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Determination of Digestibility of Artificial Diets for the Silkworm (Bombyx mori) by Using Magnetite Sand

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Abstract
A method for determining the digestibility of diets for the silkworm (B. mori) by using magnetite sand was developed with sea sand as a labelling substance. The larvae were reared on the artificial diet containing purified magnetite sand. The values for the ingestion and digestion were estimated by weighing the magnetite sand which was collected from the feces of the silkworms. In the above process, no chemical analyses were required. Suitable quantity of magnetite sand required for the mixing in the artificial diet was 1% on a dry matter weight basis of the diet. The natural sea sand was purified to obtain a better attraction of sand to the magnet. The values of digestibility calculated by using the magnetite sand method were similar to those obtained by using other conventional methods.

Discipline: Sericulture
Additional key words: labelling substance, purified magnetite sand

Introduction

Substantial improvement in the composition of artificial diets has enhanced both the quality and quantity of cocoon crops as well as the fecundity of the silkworms. The cost effectiveness of producing cocoons by using artificial diets for silkworm rearing has been investigated[3]. It is furthermore necessary to increase the efficiency of the diets through increased digestibility for the silkworm. The authors who studied improvements in the composition of artificial diets considered that the measurement of the digestibility was the optimum method to detect a high digestion ratio in the constituents of the diet.

There are several methods for estimating the digestibility of diets in the silkworms (*B. mori*). One of the methods reported by Hiratsuka[1] is frequently used for determining the digestibility in silkworms fed with mulberry leaves. On the other hand, in the case of cattle, labelled substances were added to the feed to calculate the ingestion and digestion ratios [5]. These methods are used commonly due to the simple and rapid procedures for the estimation. Chromium oxide[4] was used as a labelling substance in cattle and silkworms reared on artificial diets[2]. Materials such as cellulose, lignin, chromogen, polyethylene glycol and radio isotopes which were also used as labelling substances[6], have not yet been evaluated for the silkworm.

The authors investigated the suitability of several kinds of substances attracted by magnets as indicators. Sea sand was found to be the optimum material to determine the digestibility of artificial diets for silkworm rearing. The purpose of the present study is to evaluate the magnetite sand method for use in the estimation of the digestibility of artificial diet for the silkworm and to compare this method with conventional gravimetric methods.

Materials and methods

The parent silkworm eggs of F_1 hybrids namely

Table 1. Composition of artificial diet

Substances	Larval stage		
	1st and 2nd stages	3rd and 4th stages	Final stage
Mulberry leaf powder	50.0 g	40.0 g	30.0 g
Agar powder	7.0	6.0	5.0
Potato starch	6.0	6.5	7.0
Sugar	5.5	6.0	7.0
Safflower oil	1.0 m*l*	1.0 m*l*	1.0 m*l*
β-Sitosterol	0.3 g	0.3 g	0.3 g
Ascorbic acid	2.0	2.0	2.0
Salt mixture	3.0	3.0	3.0
Citric acid	3.0	4.0	4.0
Sorbic acid	0.2	0.2	0.2
Defatted soybean meal	22.0	28.0	34.0
Cellulose powder		3.0	6.5
Vitamin B mixture	10.0 m*l*	10.0 m*l*	10.0 m*l*
Antiseptics	10.0	10.0	10.0
Water	280.0	280.0	250.0

Japanese race J 145 × J 01 (5·1) and Chinese race C 146× C 01 (6·1) were used for the study. The artificial diet (Table 1) which was developed by the authors for rearing the parent silkworms was used. This artificial diet was fed from the period of brushing to 4th instar larvae and then newly exuviated 5th instar larvae were used for the treatment. The larvae tested were selected carefully to obtain scattered weights within differences of ±0.005 g. The weight of the newly exuviated 5th instar larvae of Japanese females and males was 1.08 and 1.00 g and that of the Chinese females and males was 1.11 and 1.00 g, respectively. Fifty female and 50 male larvae from each parent were reared separately in each test batch.

The indicator substances were selected based on their attraction to the magnet. Hence, materials such as nickel, stainless steel, triiron tetroxide, ferrite and sea sand were considered. Each of these substances was examined for the effect on the growth of the silkworms and their recovery and then their suitability was estimated for determining the digestibility. These indicator substances were put into the artificial diet at a rate of 1% in each diet after steaming and the diet was stirred thoroughly to disperse homogeneously the substances. The feces were collected at 24 hr intervals from the first feeding in 5th instar larvae and the labelling substances were recovered from the feces as follows : a homogenizer was put into the beaker and the separated labelling substances were attracted together to the magnet which was set at the bottom of the beaker. Thus the recovered substances were dried and weighed. Digestibility was calculated by the following equation according to the quantity of the substances recovered.

Quantity ingested = Quantity of labelling substances recovered from feces × 100,

Quantity digested = Quantity ingested − (Dry matter content of feces − Quantity of labelling substances contained in feces),

$$\text{Digestibility } \% = \frac{\text{Quantity digested}}{\text{Quantity ingested}} \times 100.$$

Conventional gravimetric method was also applied at the same time to calculate the approximate digestibility and the results were compared with those obtained by the new method.

Results and discussion

Several substances were screened out of which fine materials were selected based on their attraction to the magnet and low magnetic susceptibility.

Table 2. Labelling substances and their effect on larval growth

Labelling substances (powder)	Items			
	Doping rate (%)	Recovery	Effect on larval growth	Remarks
Nickel	1%	Imperfect	Disturbance	
Stainless steel	1%	Imperfect	Disturbance	Rusty
Triiron tetroxide	1-3%	Perfect	Slight disturbance	*
Ferrite	1-3%	Perfect	Normal	*
Sea sand (untreated)	1-3%	Imperfect	Normal	Requires purification
Sea sand (treated)	1-3%	Perfect	Normal	74-177μ were used.

*Suitable size of granule could not be easily obtained.

Sea sand (300 g)
├─Wash with water (3 times)
Boil 30 min
├─Dry
Eliminate granules more than 177 μ and less than 74 μ in size by using 80 and 200 mesh filter
│
Wash with ether
├─Dry (Approximately 100 g)
After 10 min treatment by dispersion and mixing in water, floating granules are eliminated by decantation
├─Dry
Add heavy solution (CH_2I_2 $d^{20}=3.3254$) to eliminate the floating granules.
│ Heavy solution is filtered for recycling.
├─The same as above (3 times)
Wash with ether
├─Dry
Eliminate granules less than 74 μ in size by using 200 mesh filter
│
Purified sea sand (Approximately 80 g)

Fig. 1. Flow chart of purification method of natural sea sand

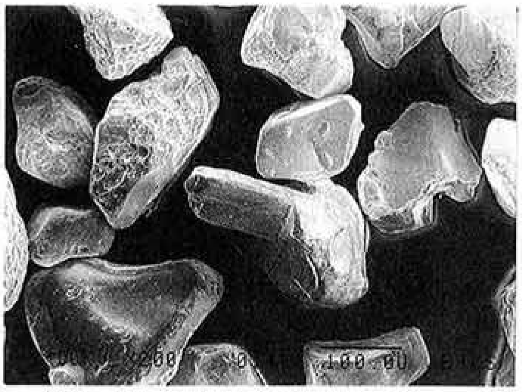

Plate 1. Natural sea sand (×200)

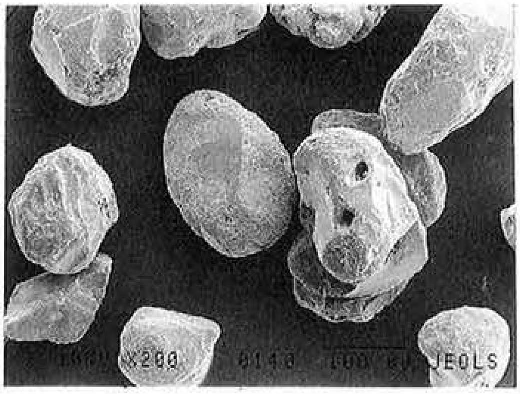

Plate 2. Purified sea sand (×200)

These substances were mixed into the artificial diet and their effect on the growth and development of the silkworms as well as their relative recovery from the feces was investigated. The results are shown in Table 2.

Nickel, stainless steel and triiron tetroxide used in the diets had an adverse effect on the development and the larval growth was retarded. Relative recovery of stainless steel from the feces was lower compared to the other materials. As a result, the above substances were eliminated due to their unsuitability as indicators. The recovery of ferrite was slightly better and it did not adversely affect the growth. However, suitable sizes of the granules could not be obtained and the granules were too fine. Natural sea sand was first used as an indicator. It did not adversely affect the larval development but the relative recovery was low and the rearing results were unstable. Examination of the sea sand by an X-ray micro-analyzer, revealed the presence of crystals of silicic anhydride on the surface of the sand granules (Plate 1).

The author considered that these crystals may have disturbed the attractivity to the magnet, thereby reducing the recovery of the sand. Therefore, natural sea sand was purified as indicated in Fig. 1.

The crystals of silicic anhydride were removed from the surface of the granules by using a dispers-

Table 3. Diet digestibility in 5th instar larvae measured by the gravimetric method and by using magnetite sand as indicator substance

Races	Gravimetric method			Magnetite sand method		
	Ingested* (g)	Digested* (g)	Digestibility (%)	Ingested* (g)	Digested* (g)	Digestibility (%)
J 5·1 ♀	4.30	1.81	42.1	4.77	1.95	40.9
J 5·1 ♂	3.47	1.40	40.3	3.86	1.54	39.9
C 6·1 ♀	4.79	2.03	42.4	5.25	2.20	41.9
C 6·1 ♂	3.68	1.58	42.9	4.18	1.78	42.6

*Amount of dry matter in food.

ing mixer. Thus the detached crystals were isolated from the granules due to the differences in the sinking speed of the substances by employing methylene diiodide as a heavy solution. After the above treatments, purified magnetite sand consisting of triiron tetroxide and titanium oxide was obtained (Plate 2). The attraction to the magnet of the purified magnetite sand was stronger than that of natural sea sand and its relative recovery was high. These facts indicated that purified magnetite sand was suitable as an indicator substance.

Varying quantities of magnetite sand were added to the artificial diet. The values of digestibility were scattered when the purified sand was added to the diet at the rate of 0.5% by weight. When the diet with 1% of sand was fed, the appetite of the silkworms was not adversely affected. However the increase of the concentration to 2 or 3 % resulted in a gradual reduction of the ingestion or digestion ratios. Hence it was concluded that the addition of magnetite sand at the rate of 1% of the diet would be optimum.

The values of digestibility measured by the gravimetric method and by using magnetite sand as indicator substance (hereafter described as magnetite sand method) are given in Table 3.

The digestion ratios calculated through the magnetite sand method for the Japanese race (5·1) were in the order of 40.9 % in females and 39.9% in males whereas in the Chinese race (6·1) the values were 41.9% for the females and 42.6% for the males. The corresponding values estimated by using gravimetric method were 42.1% in the females and 40.3% in the males in race (5·1) and 42.4 % in the females and 42.9% in the males in race (6·1). Thus, the reasons why the digestibility percentages recorded by the magnetite sand method were lower by 1.0% compared to those by the gravimetric method remain to be determined. It should be emphasized, however that the small difference in the digestibility could not be attributed to the effect of the application of magnetite sand because the growth of the silkworms which were fed on an artificial diet containing magnetite sand was quite satisfactory.

It is thus concluded that the magnetite sand method is a rapid method for determining the digestibility by using indicator substances. In addition this simple method enables to determine the quantity of the indicator substance without requiring the use of any special chemical analysis.

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