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Larvae, cocoon and post-cocoon characteristics of *bombyx mori L.* (lepidoptera: bombycidae) fed on mulberry leaves fortified with Kenyan royal elly

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ABSTRACT: Fourth instar *Bombyx mori* silkworm larvae were fed on mulberry leaves to which royal jelly had been added. The impact on the larval, cocoon, shell and pupal weight, shell ratio percentage, filament length and weight, and the number of breaks during reeling were examined. The results indicate that royal jelly-enhanced diet significantly increased larval, cocoon and pupal weights, but had no significant effect on shell weights and denier. Similarly filament length, weight and filament reeling breaks were significantly different between controls and royal jelly fed groups. The cocoon shell ratio percentage was significantly higher in the control compared to the royal jelly fed groups. Results established positive trends in all the values of different parameters observed in the experimental group against the control group, apart from the cocoon shell ratio percentage. Results imply that supplementing mulberry leaves with royal jelly has the potential to enhance the commercial qualities of silk and can be used in sericulture for yield improvement. @ JASEM

The *Bombyx mori* is essentially monophagous and survives solely on mulberry leaves (*morus* sp.) which play an important role in the nutrition of the silkworms, and in turn cocoon and silk production (Nagaraju, 2002). The nutritional elements of mulberry leaves determine the growth and development of the larvae and cocoon production (Seidavi et al., 2005). The quality of the leaves has a profound effect on the superiority of silk produced by the *B. mori*. In this regard, the production of good cocoon crop is totally dependent on the quality of leaves. Leaves of superior quality enhance the chances of good cocoon crop (Ravikumar, 1988). It has also been demonstrated that the dietary nutritional management has a direct influence on quality and quantity of silk production in *B. mori* (Murugan et al., 1998).

In recent years, many attempts have been made to improve the quality and quantity of silk (Hiware and Ambedkar 2005), through enhancing the leaves with nutrients, spraying with antibiotics, juvenile hormone, plant products, with JH-mimic principles or using extracts of plants. Mulberry leaves have been supplemented with various nutrients for silkworm feeding to promote silk quality and quantity. The supplementation and fortification of mulberry leaves is a recent technique in sericulture research (Murugan et. al., 1998). It has been reported that the vitamins of B-complex group and certain essential sugars, proteins, amino acids, minerals etc. are responsible for the proper growth and development of the silkworm, *B. mori* (Faruki, 1998). A number of researchers have worked on the effects of vitamin-enriched food on the reproduction of *B. mori* females (Faruki et al., 1992; Saha and Khan, 1999).

Royal jelly is rich in nutrients and energy. It contains 12-13% protein, 12-15% carbohydrates, and 5-6% beneficial lipids, B complex vitamins including high amount of fatty acid, 10-hydroxy-2-decanoic acid (10-HDA), which is involved in growth, regulation and immunity (Vitek, 1995). Studies have been carried out on the effect of royal jelly used in artificial diets on the growth, development, weight of cocoons and the number of eggs laid by the silkworm of *B. mori* (Saikatsu et al., 1989). Murugappan et.al., (1996) has studied effects of fortification of mulberry leaves with jaggery on *B. mori*.

Nutrition is an important growth-regulating factor in silkworm, and with this background, the study investigated the growth of silkworm larvae fed on mulberry leaves enhanced with royal jelly, as well as the cocoon and post cocoon characteristics.

MATERIALS AND METHODS

Experimental insect:

Disease free layings (dfls) of bivoltine ICIPE II silkworm strain for this study were obtained from the germplasm bank of the International Centre of Insect Physiology Ecology, Commercial Insects Programme (CIP), Nairobi, Kenya. Silkworms were reared under standard recommended conditions at 26 ± 2^0 C temperature, 75 ± 5 % relative humidity under 12:12 (light: dark) photoperiod. Kanva 2 variety mulberry leaves (*Morus alba)*, harvested from the CIP mulberry garden were fed to silkworms three times a day. After the third ecdysis, larvae were randomly divided into two groups and reared under similar environmental conditions.

Leaf treatment:

Royal jelly was obtained from *Apis mellifera scutellata* colonies. Larvae were grafted at the age of 24 hours. The royal jelly was harvested after 72 hours

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and freeze dried. The concentration of royal jelly used in the experiment was 0.5gms-dried powder per 20ml-distilled water. The entire leaves were dipped in this solution and dried by fanning. The treated leaves were then fed to the larvae of the experimental group from Day 1 of the fourth instar till spinning. The larvae of the control batch were simultaneously reared on fresh mulberry dipped in distilled water and dried by fanning.

Administration of Royal Jelly:
Freshly moulted fourth instar larvae were randomly divided into two groups of 50 larvae each and fed three times a day with 40gms of matured mulberry leaves in each feed. One group was kept as control and fed with plain mulberry leaves while the experimental group was given feed to which 20ml of royal jelly solution had been added. Each treatment had three replications of 50 larvae each. All the rearing operations were carried out according to routine procedures recommended by Hiware (2001).

Data collection:
The development of the 4th instar larvae was monitored for both groups. Larval weights, temperature and humidity were recorded. For spinning purposes mature larvae were transferred to mounting frames for cocoon building. After cocooning, cocoon, pupa, shell weight; shell ratio %, average filament length and weight were taken and recorded. The following formulae were used for cocoon shell ratio and silk filament length respectively.

- Cocoon Shell Ratio (SR%) = $\frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon weight (g)}}$ X 100

- Silk Filament Length (m) = Revolutions of epprouvette X Wheel Circumference (m)

Analysis of data
The differences in larval weights, cocoon weights, shell and pupal weights, shell ratio %, filament length and weight and reeling breaks between the control and royal jelly fed groups were compared using the Student t-test and the probability $\propto = 0.05$ was taken as the critical value for all tests.

RESULTS AND DISCUSSIONS

Royal jelly fed larvae had significantly larger weights 4 days after the initiation of royal jelly enhanced diet. On day 1 of the 4th instar stage; mean larval weight for both control and royal jelly fed group were not significantly different. However, mean larval weight differences between the control and royal jelly fed group became evident from day 4 of the 4th instar and these differences continued until spinning (Table I).
These results concur with those reported by Keizo et al., (1965), who observed that royal jelly increases moulting and larval weight. It contains acetylcholine, which is required in the diet of silkworm larvae for normal moulting to occur. In another study by Saikatsu et al., (1989), it was revealed that the growth and developmental speed of the silkworm were quickened by addition of royal jelly. The number of eggs increased markedly by the feeding of raw royal jelly but not of boiled royal jelly. The evaluation was based on the economic parameters including the larval weight, cocoon and shell weights, denier of filament and number of breakages during reeling. Ebeid, (1993) reported a decrease in larval duration in larvae fed on royal jelly and also an increase in oviposition capacity in adult females due to stimulation of ovary development.

Similarly, pupal and cocoon weights from the royal jelly-fed group were significantly higher compared to the controls (Table II). Research has demonstrated that royal jelly causes enhanced absorption of consumed food. Murat, (1957) recorded reduced quantity of chick droppings, indicating better assimilation of food. This factor may have caused better assimilation of food by the silkworms fed on mulberry leaves with royal jelly in this study, resulting in heavier pupae and cocoons. Allied studies cite that disparity in cocoon weight may be influenced by other factors. Aruga (1994) points out that the cocoon weight also varies with the sex of the silkworm. Quader et al., (1992) found out that nutritional value of mulberry leaves was directly reflected on the larval growth and cocoon characters of *B. mori* silkworm. The nutritive value of mulberry leaves depends on various factors of agro-climatic nature and any deficient quantities of nutrients from leaves affect silk synthesis by silkworms. In this study, mulberry leaves of the same variety were obtained from the same field. Consequently the difference in larval weights can only be attributed to the effect of royal jelly.

No significant differences were noted in shell weights for the control and royal jelly fed groups. However, the CSR% in the control group was significantly higher than that of the royal jelly fed group (Table II).

Cocoon and shell weights are the major traits evaluated for productivity in sericulture and have been used for more than half a century. Cocoon weight is an important commercial characteristic used to determine approximately the amount of raw silk that can be obtained. Shell weight gives a better

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measure, but cannot be determined in commercial cultures because it requires damaging the cocoon. The difference between the two measures is the weight of the pupa (Gaviria et al., 2006). Yungen and Junliang (1999) confirmed that use of high protein diet effectively increases the quality of cocoon shell. However in this study no significant differences were noted in shell weights. This could be attributed to the high pupal weights observed in the royal jelly fed group as opposed to low pupal weights in the control group. This observation resulted to the CSR% in the control group being significantly higher than that of the royal jelly fed group.

The filament obtained from cocoons produced by larvae fed on royal jelly was significantly longer than the filament obtained from the control group. Likewise, the mean filament weight obtained from the control group was less compared to that of the royal jelly group. The differences were statistically different. However, the silk denier from the control group was not significantly different to that of the royal jelly fed group. Significantly fewer reeling breaks were recorded in filament obtained from cocoons produced by royal jelly fed larvae compared to the breaks recorded for the filament from the control group (Table III).

Silkworms hatched from the same dfls were used in this study to minimize varietal differences in performance. The study discovered that significantly longer filaments were obtained from cocoons produced by larvae fed on royal jelly. This can be attributed to the significantly fewer reeling breaks recorded in filaments obtained from the same group. Increases in filament length and non-breakable filament length of silk produced are the most important commercial characters in the improvement of silk quality and yield (Kamimura, 1998).

In this study positive trends were observed in most values of different parameters except for the CSR% in the experimental group, when compared to the control group. The LW, CW, and PW were increased by 13.57%, 13.25% and 16.92% respectively. A minimal increase of 0.56% was noted in the SW; whereas FL and FW recorded a positive increase of 2.93% and 8.87% respectively. However a negative trend of 11.62% was recorded for the CSR%, an indication that reeling breaks in the experimental group were lower than in the control group.

Hiware (2006) results indicated positive results in larval and shell weights, and filament length upon feeding larvae on mulberry leaves treated with *Nux vomica*. Cocoon and pupal weights and average denier had negative results. In related studies administration of a JH analogue, Murakoshi et al. (1972) reported an enhanced yield of 20-35 % in cocoon and pupal weight. However, the increase was always accompanied by a prolonged feeding period. Muroga et al., (1975) was able to get only an enhancement of 9-14 % but without any change in the larval period. Trivedy et al., (1997) got considerable increase in the cocoon and shell weight on administration of minute quantities of a strong JH mimic. These reports make it clear that the response of silkworm in terms of improvement in economic traits varies with the compounds used, silkworm races and geographical region (Nair et al., 2003). Their study also infer increased cocoon shell weight is variably converted to the end product, the reelable silk filament.

In correlated studies on animals, royal jelly has been found to have a growth promoting effect as it increased the body weight in male and female mice fed on a diet enriched with royal jelly (Ebeid, 1993). Rabbits reacted to a normal diet supplemented with 100 – 200mg of royal jelly per kilogramme of body weight with increased fertility and embryonic development (Khattab et al., 1989). The Japanese quail reached sexual maturity sooner and laid more eggs after supplementation of diets with high doses (0.2g) of lypholized (freeze dried) royal jelly (Csuka et al., 1978). In related studies, other forms of life including higher animals showed remarkable gains in growth, weight and sexual activity after being fed or/and injected with royal jelly.

In conclusion it is noted that treatment of late age silkworm larvae with royal jelly elicits favourable response in improving the commercial qualities of silk fibre and can be used in sericulture for yield enhancement.

Table I: Mean Larval weights showing the effect of mulberry leaves fortified with royal jelly on *B. mori* silkworms.

Treatment	4^{th} Instar			5^{th} Instar		
	Day 1	Day 4	Day 7	Day 1	Day 3	Day 8
Control group	0.402±0.069a	0.545 0.022a	0.623±0.004a	0.700±0.113a	1.440±0.253a	3.63±0.537a
Royal jelly group	0.402±0.058a	0.619±0.058b	0.784±0.002b	0.806±0.165b	1.750±0.404b	3.80±0.475b

Means (± S.E) followed by same letters within the same column are not significantly different (t-test, $\propto = 0.05$).

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Table II: Effect of mulberry leaves fortified with royal jelly on various cocoon characteristics of *B. mori*.

Treatment	CW	PW	SW	CSR %
Control group	1.66±0.223a	1.30±0.211a	0.354±0.003a	21.5±2.82a
Royal jelly group	1.88±0.106b	1.52±0.105b	0.356±0.002a	19.0±1.39b

Means (±S.E) followed by same letters within the same column are not significantly different. (t-test, $\propto = 0.05$). CW: Cocoon Weight; PW: Pupal Weight; SW: Shell Weight; CSR%: Cocoon Shell Ratio percentage

Table III: Effect of mulberry leaves fortified with royal jelly on various filament characteristics of *B. mori*.

Treatment	FL	FW	D	RB
Control group	1056±32.1a	0.296±0.015a	2.67±0.031a	2.32±2.28a
Royal jelly group	1087±79.5b	0.322±0.024b	2.67±0.069a	1.57±1.99b

Means (±S.E) followed by same letters within the same column are not significantly different. (t-test, $\propto = 0.05$). FL: Filament Length; FW: Filament Weight; D: Denier; RB: Reeling Breaks

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