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Chromogen-Ratio Method for Determining Digestibility of Plants by Grasshoppers

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Abstract
The concentration of plant pigments in excreta vs. food provided a measure of digestibility of most plants by grasshoppers. Digestibility values obtained with the chromogen-ratio method and consumption-excretion ratios were highly correlated (r = 0.789**). Greatly disparate results were obtained in 4 cases. The chromogen method, when applicable, gave better duplicability and eliminated many of the errors and much labor associated with consumption-excretion ratios.

The chromogen-ratio method is based on the assumption that nonabsorbable substances in a forage may be used as markers and that the concentration of these substances in the excreta will be proportional to the digestibility of the forage. Inert substances such as chromic oxide have been successfully utilized in insect-nutrition studies (McGinnis and Kasting 1964). Most plant pigments would seem applicable to such a technique and are naturally incorporated into insect diets in uniform quantities. Reid et al. (1950) first reported a method for using plant pigments as markers in digestibility studies with dairy cows. Smart et al. (1954) modified Reid's method by introducing copper into the porphyrin ring of chlorophyll and pheophytin, thus stabilizing these pigments to light, acid, and alkali, and at the same time largely eliminating the carotenoids. This paper reports results obtained with the chromogen-ratio method in digestibility studies involving insects.

Methods

Several species of grasshoppers served as test insects. These were field collected, sexed, and confined to small cages constructed of wood and fiberglass screening. In most tests 6 grasshoppers/cage were used.

Plants were oven dried and uniformly ground to ensure that the plant material used for chromogen extraction was a representative sample of that eaten. The ground plant material was fed in shallow aluminum dishes. Water was provided ad lib. in cotton-stoppered vials.

These trials were conducted over a considerable period. No attempt was made to control environmental variables in the laboratory. Grasshoppers of different ages and plants of varying maturity were used. Dry plant parts are not normally ingested by the species of grasshoppers used in this study. Thus it is impossible, and not the purpose of this paper, to make any precise statements about the relative digestibility of different plants. These tests only compared the effectiveness of the chromogen-ratio method with consumption-excretion ratios for determining digestibility.

Most trials were of 1-day feeding duration, but in a few cases longer time intervals were used to obtain sufficient excreta for analysis. Each treatment was replicated 2 or 4 times on a single day. Additional replicates were obtained by repeating the test. A l-day starvation period was necessary between tests to ensure that all feces were voided from the preceding treatment. The same cage of grasshoppers was used for several tests but treatments were tried alternately on different groups.

The chromogen-ratio method of Smart et al. (1954) was used for analysis. Optical densities of chromogen extracts were read with a Beckman spectrophotometer at 420 mμ. When available, 200 mg of food and 100 mg of excreta were analyzed. Excellent duplication was obtained with as little as 20 mg of excreta, but quantities smaller than 50 mg often gave optical densities below the optimum range of sensitivity of the spectrophotometer used. When possible, all readings were made in the range 0.30–0.35 optical density by appropriate dilutions. Consumption-excretion ratios were simultaneously obtained for comparison.

Results

Variation was slightly greater for males but digestibility of plants did not differ significantly for the 2 sexes of any species of grasshopper used in these tests. Table 1 is a partial summary of the combined results for both sexes and the 2 methods used. Digestibility values obtained by the chromogen method were highly correlated with consumption-excretion ratios (r = 0.789**).

The chromogen method resulted in a lower value for digestibility of alfalfa flowers. Chlorophyll is probably the most important pigment measured by this chromogen method and the technique may have been unreliable for these flower pigments which are primarily anthocyanins. Unexplainable disparities between the 2 methods occurred with prairie sandreed, sunflower, and prickly lettuce. Both methods gave similar results for all other plants. Even materials such as sorghum seeds, although containing little or no chlorophyll, were correctly evaluated by the chromogen method.

Table 1. Digestibility of plants by grasshoppers as determined by 2 methods

Plant species	Grasshopper species[a]	Percent digestibility	
		Consumption-excretion	Chromogen
Sedge, *Carex* sp.	Md	8.77	11.98
Little bluestem, *Andropogon scoparius* Michx.	Md	15.82	11.98
Big bluestem, *Andropogon gerardi* Vitman	Md	29.23	32.03
Sorghum (variety Atlas), *Sorghum vulgare* Pers.	Md	20.09	16.13
Sorghum (variety Kafir)	Md	23.24	23.86
Sudan grass, *Sorghum vulgare* Pers.	Md	30.15	26.79
Leadplant, *Amorpha canescens* Pursh.	Md	9.85	5.57
Sunflower, *Helianthus annuus* L.	Md	11.10	44.23[b]
Beeplant, *Cleome serulata* Pursh.	Md	11.93	14.81
Smartweed, *Polygonum pennsylvallicum* L.	Md	15.51	22.11
Smallflower gaura, *Gaura parviflora* Dougl.	Md	16.18	15.31
Prickly lettuce, *Lactuca scariola* L.	Md	24.88	13.20[b]
Snow-on-the-mountain, *Euphorbia marginata* Pursh.	Md	25.37	23.92
Bush morningglory, *Ipomoea leptophylla* Torr.	Md	27.15	25.77
Alfalfa (stems), *Medicago sativa* L.	Md	13.06	14.83
Alfalfa (leaves, pre-bloom stage)	Md	29.87	34.86
Alfalfa (leaves, bloom stage)	Md	25.10	34.39
Alfalfa (flowers)	Md	44.05	30.44[b]
Needleandthread, *Stipa comata* Trin. & Rupr.	Ms	14.28	11.68
Prairie sandreed, *Calamovilfa longifolia* (Hook.)	Ms	14.40	–0.02[b]
Sorghum (seeds, variety RS608)	Mb	69.36	70.47

a. Md = *Melanoplus differentialis* (Thomas), Ms = *Melanoplus sanguinipes* (F.), Mb = *Melanoplus bivittatus* (Say)
b. Significantly different from consumption-excretion value at the 0.05 level.

Daily variation in digestibility was highly significant, but good duplication was obtained between replicates within days. One trial was conducted to determine possible causes of this daily variation. Nymphs of the red-legged grasshopper, *Melanoplus femurrubrum* (De Geer) , were fed 2 species of plants in both dry and fresh forms and with continuous food vs. food only on alternate days. Results, presented in Table 2, suggest that the use of dry food contributed to high daily variation. When fresh plants were used, digestibility remained relatively uniform throughout the test period regardless of feeding interval. When dried plants were used, digestibility became variable and tended to decrease with time, irrespective of feeding interval. Since dried plants for the entire test were prepared in 1 batch, this variation was due to the grasshopper and not to actual differences in digestibility of the plant over the time interval involved.

To determine if daily variation in the ability of grasshoppers to digest plants might also exist in the field, 4th instar to adult *Phoetaliotes nebrascensis* (Thomas) were collected from a nearly solid stand of western wheatgrass, *Agropyron smithii* Rydb., for 8 consecutive days. Excreta voided within 4 hr of collection were held in a deep freeze until conclusion of the sampling period. Daily plant samples were collected from the same area and all samples were analyzed simultaneously in duplicate. Digestibility varied significantly, ranging from

a high of 43.0% on the 3rd day to 23.5% on the 5th. Whether this variation was due to the grasshopper or to actual changes in digestibility of the plant was not determined, but results confirmed that daily variation in digestibility may occur under field conditions over short time intervals.

Table 2. Effect of continuous and alternate-day feeding on digestibility of 2 plant species by *Melanoplus femurrubrum* nymphs

Plant	Feeding interval (days)	Percent digestibility on consecutive days										Mean daily consumption (mg)
		1	2	3	4	5	6	7	8	9	Mean	
Alfalfa, fresh	1	52	53	45	58	59	60	56	48	52	54	8.35
	2	52		60		59		58		57	57	9.41
Alfalfa, dry	1	46	50	55	54	58	36*	30*	0*	46*	46	5.84
	2	49		52		54		36		4	39	7.62
Bluegrass, fresh	1	67	69	51	46	57	40	49	41	51	57	6.00
	2	62		58		56		41		57	55	6.55
Bluegrass, dry	1	41	51	42	27*	47		18	24	33	36	3.92
	2			56		48		0		26	32	6.79
Daily means		53	56	52	49	55	51	36	32	40		

* Data from only 1 replicate.

Conclusions

The chromogen-ratio method proved an accurate means for determining digestibility of most plants by grasshoppers. Better duplication was obtained with the chromogen method than with consumption-excretion ratios. Much of the labor and many of the errors involved in digestibility trials were avoided with the chromogen method. Accurate weighing of samples of food, preliminary moisture determinations, and complete recovery of uneaten food and excreta are unnecessary when the chromogen method is used. Since the quantity of excreta produced need not be determined, there is no need for a starvation period. Excreta produced for 1 day following start of any diet can be discarded. Mortality of insects containing food in their stomachs, cannibalism, and ingestion of extraneous items (such as cotton from the water vials) may bias consumption-excretion ratios. Fecal pellets containing other than undigested plant parts arc easily recognized and can be discarded in toto without biasing results obtained with the chromogen method. When results from the 2 techniques were comparable, we believe those obtained with the chromogen method were more precise. However, since unusually disparate results were obtained in a few cases, further tests are needed to determine the limitations of the chromogen method.

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