

## Silkworm (*Bombyx mori*) Response to Differently Formulated Artificial Diets

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**Artificial diet (AD) has shown with different advantages over mulberry leaves (ML). Various types of such feed for silkworm have been developed and proposed. The aims of this study were possibility rearing whole instar of silkworm on AD and to find some economic formulation for rearing silkworm, compare to that ML. Eleven ADs (D<sub>1</sub> to D<sub>11</sub>) were prepared with different percentage of mulberry leaf powder, and other ingredients such as soybean meal, cellulose, potato starch, agar, wheat flour, rice bran, etc. Five formulated diets (D<sub>1</sub>, D<sub>2</sub>, D<sub>5</sub>, D<sub>10</sub> and D<sub>11</sub>) appeared to have similar response to that ML (D<sub>m</sub>). There were no significant differences for duration period between ADs, all of which recorded with higher duration than mulberry leaves. Compared to other diet used in the present study D<sub>1</sub> and D<sub>10</sub> showed better growth development and survival rate. Larvae fed D<sub>1</sub>, D<sub>2</sub>, D<sub>5</sub>, D<sub>10</sub> and D<sub>11</sub> grew faster and produced heavier and more useful cocoons.**

**Key words:** Silkworm, *Bombyx mori*, Artificial diet

### Introduction

The silkworm, *Bombyx mori*, has long been considered as a monophagous insect with mulberry leaves as a known feed for along time. Since some limitation may be affected the growing of mulberry leaves, therefore artificial diet has been proposed as an alternative for silkworm.

Study on the artificial diets for silkworm began in 1960s and Fukuda in 1953 first reared the silkworm from egg to moth on an artificial diet successfully. However the compo-

sition of diets used at that time was rather simple, due to lack of information on the nutrients requirements. As a result larvae growth and development on these diets were retarded, small cocoons appeared, and the adult laid only a small number of eggs (Tazima, 1978; Djavanshir, 1995). Consequently, it has been found that the dietary composition influencing of blood sugar, proteinase and sucrase activity (Moon, 1973). There is considerable evidence that proteins are the basic biomolecules for constructing silkworm body. It has been shown that, there is a correlation between protein content (up to 50%) of artificial diets and growth, development, and reproduction of silkworm, as well as, cocoon weight and cocoon shell weight (Lee and Sohn, 1985; Yungen, 2000).

Lee and Sohn (1985) demonstrated that protein content affected duration of the larval period. The results of other studies indicated that body weight gain, cocoon quality and feed efficiency of the 5th instar larvae were significantly affected by the level of protein and carbohydrate (Moon, 1974) cellulose (Moon, 1977; Tazima, 1978) and water (Yungen, 2000).

Since 1970, several improvement have been obtained with artificial diet. At present time there is little difficulty of rearing the silkworm on artificial diet in obtaining cocoon of good quality (Shinbo and Yanagawa, 1994; Hori, 1995). Artificial diets in sericulture are reducing the labor, pathological risk and enable silkworm rearing in all seasons. In addition, when rearing on artificial diet, the whole instar will greatly contribute to the safety production of silkworm eggs from the point of pebrine elimination (Hori, 1995). The aim of present study was to investigate the extent to which artificial diet could be replaced with mulberry leaf and its effects on the silkworm performances.

### Materials and Methods

Two hundred of silkworm larvae, *Bombyx mori*, hybrid

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**Table 1.** Ingredients of different artificial diet used for silkworm rearing (%)

| Ingredient       | D <sub>1</sub> | D <sub>2</sub> | D <sub>3</sub> | D <sub>4</sub> | D <sub>5</sub> | D <sub>6</sub> | D <sub>7</sub> | D <sub>8</sub> | D <sub>9</sub> | D <sub>10</sub> | D <sub>11</sub> |
|------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|
| Mulberry leave   | 40             | 45             | 35             | 25             | 25             | 20             | 10             | 5              | 0              | 25              | 20              |
| Soybean meal     | 15             | 10             | 15             | 23             | 20             | 25             | 30             | 32             | 30             | 33.2            | 15              |
| Cellulose        | 7              | 12             | 5              | 0              | 0              | 6.4            | 5              | 3              | 7              | 10              | 20              |
| Sacarose         | 10             | 10             | 15             | 20             | 14             | 15             | 15             | 19.4           | 15             | 5               | 10              |
| Potato starch    | 15             | 10             | 12             | 10             | 9              | 13             | 15             | 10             | 14.4           | 10              | 20              |
| Agar             | 4              | 0              | 0              | 2              | 5              | 2              | 2              | 5              | 7              | 4               | 0               |
| Wheat            | 0              | 4              | 2              | 0              | 0              | 5              | 0              | 8              | 5              | 6               | 4.7             |
| Inositol         | 0.2            | 0.2            | 0.2            | 0.2            | 0.5            | 0.5            | 0.5            | 0.5            | 0.5            | 0.2             | 10.5            |
| Salt mix         | 3              | 3              | 4              | 4              | 5              | 3              | 4              | 4              | 5              | 2               | 1               |
| Organic acid mix | 3.75           | 3.6            | 3.6            | 3.6            | 2.9            | 3              | 3.5            | 3              | 3              | 2               | 1.2             |
| Vitamin mix      | 2.05           | 2.2            | 2.2            | 2.2            | 2.2            | 1.7            | 2.6            | 3.1            | 3.1            | 2.6             | 2.6             |
| Corn             | 0              | 0              | 0              | 0              | 0              | 0              | 5              | 7              | 10             | 0               | 0               |
| Rice bran        | 0              | 0              | 6              | 10             | 10             | 5.4            | 7.4            | 0              | 0              | 0               | 5               |

polyphagous were supplied by local sericultural company, and grouped into 11 treatment. The larvae were placed on rearing tray and offered one of 11 artificial diet (D<sub>1</sub> to D<sub>11</sub>) as well as fresh mulberry leaves (D<sub>m</sub>). Table 1 shows the composition of differdiets diets. Aritificial diets making-up, rearing methods and rearing condition were according to Yungen(2000). Briefly, powderd raw material of artificial diet were prepared using mulberry leaves, cellulose powder, minerals mix, vitamins mix, water and two different aggregators as agar agar and pectin. Dough were prepared with proper water and was heated with steam for 45 – 50 min. The pieces of feed were then cut to convenient size and shape for each instar. Subsequently, 100 newly ecdysed 5th instar larval were separated for measuring body. weight,survival rate during the larvae period and economic characteristics of cocoons.

## Results and Discussion

### Attracting factors

When rearing silkworm on an artificial diet, attractant material in diets which seems to appeared as smell and taste stimulators, are considered as two important factors. Attractive and feeding response of newly hatched silkworm larvae to the diets were documented approximately 4 – 5 hrs. Table 2 summarized the feeding respond of lar-

vae to diets containing different percentage of mulberry leaf. The result of this study clearly showed that excluding of mulberry leaf powder in one treatment lead to failed larvae attraction which was similar to results obtained in other investigations(Asaoka, 2000; Cui *et al.*, 2001).

As Tabel 2 shows, 5 out of 11 formulated diets (*i.e.*, D<sub>1</sub>, D<sub>2</sub>, D<sub>5</sub>, D<sub>10</sub> and D<sub>11</sub>) appeared to have relatively similar response to mulberry feed. Other diets showed either less positive or negative response. It seems highly likely that decreasing of mulberry leaf in diets assist in such a negative attraction. These results suggests that feeding stimulants and deterrents are two most important factors of aritificial diets for silkworm as it supported by other study (Asaoka, 2000). As it appeared in present study with 4 formulated diets (namely D<sub>6</sub>, D<sub>7</sub>, D<sub>8</sub> and D<sub>9</sub>) a minimum amount as 25% mulberry leaf is necessary for larvae positive attraction, though at the same time the presence of repellents should also be considerd (Homamoura, 2000).

### Larvae biting

Physical nature of the diet such as, hardness and softness also have a large influence on larvae feed intake. Low water cause the diet to dry and too much water will produced very soft dough with no suitable physical shap. Agar and carragheean are two materials which are usally used for such a purpose to improve the physical properties by solidification and to keep diets with optimal moisture

**Table 2.** Feeding respons of larvae to different diets

| Diet             | D <sub>1</sub> | D <sub>2</sub> | D <sub>3</sub> | D <sub>4</sub> | D <sub>5</sub> | D <sub>6</sub> | D <sub>7</sub> | D <sub>8</sub> | D <sub>9</sub> | D <sub>10</sub> | D <sub>11</sub> | D <sub>m</sub> |
|------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|----------------|
| Feeding Response | +              | +              | ±              | ±              | +              | -              | -              | -              | -              | +               | +               | +              |

+ : accepted, - : do not accepted, ± : partialy accepted

content. Agar in diets may varies according to varying dietary compositions though, 5% agar is added as gelling agent to keep water content at a constant percent as 70% – 75% (Sinbo and Yanagawa, 1994; Hori, 1995; Yungen, 2000).

In this study pectin was also used as a substance with gelling characteristics, to see extend to which this natural product could be replaced with agar. In fact, the main reason for such substitution was the benefits which pectin may offer over other elasticviscous Jellification ingredient. It was observed that compared to agar, pectin based diets (D<sub>2</sub> and D<sub>11</sub>) led to no significant differences in either feed texture or larvea responos (Table 2). This result suggest that pectin could be replaced by agar in silkworm artificial diet without adversely affecting performance.

### Duration of the larvae period

It has been established in silkworm that protein content of diet affect larvae duration period (Lee and Sohn 1985). In this study, time from the instant of hatched larvae, till end of molting was recorded and its average for each group considered as the duration of the larvae period. The results showed that duration period for D<sub>1</sub>, D<sub>2</sub>, D<sub>5</sub>, D<sub>10</sub>, D<sub>11</sub> and mulberry leaves (D<sub>m</sub>) were 31.3, 31, 33.9, 32.5, 33.4 and 20.2 days respectively, which indicate to considerable

reduction in larvae duration periods reared with mulberry leaves, though no significant differences were obserred between various artificial diets (Table 3).

### Larvae survival, growth and development rate

The material particle shape, size, mulberry leaf powder content, soybean meal, corn, and water content are considered as important in silkworm artificial diets. These factors in turn affected survival, growth and development rate of larvae (Sinbo and Yanagawa, 1994; Hori, 1995; Yungen, 2000).

In present study, survival and growth rate of larvae reared with different artificial diets and mulberry leaves from early 5th instar to end of molting were documented and are summericed in Table 4. Whitin the diets used in the present study, D<sub>1</sub> and D<sub>10</sub> showed better growth development and survival rate, however, fresh mulberry leaves (D<sub>m</sub>) still produced higher uniformity, growth and survival ratio, which confirm results obtained by others (Sinbo and Yanagawa, 1994; Hori, 1995; Yungen, 2000).

### Some economic characters of cocoon

Cocoon with complete spinning were prepared using mature larvae. To do this larvae were selected and transferd to molting place at 25°C. Eight days after spinning,

**Table 3.** Effects of different artifical diets and mulberry leave on the larvae duration period time\*

| Treatment       | 1st instar (hr) | 2nd instar (hr) | 3rd instar (hr) | 4th instar (hr) | 5th instar (hr) | Spining (hr) | Whole <sup>1</sup> (days) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------------|---------------------------|
| D <sub>m</sub>  | 78              | 51              | 81              | 111             | 144             | 20           | 20.2 <sup>A</sup>         |
| D <sub>1</sub>  | 144             | 80              | 110             | 130             | 168             | 120          | 31.3 <sup>B</sup>         |
| D <sub>2</sub>  | 148             | 90              | 120             | 144             | 170             | 72           | 31 <sup>B</sup>           |
| D <sub>5</sub>  | 144             | 80              | 110             | 156             | 180             | 144          | 33.9 <sup>B</sup>         |
| D <sub>10</sub> | 148             | 80              | 110             | 120             | 180             | 132          | 32.5 <sup>B</sup>         |
| D <sub>11</sub> | 148             | 80              | 110             | 145             | 180             | 144          | 33.4 <sup>B</sup>         |

\*Means within a column with no comman superscript are significantly different.

<sup>1</sup>Whole larvae duration period (days)

**Table 4.** Effects of different artificial diet on larvae weigh, development, uniformity, and survival rate

| Treatment       | Weigh of larvae in the 5th instar (g/larva) |            | Survival rate (%) |       | Uniformity   |              |
|-----------------|---|------------|-------------------|-------|--------------|--------------|
|                 | Early instar                                | End instar | Larvae            | Pupae | Young larvae | Grown larvae |
| D <sub>m</sub>  | 1.1   | 4.6        | 100               | 100   | +            | +            |
| D <sub>1</sub>  | 0.95  | 4.6        | 76.9              | 82.2  | -            | +            |
| D <sub>2</sub>  | 0.93  | 4.5        | 69.3              | 55.6  | +            | +            |
| D <sub>5</sub>  | 0.55  | 3.9        | 65.5              | 73.7  | +            | -            |
| D <sub>10</sub> | 0.71  | 4.1        | 93.7              | 89.9  | +            | +            |
| D <sub>11</sub> | 0.55  | 3.9        | 81.5              | 71.2  | -            | +            |

+ : Uniform, - : not uniform

**Table 5.** Means\* cocoon weight, layer weight and cocoon shell produced by silkworm reared on different artificial diet<sup>1</sup> and mulberry leaves (D<sub>m</sub>)

| Diets           | Cocoon weight (g)  | Weight of layer (g) | Cocoon shell (%)   |
|-----------------|--------------------|---------------------|--------------------|
| D <sub>m</sub>  | 55.23 <sup>A</sup> | 11.27 <sup>A</sup>  | 20.79 <sup>A</sup> |
| D <sub>1</sub>  | 26.90 <sup>B</sup> | 3.80 <sup>B</sup>   | 14.01 <sup>C</sup> |
| D <sub>5</sub>  | 8.32 <sup>C</sup>  | 1.098 <sup>C</sup>  | 13.42 <sup>C</sup> |
| D <sub>10</sub> | 24.10 <sup>B</sup> | 4.05 <sup>B</sup>   | 16.70 <sup>B</sup> |
| D <sub>11</sub> | 12.09 <sup>C</sup> | 1.68 <sup>C</sup>   | 13.75 <sup>C</sup> |

<sup>1</sup>No sufficient cocoon was obtained with D<sub>2</sub>.

\*Means within a column with no common superscript are significantly different.

some items were measured as followed. 1-Cocoon weight as separate sex in four classes: good, average, double and bad. 2-Cocoon layer weight as separate sex in four classes as good, average, double and bad. 3-Cocoon shell percent. This item was calculated using equation as : cocoon shell percent (%) = Cocoon shell weight/Cocoon weight. Table 5 summarized results obtained with different treatments.

According to the results, larvae reared on diets D<sub>1</sub>, D<sub>2</sub>, D<sub>5</sub>, D<sub>10</sub> and D<sub>11</sub> appeared with faster grow and heavier cocoon. It was observed that sex has no significant effect on cocoon characters, which is not in good agreement with that reported by Cui *et al.* (2001). In conclusion, these results clearly demonstrated that, silkworm rearing on artificial diet may offer some advantages, though economically sound approach still need more studies.

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