The effect of feeding various species of mulberry (Morus spp.) on the growth of silkworm and quality of cocoon hybrid BS 09

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Abstract. Hybrid silkworm varieties, mulberry species, environmental factors, and rearing factors are essential for the cocoons' quality. The species of silkworm feeding is a key factor in the sericulture industry, so the suitability of mulberry species for each silkworm hybrid species needs to be known. This study aimed to determine the effect of mulberry species on silkworm growth and quality of cocoons BS 09. The research method used was a randomized block design with four species of mulberry: Morus bombycis var Lembang, M. cathayana, M. multicaulis, and M. alba var Kanva 2 and one control species (mixed mulberry species). The research was carried out at Dramaga Research Forest, Bogor Regency. The determined parameters were the percentage of rearing, larva weight, cocoon weight, cocoon shell weight, and percentage of cocoon shell. The experiment results showed that the percentage of rearing was 97.5%-100% for all species of mulberry. The best result of the cocoon weight was feed treatment of M. multicaulis (1.89 g), but it was not significant with feed treatment of M. bombycis var Lembang or M. cathayana or M. alba var Kanva 2. M. cathayana feed treatments give the best results for the cocoon shell weight (0.44 gram) and percentage of cocoon shell (22.82%).

1. Introduction
Silk has always been a symbol of luxury and expensive. This causes the demand for national silk threads to increase continuously, while the raw material products, namely cocoons and silk threads, decrease. The gap between the increasing demand for silk and the reduced supply of raw materials is a factor that causes sericulture activities in Indonesia to possess the prospect of being developed.

The sericulture activities are developed in Indonesia territory because of its agro-climatic conditions that support the cultivation of the mulberry and silkworm Bombyx mori L. It can absorb household-scale labor because men, women, and children can carry out the activities. In some areas, silk is a culture, especially during traditional events [1, 2]. For example, in South Sulawesi Province, silk cloth is a tradition in people's daily activities. Meanwhile, for some circles, the use of silk cloth can increase the "prestige" of the wearer so that consumers are relatively large even though the price is high.

There are two activities in sericulture activities, namely mulberry cultivation and rearing of the silkworm. Bombyx mori L silkworm eats on the leaves of Morus spp. only. The reason the theoretical genetic base for Bombyx mori L silkworms eats only mulberry leaves is still unknown. However, [3] have identified that the GR66 gene is the main factor causing Bombyx mori L silkworms to eat only mulberry leaves.

Furthermore, this mutation in GR66 can reduce the ability to eat mulberry leaves and the possibility of eating other species of plants. Research on the eating preferences of bombyx mori apart from mulberry
leaves are useful because, in some areas which are not suitable for mulberry plant growth, the suboptimal production of mulberry leaves is an obstacle in silkworm maintenance activities. In addition, other feed species can make silkworm maintenance continue because they do not have to wait for the pile of feed at the farmer, whose garden area is usually small.

According to [4], several factors that contribute to the success of cocoon harvest are feed (38.20%), climate (37.00%), silkworm cultivation techniques (9.30%), and hybrid species of silkworm (4.20%). The quality, quantity, and species of mulberry will affect the growth of caterpillars, cocoon production, and silk thread. Mulberry belongs to the Morus genus of the Moraceae family, which includes Morus nigra, M. multicaulis, M. australis, M. alba, M. alba var macrophylla and M. bombycis [5]. According to [6], mulberry plants do not need difficult plant growing requirements, and they can grow on all types of soil as long as there is sufficient water in the soil. The species of mulberry cultivated by silk farmers in Indonesia are conventional mulberry species. These conventional species of mulberry are the species of mulberry that are commonly kept by farmers and are of good quality, including M. cathayana, M. alba, M. multicaulis, and M. alba var Kanva 2.

Besides the quality of mulberry, the silkworm species also affect the growth of the larvae, the productivity of cocoons, also the quality of cocoons and threads. BS 09 is a hybrid of sericulture research results at the Forest Research and Development Center. The production of BS 09 hybrid seeds has also been collaborated with Perum Perhutani to become a commercial hybrid so that farmers have an alternative to silkworm seeds besides the C 301 hybrid produced by Perum Perhutani. The advantages of hybrid BS 09 are high hatchability (more than 90%), cocoon shell percentage 21-23%, filament length 1000-1200 m, filament percentage 16-19%, and filament thickness 3-3.3 denier [7].

The interaction between silkworm hybrid species and species of feeding and other factors, namely the conditions of maintenance and the skills of the silk farmers, will determine the productivity and quality of the produced cocoons and threads. Because the species of silkworm feeding is a key factor in the sericulture industry (38.20%), mulberry species’ suitability for each silkworm hybrid species needs to be known. Furthermore, the species of mulberry for silkworm feed must be selected with good quality so that the superior silkworm hybrid produces an optimal cocoon quality. Therefore, this study aimed to determine the effect of mulberry species as feed for BS 09 hybrid silkworms on the growth of larvae and the quality of cocoons.

2. Materials and Methods

2.1. Location and time of research
The research was conducted in Dramaga Research Forest, Bogor, during the silkworm rearing period from February to March 2012.

2.2. Materials and tools
The materials used in this study were mulberry leaves, two months old pruned from four varieties of M. alba var Kanva 2, M. cathayana, M. multicaulis, M. bombycis var Lembang, and silkworm hybrid of BS 09 4th instar until cocoon harvest. Instar is the stage of larva development characterized by a "sleep" phase and molting. For example, Bombyx mori L larvae have five instars before turning into cocoons.

The auxiliary equipment used were maintenance equipment, seriframe, tools for monitoring and reporting larvae and cocoons’ growth, analytical scales, cutters, rulers and calipers, books, and stationery.

2.3. Research procedures

2.3.1. Field preparation. Intensive maintenance of mulberry plants used in research for optimal production included weeding, hoeing, pruning, controlling mulberry pests and diseases, intensive fertilization, and protecting plants from drought/flooding. The mulberry leaves used are mulberry plants that have been pruned at two months old, according to the needs of the fourth and fifth instar larvae.
2.3.2. *Taking the mulberry and feeding the silkworm.* Each species of mulberry leaves from 2-month old plants, stored in plastic bags to keep leaf moisture. The number of leaves harvested was adjusted to the needs of the larvae per day. For example, the experience of silkworm egg producers in Perum Perhutani Candirototo, the average feed requirement for silkworms in the 4th instar was 0.14 g head⁻¹ day⁻¹, and in the 5th instar, it required an average of 4 g head⁻¹ day⁻¹ [8]. Thus, feeding was done three times a day, just the leaves without the stems.

2.3.3. *Rearing of the silkworm.* The silkworm cultivation (*Bombyx mori* L.) begins after the eggs hatch until they become cocoons. Before silkworm cultivation, the room and equipment were disinfected using a solution of formalin, chlorine, or Neo P.P.S (through fumigation) [4].

Silkworms are cleaned from food scraps and dirt every day and changed to rearing mats. The function of mats training is to reduce pests and diseases and to provide a place for maintenance. The larvae sleep for three days during the development of the fourth silkworm instar to the 5th instar. The larvae on the 5th instar were given a 5% chlorine mixture as a disinfectant. At the end of the 5th instar, the larvae were ready to be transferred to the seriframe and will be cocooned for 6-7 days. Cocoon harvesting was done manually from seriframe.

2.4. *Research design*

This study used a randomized block design with five treatments (four species of mulberry and one control for mixed mulberry), and each treatment was repeated four times. Each replicate consisted of 100 larvae. The position of the silkworm's mats was grouped per larvae rack.

2.5. *Parameters of research*

The parameter of silkworm growth is the percentage of cocoon yield and larva weight of the fifth instar on the seventh day. The percentage of cocoon yield is the number of cocoons divided by the number of initial larvae on the 4th instar, multiplied by 100%.

Parameters of cocoon quality are cocoon weight, cocoon shell weight, and cocoon shell proportion. Measurement of cocoon weight (in g) was done by weighing the cocoon. Measuring the weight of the cocoon shell (in grams) was done by weighing the cocoon without the pupa. The percentage of cocoon shell (%) is the ratio between the cocoon shell's weight and the cocoon's weight.

2.6. *Data analysis*

Diversity analysis was carried out using the F test to determine the effect of feeding various species of mulberry. If the results had a significant effect, the mean difference test was carried out using the Least Significant Different Fischer's (LSD).

3. *Results and Discussion*

Cocoon harvest was done on the 6th or seventh day after a larva started making a cocoon. The characteristics of ready-to-harvest cocoons are that the cocoon shell is hard. Therefore, harvesting was done manually, taking one by one from the seriframe. The following process is to clean the fibers at the cocoons' skin used by the larva to attach to the seriframe. Then, in production activities, clean cocoons are ready to be dried or spun directly into yarn. The results of observations of larvae and cocoons in this study are presented in Table 1.
Table 1. Growth of silkworm and quality of cocoon hybrid BS 09 fed using various species of mulberry.

| Treatments                      | Growth of silkworm | Quality of cocoon |
|---------------------------------|--------------------|-------------------|
|                                 | Cocoon yield       | Larva weight      | Cocon Weight | Shell Weight | Percentage of Cocoon Shells |
|                                 | percentage (%)     | (g)               | (g)          | (g)          | (%)                       |
| *M. bombycis* var Lembang       | 98.50\( ^a \)     | 1.78\( ^a \)     | 1.83\( ^a \) | 0.44\( ^a \) | 23.80\( ^a \)          |
| *M. cathayana*                  | 99.50\( ^a \)     | 1.73\( ^b \)     | 1.76\( ^a \) | 0.44\( ^a \) | 24.82\( ^a \)          |
| *M. multicaulis*                | 100.00\( ^a \)    | 1.70\( ^b \)     | 1.89\( ^a \) | 0.42\( ^a \) | 22.29\( ^b \)          |
| *M. alba* var Kanva 2           | 100.00\( ^a \)    | 1.83\( ^a \)     | 1.84\( ^a \) | 0.43\( ^a \) | 23.10\( ^a \)          |
| Mixed mulberry                  | 97.50\( ^a \)     | 1.90\( ^a \)     | 1.69\( ^b \) | 0.38\( ^b \) | 22.63\( ^b \)          |

Remarks: Values in a column followed by the same letter indicate no difference at the Least test 1% Significant Different Fischer's (LSD).

Table 1 shows that the species of mulberry has a significant effect on larva weight and quality of cocoon includes the cocoon and the shell weight, as well as percentage of cocoon shell. The species, quality, and quantity of *Bombyx mori* L. silkworm feed are very important factors in the rearing of silkworms. The quality of nutrients in mulberry leaves will affect the growth and development of larvae, affecting the quality of the cocoons [9]. The results of the proximate test conducted by [10] on four species of mulberry plants, according to the species of mulberry used in this study, are presented in Table 2.

Table 2. Results of the proximate analysis of *M. multicaulis, M. cathayana, M. alba* var Kanva 2 and *M bombycis* var Lembang.

| Composition of chemical compounds (%) | *M. multicaulis* | *M. cathayana* | *M. alba* var Kanva 2 | *M. bombycis* var Lembang |
|---------------------------------------|-----------------|----------------|-----------------------|---------------------------|
| Water                                 | 72.67           | 71.88          | 72.45                 | 73.10                     |
| Protein                               | 20.92           | 24.56          | 21.26                 | 22.25                     |
| Fat                                   | 3.51            | 4.00           | 4.46                  | 4.46                      |
| Fiber                                 | 9.77            | 7.90           | 8.22                  | 9.18                      |
| Ash                                   | 7.52            | 8.71           | 6.11                  | 8.34                      |
| Calcium                               | 1.55            | 1.75           | 1.50                  | 2.05                      |
| Phosphor                              | 0.22            | 0.21           | 0.16                  | 0.19                      |
| BetN                                  | 35.61           | 32.95          | 37.50                 | 32.67                     |
| Carbohydrate                          | 45.38           | 40.85          | 45.72                 | 41.85                     |

Source: [9]

The nutrient content in mulberry leaves will vary based on species of mulberry (genotypic characteristics), soil conditions (nutrients, biotic and abiotic components), environmental factors, plant age, how to harvest leaves, and plant cultivation [11, 9, 12]. This means that the success and profits of the sericulture business are closely related to the productivity and quality of mulberry leaves. In other studies, mulberry leaves that are attacked by pests and diseases will reduce the leaf productivity and affect the sericulture productivity, cocoon quality, and produced fiber from silkworm rearing [13].

3.1. Growth of silkworm
The observed growth of silkworms was the percentage of cocoon yield and larvae weight which were important factors related to silkworms’ health. The observation showed a significant difference between species of mulberry by larvae weight but not significantly different in cocoon yield percentage.

The growth and development of larvae were built by the climate during rearing, the species of mulberry leaves, and disease attacks [5]. A study that assessed the effect of mulberry species on the
quality of larvae, cocoon silk, and filaments indicated that in the same environmental conditions, the mulberry species has a high proportion on the rearing of silkworm [14].

3.1.1. Cocoon yield percentage. The cocoon yield percentage is an important parameter because a higher cocoon yield percentage means lower mortality and positively correlates with cocoon productivity. Cocoon yield percentage is the ratio of the number of the cocoon and the total number of larvae reared (initial fourth instar). The measurement of rearing percentage was conducted on each instar, starting from the initial fourth instar to the 5th instar on the 7th day (before cocooning).

The cocoon yield percentage was significantly different in all species of mulberry. However, all the mulberry species that were given provided adequate nutrition to the caterpillars and were relatively favorable because the cocoon yield percentage was more than 98%. This was an indication that the larvae were healthy so that the larvae mortality was under 3%, meaning that the mortality rate and failure of the rearing were small. The effect of feed on maintenance depends on how to offer feed based on the larva needs and instar stage of larvae [15]. For example, larvae at the 1st, 2nd, 3rd instars were fed with mulberry leaves aged ±30 days through thin slices, and larvae at the 4th, 5th instars were fed mulberry leaves aged 60-90 days with whole leaves [16].

3.1.2. Larva weights. Mixed mulberry species, *M. alba* var. Kanva 2 and *M bombycis* var Lembang gave a better yield of larvae than *M cathayana* and *M multicaulis*. The optimal growth of larvae will affect the cocoons' quality and produced fiber. Mixed feed also gave the best larvae weight due to variations in the compound content and taste of the feed, although on other parameters, mixed mulberry gave the lowest value. Feed with mixed varieties of leaves and frequency of feeding will affect the health of silkworms [12]. Changes feed in the species of mulberry for small larva (1st, 2nd and 3rd instar) and large larvae (4th and 5th instar) will affect larva growth, and feeding mixed mulberry at all instar will have a better effect than single mulberry species [17].

The growth of silkworm also takes advantage of the content of macronutrient and micronutrient compounds in mulberry leaves, for example, water, fiber, carbohydrates, fat, etc. *M. bombycis* var Lembang and *M. alba* var Kanva 2 gave better results than *M. cathayana* and *M. multicaulis* because they have better water, carbohydrate, and fat content although *M. cathayana* has a better protein content. Silkworms that eat mulberry with high protein, water, carbohydrate, and starch content, have better silkworm growth and a better proportion of leaf consumption [18, 19].

3.2. Quality of cocoons

The quality of the cocoons is influenced by the species of a hybrid silkworm, feed factors, and the rearing conditions. The quality of cocoons is an important parameter because it affects the productivity and economic value of cocoons. According to [20], cocoon weight, cocoon shell weight, and percentage of cocoon shell will vary according to the age and species of hybrid silkworms. Parameter of larva weight and several parameters of the economic value of cocoons, namely cocoon weight, cocoon shell weight, and percentage of cocoon shell, indicate that the rearing activities have been carried out properly and following objectives of the silkworms rearing [21, 22].

The results of the cocoon quality analysis showed that there were significant differences between the species of mulberry. In total, silkworm with feed mixed mulberry gave the values of cocoon weight, cocoon shell weight, and percentage of cocoon shell showed the lowest value compared to other feed species. On the other hand, the species of *M. bombycis* var Lembang, *M cathayana*, *M multicaulis*, and *M. alba* var Kanva 2 did not show significant differences in all parameters, except for the percentage of cocoon shell feed *M. multicaulis*.

Three parameters of cocoon quality will affect its economic value. Cocoon weight will have an effect because cocoons are usually sold wet or dry based on weight (shell with pupa). Meanwhile, the cocoon shell weight and percentage of cocoon shells will affect when the cocoons are spun. Cocoons with a higher percentage of cocoon shells are better than heavier cocoons but have thinner shells. A heavy (thick) cocoon shell will produce a longer thread.

Cocoon prices are usually set based on the weight of the cocoons (wet or dry). Based on the Indonesian National Standard (SNI), the requirements for the quality class of cocoons are presented in
Table 3. The application of Indonesian Nasional Standard (SNI)-based prices is still difficult because the maintenance conditions in silk farmers are still not intensive, and there are limited skills in the rearing of silkworms so that production and quality are still fluctuating and varying. The application of strict restrictions will reduce the interest of farmers in the rearing of the silkworm.

Table 3. SNI 01-5009.11-2002 about the requirement for the quality class of cocoons.

| Parameters                          | Class of Cocoon Quality |
|-------------------------------------|-------------------------|
| Weight of cocoon (gram)             | A           | B         | C         | D         |
|                                     | ≥2.0       | 1.7-1.9   | 1.3-1.69  | <1.3      |
| Percentage of shell cocoon (%)      | ≥23.0      | 20.0-22.9 | 17.0-19.9 | <17       |
| Percentage of deformed cocoon (%)   | ≤2.0       | 2.0-5.0   | 5.1-8     | >8.0      |

Source: [23]

3.2.1. Cocoon weight. The results of the variance analysis showed that the species of feed affected the cocoon weight. The cocoons’ weight (Table 1) among the four species of mulberry were not significantly different, with the best results being larvae fed on M. multicaulis, namely 1.89 g. The cocoon weight of the larvae at all treatment species of mulberry is <2 g, while the optimal cocoon weight is >2 g. Therefore, the cocoons’ weight has not met the standard for class A based on SNI 01-5009.11-2002 regarding the quality class of normal cocoons requirements.

In general, the cocoon weight in the 1.7-1.9 g range is relatively good, although not optimal value. The water and protein content in mulberry leaves, which varied in a range not much different from the four species of mulberry plants, were considered to increase the weight cocoons produced. [24] reported a study on the effect of mulberry leaves that inoculated with various microorganisms and biological fertilizers. It stated that there were indications of an increase in the results of the parameters of cocoon weight, cocoon shell weight, and percentage of cocoon shell on silkworms fed in the inoculated plant because their nutritional content was also increasing.

3.2.2. Shell cocoon weight. Cocoon shell weight is the weight of a fresh cocoon without pupa. It indicates that the fiber content produced. The results of the analysis of variance showed that the species of mulberry affected the cocoon shell weight. The cocoon shell weight of silkworm fed single feed four species of mulberry was not significantly different. However, the silkworm fed single feed four species of mulberry produced an average cocoon shell weight of 0.43 g, and silkworm eats mixed species of mulberry showed the lowest cocoon shell weight of 0.38 g.

The proximate content in mulberry leaves has a big effect on the formation of silkworm fibers. Other factors that affect the silk fiber content in cocoons are the silkworm species, the conditions for silkworm rearing, and the environmental conditions during the making of a cocooning process [25]. According to [5], generally, the weight of the silkworm cocoon shells is 0.32-0.55 g, and the pure line caterpillars are 0.3-0.4 g. This means that the results of silkworm rearing in this research have been optimal by implementing the maintenance according to the SOP and environmental conditions that support the silkworms to produce high-quality cocoons.

The cocoon shell weight is important because it will indicate how much silk fiber has been produced by silkworms. Sometimes, a visually large and heavy cocoon does not automatically mean that the cocoon shell is thick or contains much fiber because the pupa weight will affect the cocoon weight. The best value of cocoon weight of silkworm fed with M. multicaulis feed did not produce the best cocoon shell weight. Meanwhile, the weight of the female pupa will be heavier than the male pupa. This is because the nutrient reserves of male pupae for the formation of silk fiber are greater than the reserves of nutrients for reproduction [26].

3.2.3. Percentage of cocoon shell. The variance analysis for the percentage of cocoon shells showed that the silkworm-fed single feed M. cathayana, M. bombycis var Lembang, and M. alba var Kanva 2 were not significantly different. The M. cathayana species tended to show the best percentage of cocoon
shells, namely 24.82%. Three species of mulberry that produce the best percentage of cocoon shell contains good proximate compounds so that it affects to produce of silkworm fibers.

The 4th and 5th instar silkworms require a high protein feed to build silk fiber and silk fiber glands. Apart from macronutrients, the content of micronutrient compounds also affects the quality parameters of cocoons. [1] stated that mulberry leaves containing calcium, potassium, magnesium, and phosphorus will affect the value of cocoon shell percentage, fibroin content, and filament length. The percentage of cocoon shells can be used to reference the proportion of crude fiber (fibroin) from the cocoon to be produced. Fibroin, fine filaments produced by silkworms, is the main element in cocoons apart from sericin, which is an adhesive. The ratio of fibroin content to cocoon weight is 66.5-73.5%, and the ratio of sericin content is 26.5-33.5% [27, 28]. In general, cocoon weight has a positive correlation with the proportion of cocoon shells. The higher the cocoon weight produced, the greater the proportion of cocoon shells produced.

The percentage of cocoon shells is an important parameter because the higher the proportion of cocoon shells will affect yarn to be produced, which impacts the cocoons' economic value. The percentage of cocoon shells in silkworm treated fed with M. cathayana, M. bombycis var Lembang, and M. alba var Kanva 2 based on the SNI requirements for the Quality Class of Normal Cocoons is included in Grade A. Nevertheless, the quality class of a product is determined by the parameter with the lowest quality grade. In general, the cocoon yield in this study produced class B, except for the mixed treatment because the cocoon weight was 1.69 g.

4. Conclusion

M. cathayana, M. bombycis var Lembang, M. alba var Kanva 2, and M. multicaulis can be applied as feed for BS 09 hybrid silkworms and affect silkworm growth and cocoon quality. The growth of silkworm parameters has high values in all treatments. An important parameter of cocoon quality is the percentage of cocoon shells that are also high value, increasing from the standard proportion of BS 09 cocoon shells at officially published launching.

Species of feed for M. cathayana, M. bombycis var Lembang, and M. alba var Kanva 2 affected the parameters of cocoon weight, cocoon shell weight, and cocoon shell proportion. Mixed feed from four species of mulberry used in this study gave the best average weight of larva compared to other feed treatments, but they showed the lowest average in other parameters.

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Authors’ contribution

Lincah Andadari and Minarningsih were equally contributed as the main authors. Suwandi was the member contributor.