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Evaluation of Novel Tasar Silkworm Feed for *Antheraea mylitta*: It's Impact on Rearing, Cocoon Trait and Biomolecular Profile

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ABSTRACT

The *Antheraea mylitta* produces tasar silk having massive demand in international market. Its rearing is carried out in outdoor conditions and during course of I to III instar rearing, a major proportion (20-30%) of larvae die due to vagaries of nature, pests and predators etc. which considerably affects the production and productivity. The solution to this major bottleneck of the silkworm rearing lies in evolving a suitable tasar silkworm feed (semi-synthetic diet) for young age tasar silkworm. In present study, comparative evaluation was done among semi-synthetic diet fed; fresh leaf fed indoor reared and fresh leaf fed in natural outdoor reared insects. Data revealed that young age survival and Effective Rate of Rearing (ERR) were higher when larvae were brushed on semi-synthetic diet in contrast to indoor rearing on fresh leaf and complete outdoor rearing. Semi-synthetic diet fed larvae showed greater body weight and their cocoon showed higher weight, shell weight and shell ratio than controls. Concentration of hemolymph protein was slightly higher in semi-synthetic diet fed than outdoor reared larvae whereas, significantly lower in case of indoor reared. Hemolymph protein SDS-PAGE analysis indicates that, the semi-synthetic diet fed larvae is closer to complete outdoor reared larvae than indoor reared. Comparative assessment of rearing, cocoon trait and biomolecular profile of *A. mylitta* it is assumed that tasar feed developed by our laboratory is novel. It will be helpful in minimizing impact of unfavorable condition during I crop rearing and maintenance of precious eco-races stocks to enhance productivity of tasar silk.

Key words: *Antheraea mylitta*, cocoon, semi-synthetic diet, biomolecular and tasar silk

INTRODUCTION

The rearing of tasar silkworm is carried out in outdoor conditions where a major proportion (20-30%) of tasar silkworms die due to vagaries of nature, pest and predators etc. during course of young age (I to III instar) rearing (Kumar *et al*., 2010). The solution to this major bottleneck of the silkworm rearing lies in evolving a suitable semi-synthetic diet of tasar silkworm. The young age silkworms can be reared indoor on semi-synthetic diet and later transferred outdoor for carrying out late age rearing till spinning. This will save precious population of tasar silkworms and also help in increased production of tasar silkworm (Kumar *et al*., 2010). Recently several artificial diets has been developed for different insects with various combinations (Carpenter and Stephanie, 2002; Etebari and Matindoost, 2005; Kumar *et al*., 2010; Al-Ayedh, 2011; Savoie *et al*., 2011).

The effects of various kinds of dietary protein on growth of the silkworm, *Bombyx mori*, were determined using semi-synthetic diets. Larval growth was largely dependent on the dietary proteins

(Ito and Inokuchi, 1981; Horie and Watanabe, 1983). The amino acid patterns in the haemolymph were greatly changed according to supplementation. Larval growth rate is relatively independent of diet nitrogen; larval composition is highly dependent upon both nitrogen quantity and quality (Karowe and Martin, 1989). Characteristic profiles of hemolymph proteins during larval development of molting mutants of the silkworm, *Bombyx mori* has been also evaluated (Kawaguchi *et al*., 1993). Subsequently, it is also found that hemolymph protein content increased with increased levels of nitrogen in the supplemented diet of silkworm larvae, *Bombyx mori* (Hirayama *et al*., 1996). Protein of the haemolymph from fourth and fifth instar larvae of *Manduca sexta* reared diet were significantly higher than those for plant-reared larvae (Dahlman, 1969), The quantitative changes in haemolymph proteins from each physiological phase of the last three larval instars of the tobacco hornworm, *Manduca sexta*, means of disk electrophoresis. Total protein concentration increased from pharate third instar to late fifth instar larvae then decreased slightly in the pharate pupal stages. Li *et al*. (2006) used the proteomic approach to investigate the change in fifth instar hemolymph profile during growth and development and to improve the understanding of this important bioprocess and gene expression situation. The results implied that these proteins are related to biosynthesis of silk protein and metamorphosis preparation from larva to pupa. Wild silkmoth artificial diets were earlier developed for two *Antheraea* moths, *Antheraea yamamai* and *Antheraea pernyi* (Higuchi, 1990). Later, Akai *et al*. (1991) have been able to rear tasar silkworm *Antheraea mylitta* on artificial diet of oligidic nature. Two semi-synthetic diets, containing arjun and asan leaf powder were developed and found suitable for rearing of young age/chawki worms up to second moult of tasar silkworm (Daba BV) on a small scale. Uniform larval growth and higher larval weight was recorded in semi-synthetic diet fed larvae in comparison to control (fresh leaf). Diet can be preserved up to 90 days at 5°C. Feeding on semi-synthetic diet requires minimum handling of larvae during rearing. Labour cost is reduced (Kumar *et al*., 2008, 2010). Present study was conducted to evaluate performance of young age rearing on semi-synthetic diets fed, fresh leaf fed (indoor conditions up to 2nd instar) and fresh leaf fed in natural (*Terminalia arjuna* Bedd) outdoor condition.

MATERIALS AND METHODS

Freshly hatched 3200 first instar larvae were brushed separately on semi-synthetic diet, fresh leaves under indoor condition and complete outdoor on Asan plant, *Terminalia tomentosa* W and A. Larvae were reared on diets and fresh leaves up to 2nd instar and then transferred to Asan leaves in outdoor conditions (Kumar *et al*., 2008). Comparative evaluation of tasar silkworm feed fed (indoor conditions up to 2nd instar), fresh leaf fed (indoor conditions up to 2nd instar) and fresh leaf fed in natural (*Terminalia arjuna* Bedd.) outdoor condition was evaluated. The 3200 larvae (20 dfls) were utilized for each condition. Young age and mature larvae from each lot (diet fed, indoor and outdoor) were weighed daily (n = 30) on Electronic balance (Shimadzu, Japan).

After fourth moulting, the 0 day 5th instars (final instar) larvae from each three different bushes namely diet fed, indoor and outdoor were transferred to other bushes and early, middle and mature age larvae utilized for experimental purpose. Haemolymph from different stage/age groups of larvae was collected into pre-chilled Eppendorf tube containing 0.025% phenylthiourea. It was centrifuged for 5 min at 6000 rpm and supernatant was collected and stored at -80°C till utilize. Protein assay was done by using Bradford Method (Bradford, 1976). The SDS-PAGE was carried out according to procedure of Laemmli (1970). Its main components constitute of a 2.5% Stacking gel (pH-6.8) and a 10% resolving gel (pH-8.8). The electrode buffer used for running the

SDS-PAGE was prepared from tris-glycine with 0.1% SDS (pH-8.5). After electrophoresis the gel was separated out from the apparatus and was transferred into a gel box in coomassie blue staining solution for approximately 4 h to overnight and after de-staining gel photographs was taken. Alternatively gel was also visualized by using silver gel staining technique (Blum *et al*., 1987).

Data analysis: The data was subjected to the statistical analysis by using ANOVA with Microsoft Excel 2003.

RESULTS AND DISCUSSION

Data reveals that young age survival and Effective Rate of Rearing (ERR) were higher when larvae were brushed on semi-synthetic diet (survival 88.82% and ERR 46.95%) in contrast to indoor rearing on fresh leaf (survival 83.45% and ERR 42.81%) and complete outdoor rearing (survival 68.76% and ERR 36.56%) (Table 1). Maximum fresh body weight of the 3rd instar (day 0) was recorded in semi-synthetic diet (0.507±0.009 g) followed by 0.467±0.013 g in complete outdoor and minimum in indoor fresh leaf (0.397±0.012 g). In final instar, mature larvae (before spinning of cocoons) body weight were comparable in semi-synthetic diet and complete outdoor reared larvae and but higher than fresh leaf indoor condition (Table 1). The 3rd instar (day 0) and mature larvae (before spinning of cocoons) body weight were comparable in semi-synthetic diet and complete outdoor reared larvae in but higher than fresh leaf indoor condition (Table 1). Semi-synthetic diet fed larvae showed higher cocoon weight, shell weight and shell ratio (12.643±0.309 g, 1.659±0.064 g and 13.113±0.367%, respectively) than complete outdoor fed larvae (12.568±0.448 g, 1.429±0.057 g and 11.551±0.506%, respectively) whereas minimum when larvae were fed fresh leaf in indoor condition up to II stage (11.671±0.331 g, 1.364±0.051 g and 11.776±0.414%, respectively) (Table 2). Till III Instar day 1 haemolymph protein concentration was recorded significantly high in semi-synthetic diet fed larvae in comparison to fresh leaf fed larvae in indoor condition and leaf fed larvae in outdoor condition (Fig. 1). When larvae were transferred from indoor to outdoor and allowed them to feed on leaf till 5th instar, diet fed larvae given higher protein than indoor leaf fed larvae (Fig. 2). Protein concentration of hemolymph was slightly higher in semi-synthetic diet fed than outdoor reared larvae whereas, lower in case of indoor reared (Fig. 1 and 2). Haemolymph protein SDS-PAGE analysis showed the semi-synthetic diet fed larvae is closer to outdoor reared larvae profile than indoor reared larvae (Fig. 3a, b). The pattern of

Table 1: Rearing performances of young age larvae fed on diet, fresh leaf (indoor condition) and complete outdoor rearing

Particulars	No. of larvae brushed	Chawki survival (%) after II moult	Larval weight (g) after II moult	Larval weight (g) before gut purge	Effective rate of rearing (%)
Diet fed	3200	88.80	0.507±0.009	40.173±0.756	46.90
Fresh leaf fed (Indoor)	3200	83.40	0.397±0.012	36.365±1.054	42.80
Complete outdoor	3200	68.70	0.467±0.013	39.013±1.016	36.50
CD at 5%	-	-	0.038±0.000	3.180±0.000	-

Table 2: Cocoon traits of young age larvae fed on diet, fresh leaf (indoor condition) and complete outdoor rearing

Particulars	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
Diet fed	12.643±0.309	1.659±0.064	13.113±0.367
Indoor leaf fed	11.671±0.331	1.364±0.051	11.776±0.414
Complete outdoor	12.568±0.448	1.429±0.057	11.551±0.506
CD at 5%	1.228	0.193	1.448

Values are as Mean±SE

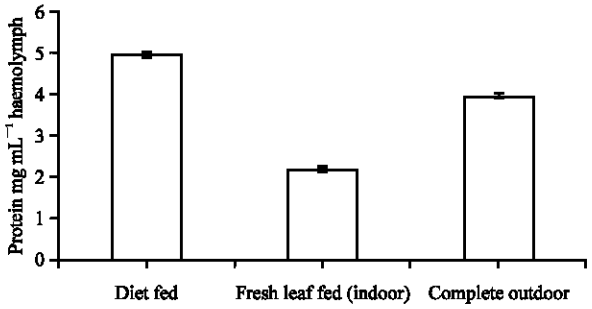

Fig. 1: Haemolymph protein of young age larvae fed on diet, fresh leaf (indoor condition) and complete outdoor rearing

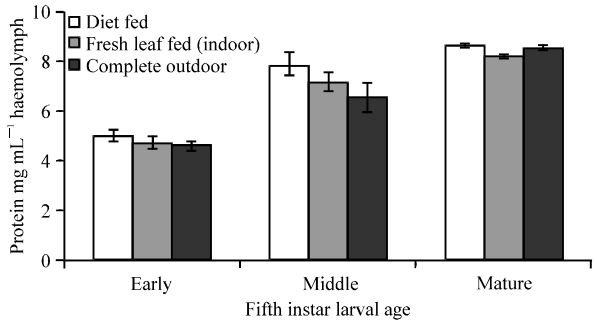

Fig. 2: Haemolymph protein of final instar of young age larvae fed on diet, fresh leaf (indoor condition) and complete outdoor rearing

protein band showed that semi-synthetic diet is comparatively more suitable than indoor leaf fed larvae (Fig. 3a, b). Based on comparative evaluation performance of rearing, cocoon trait and biomolecular profile of *A. mylitta* D it is concluded that tasar feed is novel (Fig. 4).

It is apparent that young age rearing on semi-synthetic diet gave better results and improvement in cocoon characteristics than the indoor rearing on fresh leaf up to II instar. It is also help full to avoid unfavorable condition during I crop rearing and maintenance of stock of precious ecoraces. Wild silkmoth artificial diets were earlier developed for two *Antheraea* moths, *Antheraea yamamai* and *Antheraea pernyi* (Higuchi, 1990). Later, Akai *et al.* (1991) have been able to rear tasar silkworm *Antheraea mylitta* on artificial diet of oligidic nature. In recent past several types of semi-synthetic diet has been developed for different insects (Carpenter and Stephanie, 2002; Etebari and Matindoost, 2005; Kumar *et al.*, 2010; Al-Ayedh, 2011; Savoie *et al.*, 2011) and utilized at small scale. Two semi-synthetic diets, containing Arjun and Asan leaf powder were developed and found suitable for rearing of young age/chawki worms up to second moult of tasar silkworm (Daba BV) on a small scale. Uniform larval growth and higher larval weight was recorded in semi-synthetic diet fed larvae in comparison to control (fresh leaf). Diet can be preserved up to 90 days at 5°C. Feeding on semi-synthetic diet requires minimum handling of larvae during rearing. Labour cost is reduced (Kumar *et al.*, 2008, 2010). The effects of various kinds of dietary protein on growth of the silkworm, *Bombyx mori*, were determined using semi-synthetic diets. Larval growth was largely dependent on the dietary proteins (Ito and Inokuchi 1981; Horie and Watanabe, 1983). The amino acid patterns in the haemolymph were greatly changed according to supplementation. Larval growth rate is relatively independent of diet nitrogen, larval composition

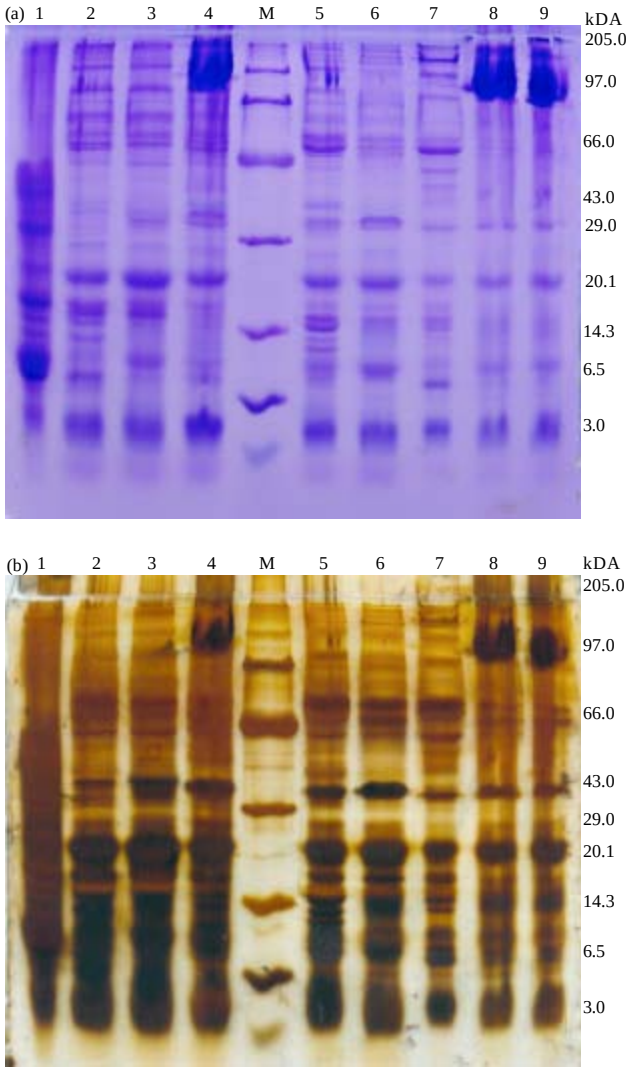

Fig. 3(a-b): SDS-PAGE analysis showing (a) Coomassie and (b) Silver stained gel of haemolymph protein of V instar larvae of *A. mylitta* fed on semi-synthetic diet, indoor and outdoor reared, respectively. Lane 1-3: Haemolymph protein profile of early V instar larvae, fed on semi synthetic diet, indoor leaf fed and outdoor leaf fed, respectively, Lane 4-6: Haemolymph protein profile of middle V instar larvae fed on semi synthetic diet, indoor leaf fed and outdoor leaf fed, respectively Lane 7-9: Haemolymph protein profile of mature V instar larvae fed on semi synthetic diet, indoor leaf fed and outdoor leaf fed, respectively

is highly dependent upon both nitrogen quantity and quality (Karowe and Martin, 1989). Characteristic profiles of hemolymph proteins during larval development of molting mutants of the silkworm, *Bombyx mori* has been also evaluated (Kawaguchi *et al*., 1993). Haemolymph protein content increased with increased levels of nitrogen in the supplemented diet of silkworm larvae,

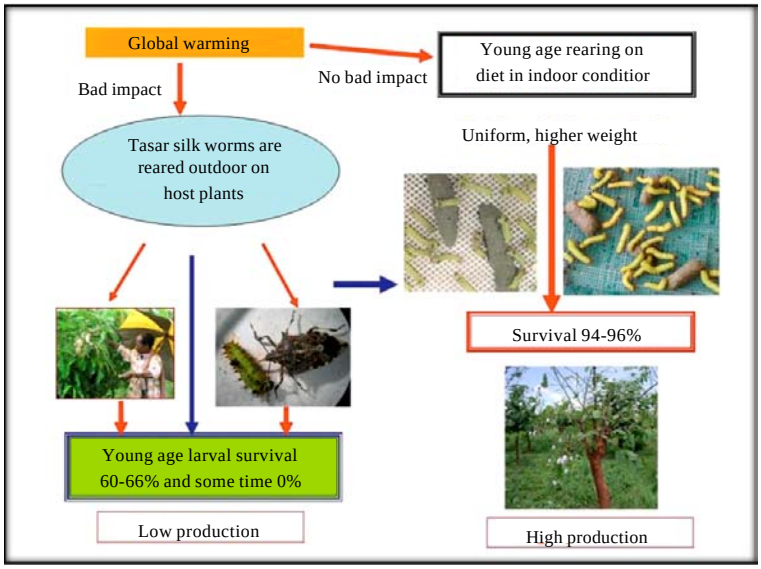

Fig. 4: Diagrammatic representation of rearing performance of *A. mylitta* larvae in outdoor condition on leaves and indoor condition on tasar silkworm feed (semi-synthetic diet)

Bombyx mori Hirayama *et al.* (1996). Protein of the haemolymph from fourth and fifth instar larvae of *Manduca sexta* reared diet were significantly higher than those for plant-reared larvae (Dahlman, 1969). The quantitative changes in haemolymph proteins from each physiological phase of the last three larval instars of the tobacco hornworm, *Manduca sexta*, means of disk electrophoresis. Total protein concentration increased from pharate third instar to late fifth instar larvae then decreased slightly in the pharate pupal stages. Sumida *et al.* (1995) reported that developmental changes in urea concentrations in the haemolymph of Daizo (T), an original strain of the silkworm, *Bombyx mori* reared on an artificial diet and on fresh mulberry leaves.

Li *et al.* (2006) used the proteomic approach to investigate the change in fifth instar hemolymph profile during growth and development and to improve the understanding of this important bioprocess and gene expression situation. It is reported that these proteins are related to biosynthesis of silk protein and metamorphosis preparation from larva to pupa (Li *et al.*, 2006). SDS-PAGE Protein profile of the semi-synthetic diet fed larvae is nearer to out door reared larvae in comparison to indoor reared larvae. It is expected that Tasar feed will be helpful in minimizing bad impact of unfavorable condition during I crop rearing of tasar silkworm. In addition, it will be also helpful in maintenance of stock of precious eco-races to increase production and productivity of tasar cocoons.

Present finding suggests that semi-synthetic diet is comparatively more suitable than indoor leaf fed larvae. We hypothesize that (1) In indoor condition on leaf larvae get to expose with repeated touch and handling by rearers. Since *A. mylitta* is a wild type insect hence that human interference might be affecting performance of the larvae which cause change in haemolymph protein profile and (2) The semi-synthetic diet several necessary micro-and macro nutrients are supplemented sufficient amount hence diet fed insect's are healthier than leaf fed indoor larvae.

CONCLUSIONS

Comparative evaluation of tasar silkworm feed fed (indoor conditions up to 2nd instar), fresh leaf fed (indoor conditions up to 2nd instar) and fresh leaf fed in natural (*Terminalia arjuna* Bedd.) outdoor condition was evaluated. Data reveals that young age survival and Effective Rate of Rearing (ERR) were higher when larvae were brushed on semi-synthetic diet in contrast to indoor rearing on fresh leaf and complete outdoor rearing. The 3rd instar (day 0) and mature larvae (before spinning of cocoons) body weight were comparable in semi-synthetic diet and complete outdoor reared larvae in but higher than fresh leaf indoor condition. Semi-synthetic diet fed larvae showed higher cocoon weight, shell weight and shell ratio than complete outdoor fed larvae whereas minimum when larvae were fed fresh leaf in indoor condition up to II stage. Protein concentration of hemolymph was slightly higher in semi-synthetic diet fed than outdoor reared larvae whereas, lower in case of indoor reared. Hemolymph protein SDS-PAGE analysis showed the semi-synthetic diet fed larvae is closer to outdoor reared larvae profile than indoor reared larvae. Based on comparative evaluation performance of rearing, cocoon trait and biomolecular profile of *Antheraea mylitta* D it is concluded that tasar feed is novel. It is expected that Tasar feed will be helpful in minimizing bad impact of unfavorable condition during I crop rearing of tasar silkworm. In addition, it will be also helpful in maintenance of stock of precious eco-races to increase production and productivity of tasar silk.

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