly the apparatus was ready to receive the experimental animal. (N.B. In an attempt to avoid "conditioning" to any of the test grasses experimental animals were kept immediately prior to the trial — at least 2 days — on a diet of laboratory pellets).

9. The randomly chosen experimental animal was weighed to the nearest 0.1 g on a "Pesola" spring balance and its sex noted.

10. The test individual was placed in the central nest box and the food-hopper/nest-box roof fixed in position. Water bottles were added.

11. The whole set-up was covered by a damp cloth in an attempt to ensure that the food supply was kept in a pristine condition.

12. During the course of each trial (48 to 72 h) the cloth cover was kept damp by sprinkling it with water. Depleted food resources in the hoppers were replenished regularly from the "spare food" compartments.

The post-trial procedure can be summarised as:

13. Removal and reweighing of the test animal before returning it to a holding cage.

14. Removal of the "yellow" hay from the nest box, at the same time ensuring that the green grass mixed with it was returned to the appropriate food compartment.

15. Removal of nest box.

16. Noting the maximum/minimum temperatures recorded during the trial period.

At this point the partly dismantled apparatus was left for 3 to 4 days to allow urine to evaporate and faeces to dry.

17. Sequential dismantling of the remaining apparatus (E to A in Fig. 1) allowed collection of the surplus food, food remains by grass species, and their allocation to appropriately labelled perforated containers for drying at 70 to 80°C over 24 h. The total faeces production was also collected and similarly dried.

18. The dry weights of the non-consumed portions of each of the preffered grass species were determined to the nearest 0.01 g, as were the dry weights of the faeces produced.

19. The "control" materials were used to determine the wet weight-dry weight relationships of each of the four test grasses.

Given the above information it was possible to calculate (a) the per-cent water content of the test grasses, (b) consumption of both separate and combined food items per individual vole per unit time in terms of dry and wet weights, (c) total

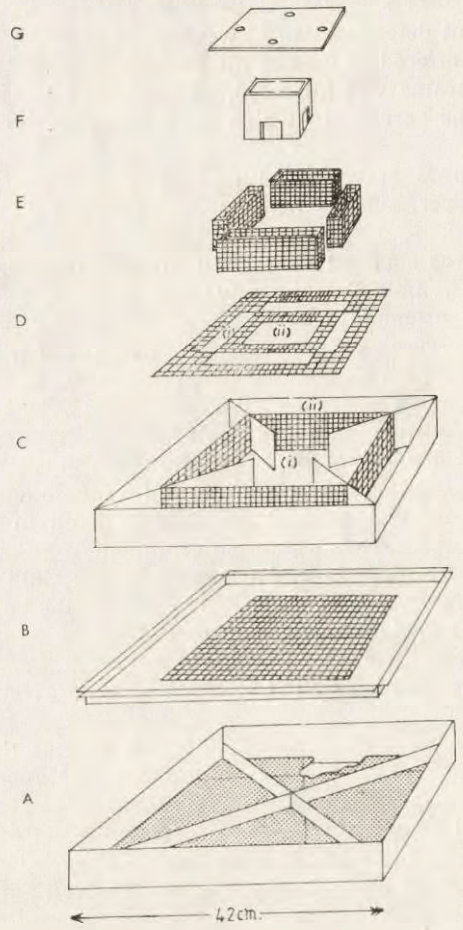

Fig. 1. An exploded view of the food preference apparatus.

A. The 42×42×6 cm collecting tray with a false bottom of muslin which retains faecal pellets and grass remains whilst allowing urine to drain away. The 3 cm high dividers separate the grass remains originating from the four feeding arenas. B. The 41×41×3 cm arena floor with a central 30×30 cm area of metal grid through which faecal pellets and food remains fall. C. (i) The four feeding arenas separated by diagonal sheet metal walls which leave a central space for the nest box (ii) the "spare food" compartments. D. The 30×30 cm metal mesh arena roof with apertures for (i) the four food hoppers and (ii) the central nest box. E. The 16×4×8 cm four metal food-hoppers. F. The 10×10×8 cm central nest box with a single opening on each of the four sides, thereby allowing free access to the feeding arenas. G. The 18×18 cm nest box and food-hopper roof with apertures for water bottles.

faeces production as dry weights, and (d) overall dry weight digestibility coefficients

$$\frac{/\text{Total consumption} - \text{total faeces production}/\times 100}{\text{total consumption}}$$

Identification of Food Items in Faeces

Correct identification of the food items appearing in the faeces required the preparation of a reference collection of different grass parts. Permanent slides of the abaxial and adaxial cuticles of the leaf blades of *B. pinnatum*, *B. erectus*, *A. elatius* and *D. glomerata* were prepared according to a procedure based on, but modified from, Metcalfe (1960). In brief it consisted of:

1. The selection of a 1.5 to 2.0 cm piece from the middle section of a fresh leaf blade. Where the mid-vein was very prominent it was removed.
2. The softening of the chosen piece in water for 10 to 30 minutes.
3. Separation of the cuticle from the "softer" materials by scraping gently with a sharp, straight-edged scalpel. During this process the tissue was flooded from time to time with hypochlorite solution to ensure bleaching.
4. Washing the cuticle in water to remove all traces of hypochlorite solution.
5. Staining in Delafield's haematoxylin for 5 to 10 minutes, with subsequent washing and dehydration.
6. Preparation of the permanent slide with Euparal as mountant.

All preparations were photographed at a variety of different magnifications. The resulting prints were used in the identification of specific food items in the faeces produced during the food preference trials.

Preparation of Slides of Faecal Material

Slides of faecal material were prepared from individual pellets as follows:

1. Lightly grinding the dried faecal pellet in a miniature pestle and mortar.
2. Transferring the ground material, after the addition of distilled water and three drops of surfactant (= detergent), to a centrifuge tube and shaking well.
3. Removal of the supernatant fluid by centrifuging for approximately 7 minutes.
4. Two washes with distilled water, each followed by 7 minutes centrifugation.
5. Two washes with absolute alcohol, each followed by 7 minutes centrifugation.
6. Transference of the dehydrated material to a microscope slide, the effective cover slip area being 22×50 mm.
7. Preparation of a permanent mount with Euparal.

Analysis of Faecal Preparations

Using a combination of light-dark field microscopy each preparation was examined means of a ×16 objective and a ×8 ocular provided with a square grid consisting of 100 equal sized grid-cells. Each grid covered 0.36 mm² of the slide and hence each grid-cell was equivalent to 0.0036 mm². A systematic examination was made of 20 regularly spaced grids (=7.20 mm²) per slide. The spacing of the

grids was arranged to encompass the 1100 mm² of the cover slip, the locations being pre-determined by fixed co-ordinates on the mechanical stage of the microscope.

The observations made included the percentage frequency of occurrence, number and size of different categories of faecal fragments occurring within the grid fields. Where possible, fragments were categorized according to grass species, but in some instances it proved impossible to distinguish between *A. elatius* and *D. glomerata* because of the similarity in size and shape of some of their cells (see Plate XIII). A category Ae/Dg was erected to receive information about such fragments. Other pieces could not even be allocated to a species group and were recorded as either unidentifiable (mainly fibres and parenchyma cells) or miscellaneous (mainly portions of cells, small hooks and hairs). The percentage frequency of occurrence and total number of categorized fragments per 60 grid fields was determined, also the size of each fragment estimated by visual comparison with a single grid-cell (0.0036 mm²) and assessing area to the nearest quarter grid-cell (0.00045 mm²).

RESULTS

Food Preference Experiments

Table 1 summarises the results of 62 trials made on 15 separate experimental occasions. Experiments 10 and 11 were conducted at a time of the year when green *B. erectus* was in short supply in the field, this species does not therefore figure in the results for February.

In common with other food preference trials on *M. agrestis* (Chitty *et al.*, 1968; Hansson, 1971; Ferns, 1976) some animals in the present series showed a weight loss.

The mean wet weight of food consumed per day was 21.35 ± 1SD 6.55 g indiv^{-1}, but statistically significant differences occurred between experiments made on different dates ($\chi^2 = 25.7$, d.f.$= 14$, $0.02 < p < 0.05$). These differences disappeared when dry weight consumption, with a mean value of 6.29 ± 1SD 2.03 g indiv^{-1} d^{-1}, was considered ($\chi^2 = 8.57$, d.f.$= 14$, $0.80 < p < 0.90$).

Over the full series of trials the coefficient of digestibility (in terms of dry weight) equalled 52.8%. Some variation occurred seasonally, digestibility coefficients being higher in Spring (March, April, May) and Summer (June, July, August) than in Autumn (September, October, November) and Winter (December, January, February); the respective coefficients were 53.7, 58.0, 44.3 and 50.6%.

Figure 2 shows the consumption of each of the four food types as a percentage of total consumption. The pattern of utilisation of each food clearly varied with season and the nature of this variation was explored by means of a factorial anovar for interaction (Table 2). In the case of both wet and dry weight consumption the interaction between food

Table 1

Food consumption and food digestibility in the laboratory.

Bp = *Brachypodium pinnatum*, Be = *Bromus erectus*, Ae = *Arrenatherum elatius*, Dg = *Dactylis glomerata*.

Experimental details				No. of trials	Mean live wt. ± 1 SD. (g)			Wet wt. of food consumed dy^{-1} ($\bar{x}g$ ± 1 SD.)					Water content of food (%)				Dry wt. of food consumed dy^{-1} ($\bar{x}g$ ± 1 SD.)					Food digestibility	
Expt. no.	Starting date	Duration (h)	T°C (range)	n	Initial	Final	Diff.	Bp	Be	Ae	Dg	Σ	Bp	Be	Ae	Dg	Bp	Be	Ae	Dg	Σ	Dry wt. of faeces dy^{-1}	Digest. coeff.
1	7. 6.79	69	13.5-19.5	3	31.23	30.33	-0.90	11.85	5.05	5.27	4.46	26.63	61	65	79	80	4.65	1.75	1.12	0.88	8.40	3.64	56.7
					12.54	11.84	0.85	2.11	0.17	5.01	3.05	6.05					0.83	0.06	1.08	0.60	1.07	1.00	
2	20. 6.79	69	20.0-26.0	5	28.70	26.16	-2.54	5.50	11.27	1.16	2.86	20.80	67	62	68	75	1.79	4.22	0.37	0.71	7.09	2.28	67.8
					8.82	8.21	1.80	3.02	8.27	0.64	2.27	5.94					0.98	3.10	0.20	0.56	2.24	0.89	
3	29. 6.79	48	15.0-21.0	6	24.93	22.75	-2.18	4.84	4.30	2.62	5.61	17.87	65	59	66	71	1.72	1.98	0.89	1.62	6.20	2.66	57.1
					8.43	8.43	1.89	2.27	1.37	2.39	1.26	2.24					0.80	0.57	0.81	0.36	0.79	0.42	
4	6. 7.79	69	19.0-24.0	6	20.72	19.00	-1.72	3.47	4.52	5.74	7.13	20.89	66	62	69	74	1.25	1.72	1.77	1.84	6.58	2.26	65.7
					1.91	2.90	2.35	1.36	1.70	2.54	1.50	2.81					0.49	0.64	0.78	0.39	0.85	0.63	
5	23. 7.79	69	18.0-24.0	4	22.90	20.90	-2.00	2.85	3.51	8.13	8.54	23.23	58	51	58	76	1.11	1.73	3.42	2.12	8.37	4.55	45.6
					5.78	4.28	2.40	1.39	1.02	2.09	3.28	3.34					0.56	0.50	0.88	0.78	1.21	0.80	
6	12. 9.79	69	13.0-20.0	3	35.00	32.70	-2.30	2.35	1.44	5.42	7.20	16.51	50	52	69	79	1.17	0.69	1.71	1.49	5.05	2.34	53.7
					9.50	8.94	0.72	0.21	0.87	3.55	3.00	6.83					0.10	0.41	1.09	1.03	1.44	1.09	
7	12.10.79	72	17.0-19.5	4	24.63	24.33	-0.30	4.02	3.06	4.86	6.26	18.20	58	66	76	81	1.68	1.05	1.16	1.16	5.06	2.14	57.7
					6.65	7.21	2.35	1.84	1.78	3.21	3.23	3.48					0.77	0.61	0.77	0.60	0.70	0.20	
8	5.11.79	72	15.0-18.0	4	24.98	22.70	-2.28	10.04	10.93	7.62	8.08	36.67	58	66	83	83	4.58	3.49	1.26	1.38	10.70	7.11	33.5
					3.80	4.03	1.59	6.24	8.63	4.55	3.77	17.44					2.84	2.76	0.75	0.64	5.59	4.66	
9	5.12.79	72	16.0-20.0	5	25.72	22.02	-1.70	3.51	5.52	9.09	10.81	28.93	66	68	83	80	1.18	1.75	1.52	2.13	6.58	3.33	49.4
					6.81	4.40	1.61	2.10	3.87	4.54	5.77	5.21					0.71	1.23	0.76	1.14	0.84	0.75	
10	5. 2.80	49	11.5-15.5	3	22.17	22.17	0.00	4.39	-	8.28	7.94	20.61	64	-	76	76	1.56	-	1.99	1.90	5.47	2.66	47.7
					1.04	2.36	1.50	3.96		1.45	1.02	1.51					1.43		0.35	0.25	0.85	0.86	
11	20. 2.80	48	13.0-16.0	3	21.83	21.83	0.00	4.19	-	3.18	6.44	16.21	55	-	76	77	1.89	-	0.86	1.94	4.69	2.08	55.7
					2.25	3.01	0.87	0.51		2.04	3.21	4.35					0.23		0.49	0.74	1.00	0.51	
12	18. 3.80	48	9.0-13.5	4	25.13	26.00	+0.88	4.06	9.35	5.77	3.81	22.99	63	76	78	78	1.51	2.25	1.27	0.84	5.86	2.42	58.7
					2.25	3.49	2.17	2.76	1.10	1.73	2.11	4.09					0.76	0.26	0.38	0.47	1.09	0.47	
13	28. 4.80	48	18.0-19.0	4	30.13	29.25	-0.88	1.78	8.11	2.98	4.11	16.57	70	71	75	79	0.53	2.35	0.75	0.89	4.52	2.20	51.3
					3.77	4.52	0.85	1.63	1.07	2.15	0.40	4.99					0.49	0.31	0.54	0.15	1.30	0.72	
14	26. 5.80	48	21.0-23.0	5	29.00	27.60	-1.42	1.98	3.91	6.86	3.78	16.52	74	74	75	80	0.51	1.02	1.72	0.76	4.06	2.06	49.3
					4.76	4.57	3.20	1.76	1.74	7.54	3.36	12.66					0.50	0.46	1.89	0.67	3.09	1.73	
15	11. 6.80	72	16.0-25.0	3	31.17	25.17	-6.00	1.95	2.93	2.54	3.96	11.38	67	68	72	76	0.64	0.94	0.71	0.95	3.24	1.67	48.5
					2.93	6.90	4.27	1.69	4.86	2.89	2.57	10.61					0.56	1.55	0.64	0.62	3.14	2.19	
				Σ 62				$\bar{x}$ 4.46	5.73	5.24	5.52	21.35					$\bar{x}$ 1.72	1.92	1.36	1.29	6.29	2.97	52.8
								±1 SD. 3.12	3.17	2.36	2.39	6.55					±1 SD. 1.35	1.01	0.75	0.51	2.03	1.47	

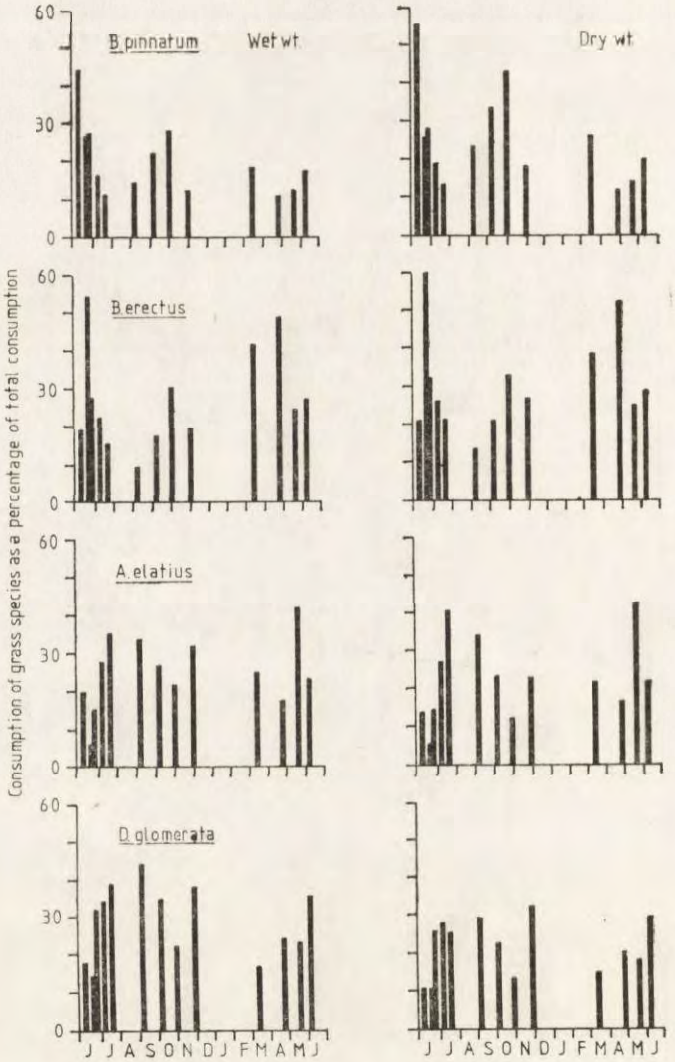

Fig. 2. Consumption of each of the four food grasses as a percentage of total consumption.

type and date proved to be highly significant. Under these conditions it was considered best to regard the series as consisting of several small experiments, one for each block or date. A separate analysis was carried out for each of the 13 blocks in which all four grass species were tested Table 3 summarises the results obtained with a) Friedman's non-parametric two-way analysis of variance (χ_r^2) and b) a parametric analysis of variance. The results of the two types of analysis are in broad

Table 2
Results of factorial anovar to test for interaction between foods (treatment) and dates (blocks).

Sources of variation	Sums of squares	Degrees of freedom	Mean - squares	F	Level of significance
			Wet weight consumption		
Treatments	50.64	3	16.88	1.49	n.s.
Blocks	493.44	12	41.13	3.63	0.01
Interaction	1033.45	36	28.71	2.54	0.01
Error	1947.63	172	11.32	-	-
Total	3525.26	223	-	-	-
			Dry weight consumption		
Treatments	15.50	3	5.17	5.02	0.01
Blocks	47.37	12	3.95	3.83	0.01
Interaction	127.30	36	3.54	3.44	0.01
Error	176.42	172	1.03	-	-
Total	366.59	223	-	-	-

Table 3
Food preferences as revealed by (a) Friedman's non-parametric two way analysis of variance (χ^2_r) and (b) a parametric analysis (Anovar).

Date	Wet weight consumption			Dry weight consumption		
	χ^2_r	Anovar	Food preference	χ^2_r	Anovar	Food preference
7 June 1979	n.s.	n.s.	-	$0.01<p<0.05$	$0.01<p<0.05$	Bp>Be>Ae>Dg
20 June	$0.01<p<0.05$	$0.01<p<0.05$	Be>Bp>Dg>Ae	$0.001<p<0.01$	$0.001<p<0.01$	Be>Bp>Dg>Ae
29 June	n.s.	n.s.	-	n.s.	n.s.	-
6 July	n.s.	$0.01<p<0.05$	Dg>Ae>Be>Bp	n.s.	n.s.	-
23 July	$0.001<p<0.01$	$0.05<p<0.01$	Dg=Ae>Be>Bp	n.s.	$0.001<p<0.01$	Ae>Dg>Be>Bp
August	-	-	-	-	-	-
12 September	n.s.	n.s.	-	n.s.	n.s.	-
12 October	n.s.	n.s.	-	n.s.	n.s.	-
5 November	n.s.	n.s.	-	n.s.	n.s.	-
5 December	n.s.	n.s.	-	-	-	-
January 1980	-	-	-	-	-	-
February	-	-	-	-	-	-
18 March	$0.001<p<0.01$	$0.001<p<0.01$	Be>Ae>Dg=Bp	$0.01<p<0.05$	$0.01<p<0.05$	Be>Ae=Bp>Dg
28 April	<0.001	$0.001<p<0.01$	Be>Dg>Ae>Bp	$0.01<p<0.05$	$0.001<p<0.01$	Be>Ae=Dg>Bp
26 May	n.s.	n.s.	-	n.s.	n.s.	-
11 June	n.s.	n.s.	-	n.s.	n.s.	-

agreement and indicate that *M. agrestis* exhibited clear food preferences in June, July 1979 and March, April 1980, but not in other months. The order of preference for the four food types varied between seasons; *B. erectus* was the preferred food in March, April 1980 and possibly June 1979, while *A. elatius* and *D. glomerata* were the most acceptable food in July, 1979.

On the basis of these results we conclude that a) *M. agrestis* exhibits a food choice in Spring and Summer, and b) consumption of different food items by *M. agrestis* is directly proportional to the availability of those foods only at certain times of the year, primarily Autumn and Winter.

Identification of Food Items in Faeces

The microphotographs shown in Plate XIII illustrate the range of cuticle-cell shapes, hooks and hairs associated with the four species of test grasses. These, and similar, photographs were used to aid the identification of grass fragments found in the faecal preparations. In the case of cuticle fragments it was relatively easy to distinguish those derived from *B. pinnatum, B. erectus* and *A. elatius/D. glomerata.*

Analysis of Faecal Preparations

To test the hypothesis that "diet composition as determined by faecal analysis reflects the rank order of food preference for the available food items" it was considered unnecessary to analyse all of the faecal pellets produced during the 62 trials. In practice we examined a random sample from the March and April 1980 trials and pellets from a contrasting trial where no food preference had been detected (May, 1980). For each of the three stated months pellets from four animals were analysed. Three slide preparations per animal were examined (*i.e.* 4×3 slides or 4×60 grid-fields per month, a total of 4×21.6 mm^2).

Table 4 shows the percentage frequency of occurrence of different food items in the faeces. Of the specifically identifiable foods it is clear that *B. pinnatum* occurred most frequently, followed by *B. erectus* and *A. elatius/D. glomerata.* As can be seen this sequence does not reflect the rank order of observed dry weight consumption.

Table 5 gives both the total number and total area of specific food categories occurring in the faecal slide preparations. As with the frequency of occurrence values the inference that *B. pinnatum* was the preferred food does not accord with the observed consumption values.

Because the mean fragment size of the different food categories varied

Table 4

Percentage frequency of occurrence of different food items in the faeces. Bp = *Brachypodium pinnatum*, Be = *Bromus erectus*, Ae—Dg = combined *Arrenatherum elatius* and *Dactylis glomerata*, Misc. = miscellaneous hooks and hairs, Unid. = unidentifiable fibres and cells.

Experimental details			Percentage frequency of occurrence in 60 grid-fields (= 21.6 mm^2)					Rank order of observed dry weight consumption (See Tables 3 & 6)
Expt. no.	Trial no.	Animal code	Bp	Be	Ae-Dg	Misc.	Unid.	
12	1	3D ♀	73.33	38.33	21.67	35.67	80.00	Bp>Ae/Dg>Be
12	2	3A ♂	73.33	43.33	31.67	75.00	83.33	Be>Bp>Ae/Dg
12	3	3B ♂	56.67	41.36	30.00	55.00	91.67	Ae/Dg>Be>Bp
12	4	3C ♂	90.00	25.00	6.67	63.33	96.67	Be>Ae/Dg>Bp
		$\bar{x}$	73.33	37.08	22.50			Be>Ae/Dg>Bp
13	1	4D ♀	61.67	30.00	15.00	45.00	80.00	Be>Ae/Dg>Bp
13	2	4A ♂	70.00	46.67	20.00	28.33	96.67	Be>Ae/Dg>Bp
13	3	4B ♂	23.33	33.33	18.33	16.167	96.67	Be>Ae/Dg>Bp
13	4	4C ♂	43.33	23.33	28.33	20.00	100.00	Be>Ae/Dg>Bp
		$\bar{x}$	52.08	33.33	20.42			Be>Ae/Dg>Bp
14	1	5D ♀	58.33	31.67	28.33	21.67	96.67	Ae/Dg>Be>Bp
14	2	5B ♂	31.67	40.00	18.33	36.67	88.33	Ae/Dg>Be>Bp
14	3	5C ♂	6.67	25.00	1.67	20.00	93.33	Bp>Be>Ae/Dg
14	4	5E ♂	33.33	21.67	35.00	17.67	93.33	Ae/Dg>Be>Bp
		$\bar{x}$	32.50	29.58	20.83			Ae/Dg>Be>Bp

(*B. pinnatum:* 0.004 mm^2, range 0.001 to 0.034 mm^2; *B. erectus:* 0.004 mm^2, range 0.001 to 0.045 mm^2; *A. elatius:* 0.008 mm^2, range 0.003 to 0.022; *D. glomerata:* 0.011 mm^2, range 0.006 to 0.013 mm^2; *A. elatius/D. glomerata:* 0.006 mm^2, range 0.001 to 0.043 mm^2; unidentifiable fibres and cells: 0.008 mm^2, range 0.001 to 0.077 mm^2; and miscellaneous hooks and hairs: 0.003 mm^2, range 0.001 to 0.027 mm^2) it is not surprising that only the miscellaneous category, with its fixed-shape items, showed a significant relationship between fragment number and area. A relationship which can be expressed by the least squares regression equation $y=0.14+0.002x$, $r^2=0.98$, $p<0.001$, where x represents number of fragments per 60 grid-fields and y, area of fragments in mm^2.

A priori reasoning suggests that fragment area should be a better indicator of food consumption than fragment number, accordingly further attempts were made to try and establish concordance between

Table 5

Total number, total area and fragment size of food categories present in the faeces. Bp = *Brachypodium pinnatum*, Be = *Bromus erectus*, Ae = *Arrenatherum elatius*, Dg = *Dactylis glomerata*, Misc = miscellaneous hooks and hairs, Unid. = unidentifiable fibres and cells.

Experimental details		Animal live wt. (g)			Total no. of fragments of each food item per 60 grid-fields (= 21.6 mm^2)								Total area of each food item per 60 grid-fields (mm^2 per 21.6 mm^2)								Mean area per fragment (mm^2)				
Expt. no.	Animal code	Initial	Final	Diff.	Bp	Be	Ae	Dg	Ae+ Dg	Misc.	Unid.	Σ	Bp	Be	Ae	Dg	Ae+ Dg	Misc.	Unid.	Σ	Bp	Be	Ae	Dg	Ae/ Dg
12	3D ♀	26.5	26.5	0.0	95	32	0	0	13	26	149	315	0.363	0.180	-	-	0.070	0.056	1.067	1.736	0.004	0.006	-	-	0.005
12	3A ♂	22.0	21.0	-1.0	112	47	2	1	21	74	206	463	0.392	0.150	0.008	0.006	0.091	0.183	0.951	1.781	0.004	0.003	0.004	0.006	0.004
12	3B ♂	27.0	27.5	+0.5	57	35	2	0	17	38	105	254	0.182	0.158	0.012	-	0.079	0.109	0.985	1.525	0.003	0.005	0.006	-	0.005
♀2	3C ♂	25.0	29.0	+4.0	105	20	0	0	4	46	103	278	0.366	0.061	-	-	0.017	0.101	0.687	1.232	0.003	0.003	-	-	0.004
13	4D ♀	32.0	31.0	-1.0	89	20	1	1	9	36	160	316	0.207	0.095	0.007	0.018	0.068	0.100	1.083	1.578	0.002	0.005	0.007	0.018	0.008
13	4A ♂	24.5	22.5	-2.0	83	38	0	0	12	19	135	287	0.258	0.140	-	-	0.049	0.043	1.024	1.514	0.003	0.004	-	-	0.004
13	4B ♂	31.5	31.5	0.0	29	26	0	0	11	10	132	208	0.129	0.100	-	-	0.070	0.022	0.892	1.213	0.004	0.004	-	-	0.006
13	4C ♂	32.5	32.0	-0.5	39	15	1	0	22	12	150	239	0.140	0.049	0.004	-	0.113	0.020	1.275	1.601	0.004	0.003	0.004	-	0.005
14	5D ♀	30.0	28.5	-1.5	60	28	2	0	19	22	113	244	0.240	0.185	0.036	-	0.147	0.057	0.839	1.504	0.004	0.007	0.018	-	0.008
14	5B ♂	34.5	35.0	+0.5	30	31	0	1	12	25	122	221	0.150	0.146	-	0.013	0.082	0.077	1.152	1.620	0.005	0.005	-	0.013	0.007
14	5C ♂	31.5	26.0	-5.5	4	21	0	0	2	12	101	140	0.015	0.113	-	-	0.005	0.040	0.979	1.152	0.004	0.005	-	-	0.003
14	5E ♂	27.0	23.0	-4.0	21	14	3	1	23	14	94	170	0.077	0.034	0.023	0.009	0.174	0.032	0.935	1.284	0.004	0.002	0.008	0.009	0.008

Table 6

Dry weight consumption of food items as a percentage of total consumption, also percentage contribution by area of each food item to both total faeces area and total area of identifiable faeces.
Bp = *Brachypodium pinnatum*, Be = *Bromus erectus*, Ae = *Arrenatherum elatius*, Dg = *Dactylis glomerata*, Misc. = miscellaneous hooks and hairs, Unid. = undentifiable fibres and cells.

Experimental details		Dry wt.consumption of food items as a percentage of total consumption				Percentage contribution by area of each food item to total faeces area							Percentage contribution by area of food types to identifiable faeces		
Expt. no.	Animal code	Bp	Be	Ae	Dg	Bp	Be	Ae	Dg	Ae+ Dg	Misc.	Unid.	Bp	Be	(Ae)+(Dg) +(Ae+Dg)
12	3D ♀	36.32	29.90	18.39	15.40	20.91	10.37	0	0	4.03	3.23	61.46	59.22	29.36	11.42
12	3A ♂	28.40	46.91	18.52	6.17	22.01	8.42	0.45	0.34	5.11	10.28	53.40	60.59	23.18	16.23
12	3B ♂	23.59	30.39	26.05	19.97	11.93	10.36	0.79	0	5.18	7.15	64.59	42.23	36.66	21.11
12	3C ♂	11.85	52.21	23.09	13.05	29.71	4.95	0	0	1.38	8.20	55.76	82.43	13.74	3.83
13	4D ♀	19.50	43.37	19.97	17.16	13.12	6.02	0.44	1.14	4.31	6.34	68.63	52.41	24.05	23.54
13	4A ♂	12.36	66.09	0	21.55	17.04	9.25	0	0	3.24	2.84	67.64	57.72	31.32	10.96
13	4B ♂	6.28	51.01	22.61	20.10	10.63	8.24	0	0	5.77	1.81	73.54	43.14	33.44	23.41
13	4C ♂	4.76	54.76	19.05	21.43	8.74	3.06	0.25	0	7.06	1.25	79.64	45.75	16.01	38.24
14	5D ♀	11.32	14.06	54.59	20.02	15.96	12.30	2.39	0	9.77	3.79	55.78	39.47	30.43	30.10
14	5B ♂	1.08	34.27	42.95	21.69	9.26	9.01	0	0.80	5.06	4.75	71.11	38.36	37.34	24.30
14	5C ♂	45.60	44.04	0	10.36	1.30	9.81	0	0	0.43	3.47	84.98	11.28	84.97	3.76
14	5E ♂	18.96	22.55	40.52	17.96	6.00	2.65	1.79	0.70	13.55	2.49	72.82	24.29	10.73	64.98

observed food preference and that inferred from faecal analysis. The dry weight consumption of each food item by each of the experimental animals was expressed as a percentage of each individual's total consumption; similarly, the area of each food category observed in each individual's faecal preparations was expressed as a percentage of the total fragment area measured. The results are shown in Table 6 where it can be seen that between 60 and 88 per cent of the faecal materials could not be assigned to a specific food type, the mean being 74 per cent. It follows that determination of "diet composition" was, of necessity, based on an average of one-quarter of the total faecal material sampled. Because of the varying proportions of specifically identifiable food items, and to facilitate comparison between food and faecal composition, the areas of each specific faecal food item were expressed as a percentage of the total area of identifiable items. Figure 3 compares percentage consumption with percentage identifiable faeces composition. In 11 out of 12 cases *B. pinnatum* was clearly over represented by the faecal

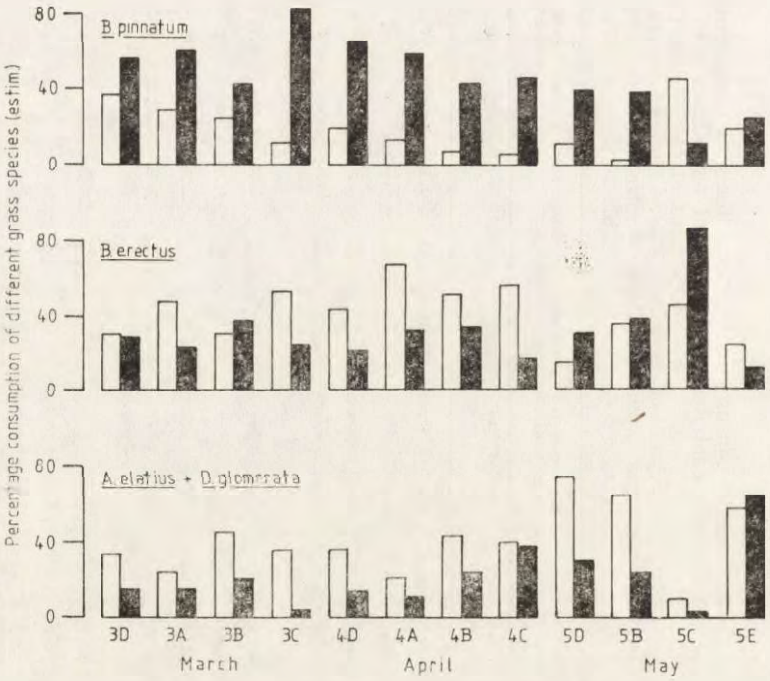

Fig. 3. Comparison of the observed percentage dry weight consumption of the different food grasses (open columns) and the percentage contribution by area of the different grasses to the identifiable portion of the faeces produced from this consumption (closed columns). The code numbers refer to individual voles, details of which are given in Table 5.

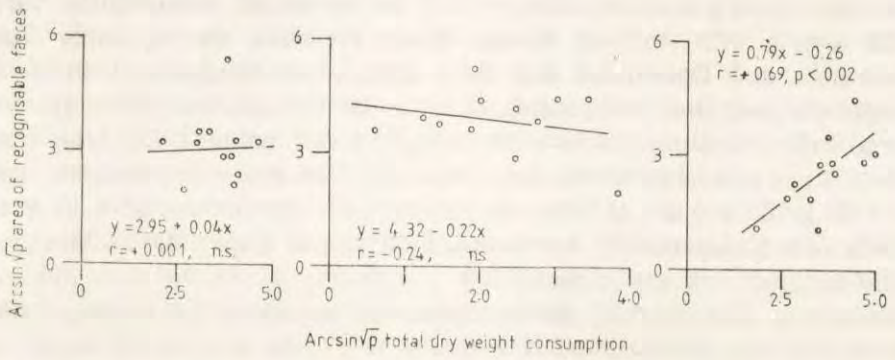

Fig. 4. Arcsin square root transformations of the percentage contribution by specifically identified food items to the total area of recognisable food items in the faeces regressed against the arcsin square root transformations of the observed dry weight percentage contribution of the different food items to total consumption.

analysis technique; *B. erectus* was under represented in 7 out of 8 March —April samples, and over represented in 3 of the 4 May ones; *A. elatius* and *D. glomerata* combined were under represented in 11 out of 12 cases. Despite the varying consumption to faeces ratio for each food type over time (see Fig. 3) it was considered worthwhile to explore the possibility of establishing an overall correction factor for each food type. Using arcsine square root transformations the area of a particular food item expressed as a percentage of the total area of recognisable fragments, was regressed against the dry weight of the same food item expressed as a percentage of total dry weight consumption (Fig. 4). No significant relationships could be established in the cases of *B. pinnatum* and *B. erectus*, although that for *A. elatius/D. glomerata* proved significant at the 2% level.

DISCUSSION

It is not surprising that most feeding studies on *Microtus agrestis* have shown grasses to be the primary food source (Summerhayes, 1941; Godfrey, 1953; Chitty, Pimentel & Krebs, 1968; Hansson, 1970 & 1971; Evans, 1973; Ferns, 1976) for it occurs most commonly among tall grasses, along field hedgerows, and in young tree plantations where the grass is lush. An exception to this general rule was provided by Myllymäki (1959), who found in southern Finland that *M. agrestis* preferred herbs to the available grasses and sedges.

In this one-year laboratory study, total grass intake by adult animals

averaged 21.35 g fresh wt. indiv.$^{-1}$ d^{-1}. Fresh weight consumption varied with season (the highest values being recorded during early June, November and December) but these differences disappeared when dry weight consumption was considered. It is not thought that the voles were specifically choosing foods with a high water content for free water was always available in the water bottles. The mean dry weight intake of 6.29 g indiv^{-1} d^{-1} is fully consistent with the values of 6.30 g and 7.10 g found respectively by Hansson (1971) and Ferns (1976). Moreover, allowing for a mean digestibility coefficient of 52.8% and applying Hansson & Grodzinski's (1970) regression equation for average daily metabolic rate (Rodent $ADMR = 19.94\ W^{-0.50}$) to a vole of mean live weight 28.67 g, it can be shown that the daily grass consumption should be *ca.* 6.07 g dry wt. at 20°C. It can be concluded that the present findings for dry weight consumption by *M. agrestis* are not untypical.

All four grass species used in this study are described by Duffey, Morris, Sheail, Ward, Wells & Wells (1974) as being coarse and characteristic of the retrogression of *Festuca* spp. sward following cessation of grazing. They are probably not an ideal food source for *M. agrestis;* indeed, in laboratory experiments Richards (1981) showed that, of the fifteen grasses occurring on Rough Common, *Festuca rubra* L. was much preferred over the four species dealt with here (see also Ferns, 1976). Even so, this species, along with *Agropyron repens* L., *Deschampsia caespitosa* L., *Helicotrichon pratense* L., *Holcus lanatus* L. and *Poa angustifolia* L. only occurred sporadically in small patches over the research area. The remaining five species, *Agrostis stolonifera* L., *Agrostis tenuis* Sibth., *Briza media* L., *Festuca ovina* L. and *Trisetum flavescens* L. were all rare (Gibson, 1976). It is highly improbable that the more palatable of these species could have collectively supported the known maximum field vole population of 100—120 ha^{-1} (Richards, 1981). It was considered justifiable therefore to assume that *M. agrestis* in the field relied upon the four most common grasses of Rough Common as their major source of nourishment, which accounts for their use in the laboratory trials of food preference.

Of particular interest in the results from this study was the order of preference exhibited for the four food grasses on offer. Only in June, July 1979 and March, April 1980 were clear preferences demonstrated, *B. erectus* being the preferred food in March, April 1980 and late June 1979, *B. pinnatum* in early June 1979, and *A. elatius* and *D. glomerata* in July 1979. Because the different food items preffered in the laboratory had equal availability it must be inferred that the various foods changed in quality over time. The nature of these changes is, as yet, unknown but there is an apparent correlation between the food preferences shown

by *M. agrestis* and the phenology of the different grass species. According to Duffy *et al.* (1974) new leaf growth in *B. erectus* occurs from late-March to May inclusive, April to May is the period of rapid growth for *B. pinnatum,* while *A. elatius* and *D. glomerata* reach their maximum growth rate during May to July. Clearly, the implication is that given the opportunity *M. agrestis* will select new, succulent growth in preference to older, tougher, and perhaps distasteful grasses. The absence of a demonstrable food choice in Autumn and Winter no doubt reflects the fact that the months March to July encompass the major growth periods for the grasses under consideration. Outside this period the quality differences between the grasses were presumably insufficient to exert a significant pressure on *M. agrestis* food selection.

Given that at certain times of the year at least, *M. agrestis* does exhibit a choice between different food items, there remains the question as to whether any method, other than direct observation, will provide reliable information about a) actual food consumption and b) food preference. Digestibility coefficients through time offer one possibility for quantitative determination of food consumption; always, of course, providing that they and faecal output can be determined accurately. A useful check on results so obtained can be carried out by using the known digestibility coefficient in conjunction with the *ADMR* regression equation of Hansson & Grodziński (1970) — see early part of discussion. A major problem associated with this approach lies in the variable nature of digestibility coefficients with both food type and time. In this study the coefficients varied between 33.6% and 67.8%, giving a mean value of 52.8%; these figures are in accord with the mean dry matter digestibility of 50% reported by Hansson (1971) and a range of 33.1% to 56.8%. In both studies higher values were noted in Spring as opposed to Winter. Clearly, a rough approximation of food consumption can be obtained by employing a digestibility coefficient of 50% and applying it to know faecal production; more desirable is the use of a realistic seasonal coefficient in conjuncton with both faecal production and the *ADMR* linear regression. Given agreement between the results of the two calculations one can be reasonably confident of having estimated consumption with a fair degree of accuracy.

More instransigent is the identification of a suitable indirect method for determining food preference in the field. Hansson (1970) recommended the use of stomach contents since the least amount of digestion occurs there, Ferns (1976) quite rightly pointed out that such material reflects only the composition of the most recent meal. With an adult full stomach fresh weight of 1.0 to 1.5 g (Hansson, 1971) it is clear that an intake of 21.35 g fresh wt of food indiv^{-1} dy^{-1} requires that the stomach be

emptied and refilled between 14 and 21 times per day. Unless many individuals are killed to provide an adequate sample on each sampling occasion it is obvious that inferences about food preference based on stomach content analyses must be treated with caution.

Both Hansson (1970, 1971) and Ferns (1976) expressed doubts about the validity of faecal analysis for the assessment of food preferences in the field, the main objection raised was the differential digestibility of different food items. Until the present work no one, to our knowledge, had subjected this proposition to experimental testing by comparing directly the composition of food intake with that of faecal output. Our own studies show that faecal analysis, whether by percentage occurrence, fragment number or fragment area, does not reflect food preference.

Aproximately 25% of the faecal material was specifically identifiable and analysis of this proportion indicated that *B. pinnatum* was the most frequently preferred food. This finding is in marked contrast to the known consumption, in that *B. pinnatum* was generally the least preferred of all the four experimental foods. Attempts to correct this, and similar anomalies were only partially successful. For each food type we separately regressed $\sin^{-1}\sqrt{P}$ of faecal proportion against $\sin^{-1}\sqrt{P}$ of intake proportion. In the case of the "tougher" grass species (*B. erectus* and *B. pinnatum*) the slopes of the regression lines did not differ significantly from zero, both species were clearly over represented in the faeces when their contribution to total intake was low and under represented when it was high. Such findings accord well with the proposition that *B. erectus* and *B. pinnatum* are only consumed in large quantities when they are "succulent" i.e. during March, April and early June. It was presumably because of this phenomenon that Chitty *et al.* (1968) found, from experiments conducted during February and March, that *B. pinnatum* was eaten extensively by *M. agrestis* in field enclosures. In fact, their results indicate that approximately 60 g fresh wt of "greenstuff" was destroyed by one adult every day; with a likely consumption of *ca.* 20 g fresh wt dy^{-1} it would appear that field voles destroy three times as much grass as they consume. The arcsine square root regression for the combined "softer" grasses (*A. elatius* and *D. glomerata*) proved to be statistically significant ($y=0.79x-0.26$, $r=+0.69$, d.f.$=10$, $p<0.02$) and could therefore be used to derive a reasonable estimate of the actual consumption of these species. Although consistently under represented in the faeces the discrepancy between faecal content and food intake is largest when these two species form a high proportion of total consumption, again an indication that the preferred food grasses are the most "succulent" ones available.

On the current evidence we conclude that without appropriate correction factors, faecal analysis gives rise to misleading inferences about food preferences. Because of the changing "quality" of food with time it is not always possible to establish such correction factors, and hence faecal analysis is unsuitable for the determination of food preferences in the field. Stomach analyses, in addition to the problems of turnover time and especially where retention time is long and digestion high, must be subject to simlar drawbacks. These conclusions clearly have relevance beyond studies of *M. agrestis* alone, namely in the wider context of food preference studies. It would appear, despite their artificial nature, that well designed "cafeteria" type experiments are the most likely indirect method of providing reliable results on food preference.

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KONSUMPCJA POKARMU U *MICROTUS AGRESTIS* I ZASTOSOWANIE ANALIZY KAŁU DO OKREŚLENIA PREFERENCJI POKARMOWEJ

Streszczenie

Autorzy postawili sobie za cel sprawdzenie dwóch hipotez: (1) czy konsumpcja różnych pokarmów zjadanych przez *M. agrestis* jest proporcjonalna do dostępności tych pokarmów, oraz (2) czy skład diety określony na podstawie analizy kału odbija preferencję zjadanego pokarmu.

Badania prowadzono w laboratorium na dorosłych osobnikach *M. agrestis*. Norniki te zjadały średnio 21.4 g świeżej trawy (=6.3 g suchej masy) na dobę. Średnia roczna strawność wynosiła u nich 52.8% (Tabela 1). Zwierzętom podawano do jedzenia, jesienią i zimą, 4 pospolite gatunki traw w takiej proporcji w jakiej występowały w warunkach naturalnych (Ryc. 1, 2). Wiosną i latem preferowały one bardziej soczyste gatunki. W ciągu roku współczynnik strawności wahał się od 33.6 do 67.8%, a najwyższe wartości osiągał również na wiosnę i w lecie.

Po przeprowadzeniu wielu różnorodnych obliczeń statystycznych (Tabela 2, 3; Ryc. 3, 4) oraz porównań z wynikami uzyskanymi przez innych badaczy odnośnie analiz pokarmu, przemian energetycznych itp. (Tabela 5, 6) autorzy konkludują, że analiza kału, bez użycia odpowiedniej poprawki daje wyniki błędne jeżeli chodzi o preferencję pokarmową. Wiąże się to głównie z niedoszacowaniem lub przeszacowaniem niektórych komponentów diety. Ustalenie tej poprawki, w oparciu o stosowane metody i rozumowanie, jest niemożliwe ze względu na ogromną zmienność „jakości" pokarmu. Zatem analiza kału nie nadaje się do oznaczania wybiórczości pokarmowej *M. agrestis* w terenie.

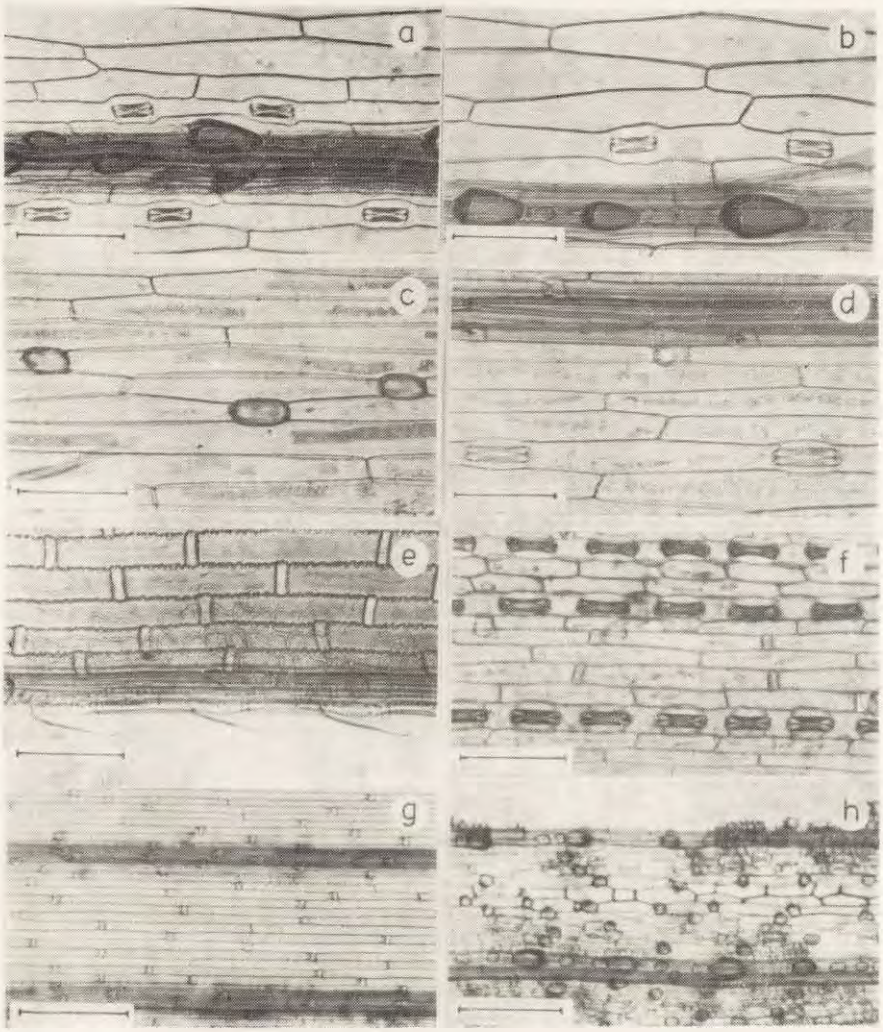

Plate XIII

Microphotographs of the four food grasses. Photographs a & b are respectively the abaxial and adaxial surfaces of *Dactylis glomerata*, c & d refer to the abaxial and adaxial surfaces of *Arrenatherum elatius*, e & f those of *Bromus erectus* and g & h those of *Brachypodium pinnatum*. The black line in the bottom left hand corner of each photograph represents 0.1 mm.

J. Phillipson *et al.* *auctores phot.*