Application of Artificial Diets in Sericulture

By TOSHIO ITO

Silkworm Physiology Division, Sericultural Experiment Station
(Yatabe, Ibaraki, 305 Japan)

An entire rearing of the silkworm, *Bombyx mori*, on artificial diets has first been achieved in 1960[1,2]. Larval growth was poor, mortality was high, development was delayed, and cocoons were small. However, with this as a momentum extensive studies have been started in the new fields, such as the analysis of nutritional and dietary requirements, the establishment of rearing system with artificial diets, the physiology and pathology of the silkworm by means of aseptic culture, and so forth.

At present there is little difficulty in rearing the silkworm on artificial diets and in obtaining cocoons of good quality in a laboratory scale. The practical use of artificial diets in sericulture has started a few years ago in Japan. As a matter of fact, younger larvae hatched from 120 thousand boxes have been reared on artificial diets in the cooperative rearing houses in 1979. One box is a unit of selling silkworm eggs and contains 20 thousand eggs or more. Thus, 120 thousand boxes amount to 2.4 billion larvae, which account for only about 5% of the total number of larvae reared in that year in the whole Japan. However, in 1980 it is expected an increase of 50% over last year. In the present paper the progress of the study on artificial diets for the silkworm as well as their application in sericulture will be reviewed briefly.

Dietary requirements[3]

There are at least four main requirements in the formulation of artificial diets for the silkworm; that is, (1) to meet both qualitative and quantitative nutritional requirements, (2) to possess the suitable physical properties, (3) to be free from the microbial contamination, and (4) not to contain any possible deterrent or repellent.

1) Nutritional requirements[4,5]

Qualitative nutritional requirements have been analyzed with synthetic or semi-synthetic diets (Table 1). Another important aspect is the quantitative requirements, since the nutritional balance reflects largely the rate of growth and silk production. Minimum optimum levels of indispensable nutrients have been determined, and it is found that these values are the same order of magnitude as those of the analytical data of mulberry leaves. Thus, mulberry leaves are considered to satisfy the nutritional requirements, both qualitatively and quantitatively.

Dose and balance of the dietary ingredients are important in the formulation of diets. The dietary level of amino acid mixture or soybean meal must be increased to some level to get a higher silk production. Furthermore, the composition of the amino acid mixture influences larval growth and silk production. A higher level of the non-essential amino acids (alanine, cystine, glycine, serine, and tyrosine) or of the acidic ones (aspartic and glutamic acids) favors the growth acceleration and the increase in silk production, whereas a higher level of the essential amino acids affects them adversely[6]. The ratio of fibroin to sericin in the cocoon layer also varies according to the amino acid composition above-mentioned. There is a preferred ratio of the amount of two acidic amino acids in the diet[7]. Furthermore, the quantitative requirements for amino acids are somewhat different between male and female larvae.

Dietary composition is necessary to be

Table 1. Qualitative nutritional requirements of the silkworm

Amino acid nutrition

Essentials
- Arginine
- Histidine
- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Valine

Aspartic and/or glutamic acids

Semi-essential
- Proline

Non-essentials*
- Alanine
- Cystine
- Glycine
- Serine
- Tyrosine

* Nutritionally important

(All L-forms)

Lipid nutrition

Sterol requirement
- β-Sitosterol, or
- Cholesterol, or
- Stigmasterol, or
- Campesterol, or
- Various plant sterols

Ergosterol (less nutritive)

Fatty acid requirement
- Linoleic acid
- Linolenic acid
- Oleic acid**
- Stearic acid**
- Palmitic acid**

** Less nutritive

Protein nutrition

Nutritive proteins (soybean, egg, milk, etc.)

Vitamin nutrition

Essentials
- Biotin
- Choline
- Folic acid
- Inositol
- Niacin*
- Pantothenic acid
- Pyridoxine**
- Riboflavine
- Thiamine

L-Ascorbic acid***

a-Tocopherol (somewhat ?)

* Nicotinamide & pyridine-3-aldehyde equally effective.

** Pyridoxal & pyridoxamine equally effective.

*** D-Araboascorbic acid almost equally effective.

Mineral nutrition

Essentials
- Calcium
- Iron
- Magnesium
- Manganese
- Phosphorus
- Potassium
- Zinc
- Not fully investigated

Carbohydrate nutrition

Highly nutritive
- Sucrose
- Glucose
- Fructose

Moderate value
- Maltose
- Lactose
- Cellobiose
- Trehalose
- Melezitose
- Sorbitol

Starch (utilized or not utilized by silkworm strains)

modified according to the larval development. For instance, the level of the dietary soybean meal must be increased up to 60% or higher on the dry weight basis of diets for the 5th instar lravae. It is reported that the efficiency of the diet, that is, the rate of silk production against the rate of food consumed is improved by increasing the level of soybean meal. However, further study on the improvement of the dietary efficiency is necessary especially in respect to the elevation of silk production.

2) Physical factors

Physical factors play often very important role in the maintenance of good quality of diets. Water content is one of the main factors, and approximately 75% level seems to be favorable (Fig. 1)[8]. However, the suitable water content must be determined by each diet, because some dietary ingredients, such as agar, sugar, cellulose powder, have a close connection with hardness or softness of diets.

Physical property is modified by the inclusion of cellulose powder or by the increase of its level in the diet. The rate of food consumption is accelerated according to the increase in dietary cellulose powder, and in consequence growth is accelerated in spite of the fact that larvae have fed the less nutritive diet[9]. Since the skilkworm cannot digest cellulose, an inclusion of optimal amount of cellulose powder in diet is considered to contribute to improve the physical property. Dietary agar also plays some important role (Table 2)[10].

According to the increase in glucose or sucrose concentration, hardness of the diet is lowered, and as a result the larval growth is retarded, and the rate of silk production is reduced[11]. Furthermore, autoclaving time in diet preparation is reported to affect the hardness of the diet. The pH value of the diet also

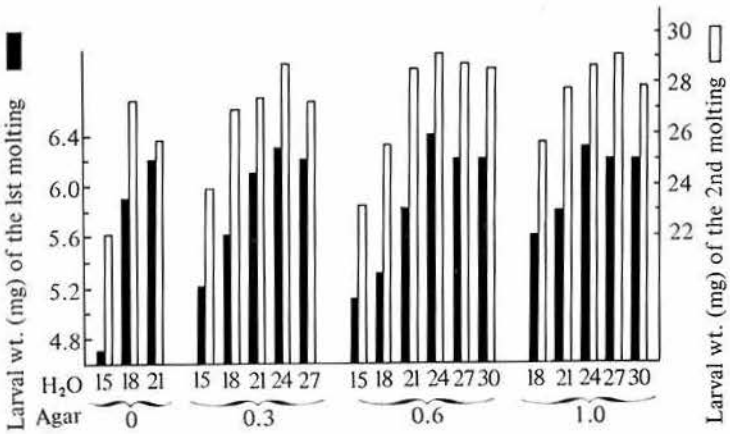

Fig. 1. Effect of water content in combination with agar content on larval growth. Amount of water added to 10.5 g dry diet varied from 15 to 30 ml[8]

Table 2. Effects of dietary levels of starch, soybean meal, agar, and cellulose powder on growth[10]

Results of analysis of variance
(started with newly hatched larvae)

	Amt. of meal	Source of variance	Body weight after		
		Main effects	5 days	10 days	14 days
Exp. 1	30 ~ 50	Starch	—	—	—
		Soybean meal	**	**	**
		Agar	—	*	—
Exp. 2	40 ~ 60	Soybean meal	*	*	—
		Cellulose powder	—	*	**
	40 ~ 70	Soybean meal	**	**	
		Cellulose powder	*	**	

** Significant at 1%
* Significant at 5%

affects the physical property of the diet.

3) Prevention against microbial contamination

Artificial diets decompose very quickly, unless any suitable antimicrobial agent is included. The larvae which have fed the highly contaminated diet will die with few exceptions. Some kinds of antimicrobal substances are harmful for the silkworm, therefore their dietary concentrations must be at the safe level. Usually, a combination of two substances or more is preferable, but it is still impossible to prevent the decomposition of diet completely under the conventional rearing conditions. In the diets which we have been using sorbic acid, propionic acid, and chloramphenicol are added at their optimal concentrations. Under the aseptic condition the bacterial contamination can be kept away, but this method is not practical in a large-scale rearing in sericulture.

Bacteriological examinations of the decomposing diets have been carried out extensively by many authors, and a few species of the bacteria belonging to *Streptococcus* group are found commonly in the diet, but the contaminations of other microorganisms are also reported. Effectiveness of antibiotics for preventing contaminations is also confirmed.

4) Palatability of diet

Since the most of the nutritive compounds have their specific taste, the role of the nutrients as either feeding stimulants or inhibitors cannot be ignored. For instance, some sugars accelerate feeding of the silkworm greatly[12], whereas some amino acids suppress feeding considerably[13]. The taste is more important than the odor for larvae to accept the diet.

Soybean meal, one of the main ingredients, contains water-soluble deterrent substance(s)[14], which is easily extracted by washing with a 90% ethanol (Table 3). The

Table 3. Deterent effect of soybean meal on feeding of the silkworm, *Bombyx mori*[14]

Feeding response of newly hatched larvae on graded doses of soybean extract

90% EtOH extract (mg/g dry diet)	Larval distribution (%)			Distribution ratio
	Experimental diet	Control diet	Filter paper	
52.7	2.4	95.9	1.6	0.03
13.2	15.0	82.2	2.8	0.18
3.3	32.3	63.7	3.9	0.51
0.82	37.1	58.6	4.4	0.63

Effect of graded doses of soybean extract on the initial growth of newly hatched larvae

90% EtOH extract (mg/g dry diet)	Mean body wt. after 22 hr rearing (mg)	Weight increase, as % of initial wt
No addition	1.235	286*
10	0.675	156
20	0.577	136
30	0.498	115
40	0.531	123

* Initial mean body weight; 0.432 mg

practical diets contain a certain amount of dried mulberry leaf powder, by which both feeding and growth are accelerated. It is possible to decrease the dietary level of the leaf powder according to the progress of larval instar, finally down to zero, without any bad effect on feeding. For drying the fresh mulberry leaves to prepare the leaf powder, a high temperature, such as 80°C, is not recommended. Although the details are unknown, larval growth is highly reduced on the diet containing such leaf powder as dried at a high temperature.

In Table 4 an example of the artificial diets for the practical purpose is shown[15].

Silkworm rearing[16]

1) *Environmental conditions*

Extensive studies have revealed that the environmental conditions required for the rearing on artificial diets are slightly different

Table 4. An example of practical artificial diet for younger larvae

Substance	Dry diet (%)
Dried mulberry leaf powder	25
Corn starch	7.5
Sucrose	8
Defatted soybean meal	36
Refined soybean oil	1.5
Soybean sterol	0.2
Salt mixture	3
Cellulose powder	15
Agar	7.5
Ascorbic acid	1
Citric acid	4
(Total)	(108.7)
Vitamin B mixture	added
Antiseptic (sorbic acid, propionic acid)	added
Antibiotic	added
Water	2.57 ml/g diet

(Modified from the report by Horie et al.)[15]

from those established for the rearing on fresh mulberry leaves. First, slightly high temperature, such as 28 or 29°C, being higher by 1–2°C than the case of mulberry-rearing, is required for a period from the 1st to the 3rd instars. At a high temperature larvae gain more weight than at a low temperature in a shorter period. Rearing temperature should be higher also in the 4th instar than the case of mulberry-rearing, but must be kept low, such as at 23 or 24°C, in the 5th instar, similar to the case of mulberry-rearing.

Somewhat high humidity in the rearing room is required in order to prevent the drying of diet. A slight air current, such as below 0.1 m per sec., has little effect on the rearing results, although the humidity in the rearing room relates mutually with the air current as for the dryness of the diet.

Although the effect of light on larvae has been rather less stressed in the mulberry-rearing, characteristic effects of photoperiodism have been reported for the rearing with artificial diets. Under the light condition, the larval period is prolonged, and larvae gain more weight. However, larvae tend to move to the dim rays of light, and escape from

light, therefore they finally move around on the rearing bed. This is very inconvenient to handle larvae in mass rearing. Some people recommend 6-hr light and 18-hr dark rhythm, while others a 24-hr darkness. Further study is necessary on the photoperiodism of the silkworm on artificial diets. Light and dark rhythms during larval period also affect the moltinism and voltinism.

2) Preparation of diets

At present a few companies are permitted to prepare and to sell artificial diets for the practical rearing under the government clearance in Japan. Some farmers purchase the prepared diets, while the others purchase the dried or pre-cooked diet from the company. In the latter case they prepare the diets by themselves in the diet-preparation center, which is built in the local area by getting the financial help. In the center various apparatus for diet preparation are equipped. Usually, newly prepared diet in the diet-preparation center, which is still hot and soft, is packel in a vinyl bag, then the bag is sealed and stored in a dark, cool room until needed.

3) Rearing in cooperative houses

In the practical mass rearing it is necessary to keep the inside of the rearing room highly clean. In the cooperative rearing house the air conditioner equipped with air filter is installed. Special caution is necessary not to bring the microbials and viruses into the rearing room from outside. Windowless rearing room is also recommended. These rearing system is already known by the name of "clean-rearing system". In the rearing house the automatic rearing machine and feeding machine are installed. In a large scale they can rear 200 million larvae or more at a time.

A few years ago the Ministry of Agriculture, Forestry and Fisheries of this country has formulated a policy to introduce the artificial diets for the practical rearing. At present, the application is confined to the rearing of younger larvae exclusively, that is, until the end of the 3rd instar at most. Under the administrative control, the rearing is carried out in the cooperative rearing houses, either newly-built or rebuilt, in the farm area, where the well-trained farmers or experts are engaged in the rearing or other sort of works. They do not need to pick up mulberry leaves any more, and have only to feed diets on every other day, by which they can save a lot of labor. So far the rearing results with artificial diets are reported to be satisfactory, comparable to those with fresh leaves. Half-grown larvae, that is, the 3rd- or 4th-instar larvae, are delivered to each farmer, where they are reared, in succession, on fresh mulberry leaves for the rest of larval period until cocoon-spinning.

The new method of rearing of the silkworm on artificial diets is a renovation technique, and its rapid expansion is expected in sericulture, even if its application is limited to only younger larvae. Both scientific and applied researches are now in progress systematically, including the establishment of the rearing system throughout the whole instars and the effective production of silkworm eggs.

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