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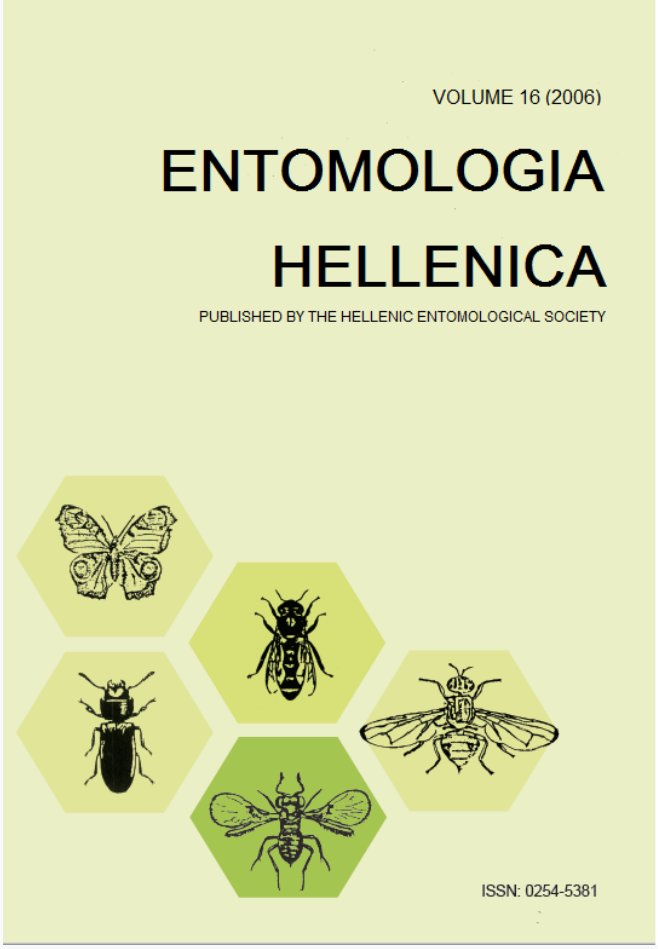

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K. Sashindran Nair, Jula S. Nair, V.A. Vijayan

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Changes in the total protein, carbohydtate and lipid contents in selected tissues of silkworm, *Bombyx mori* L. under the influence of a juvenoid R394

K. SASHINDRAN NAIR[1], JULA S. NAIR[1] AND V.A. VIJAYAN[2]

[1]*Central Sericultural Research & Training Institute, Mysore –570008, Karnataka, INDIA.*
[2]*Department of Studies in Zoology, Manasagangotri, Mysore- 570006, Karnataka, INDIA*

ABSTRACT

A juvenoid, R394 (Ethyl 9-cyclohexyl-3,7-dimethyl-2,4-nonadienoate) was applied topically to 5th instar silkworm, *Bombyx mori* L. larvae (Hybrid: KA x NB4D2) at a dose of 0.039 nl/larva at 24, 48, 72 and 96 h, for silk yield improvement. Three major selected tissues viz., posterior silk gland (PSG), haemolymph and fat body were collected from fully-grown larvae and the total protein, total carbohydrate and total lipid contents were estimated following standard procedures. The result indicated that the content of these primary metabolites varied significantly in the selected tissues depending on the time of juvenoid application. The highest protein content was observed in the haemolymph and silk gland in the larvae treated at 72 h whereas the fat body protein content was lowest for the same treatment. The total carbohydrate was recorded lowest in the 72 h treated larvae as against the highest in the control both in haemolymph and fat body with no significant change in PSG. The total lipid content did not show any notable variation in the concentration on juvenoid administration except in the silk gland treated up to 72 h which showed a decline. The results indicate that the juvenoid induces tissue-specific responses in terms of turnover in primary metabolites which commensurates with the corresponding changes observed in the cocoon weight and cocoon shell weight.

Introduction

Using juvenoid in sericulture for yield improvement has not been a concept confined to laboratories any more. Many countries led by Japan used various juvenoids to achieve 5 – 30 % increase in the cocoon production (Akai et al. 1985). But the increase in production was largely dependent on the dose of the compound applied and time of application (Chowdhary et al. 1990). Some of the treatment resulted in prolonged larval period (Akai et al. 1985) with complementary increase in the silk production, but some did not (Muroga et al. 1975). Our previous studies indicate that some plant based juvenoids do not induce a very prominent prolongation but in such cases, the increase hovers between 8 and 10% (Nair et al. 2002). The juvenoid, R394 is bioactive at extremely low concentration and can be a good candidate for field use. It is compound is also a good example for inducing varied response according to the time of application. This compound induced moderate response in terms of increased yield when treated at 24 h with no prolonged larval period. But when treated at 48 and 72h, the increase in production was greater,

accompanied by a prolongation in larval period by 24h (Nair et al. 2001). On this backdrop, this study was taken up to examine the biochemical constituents of three major tissues of juvenoid treated silkworm to understand the effect of the compound on these tissues and whether juvenoid induced increase in silk yield is commensurate with the biochemical alterations.

Materials and methods

The juvenoid, R394 (Ethyl-cyclohexyl-3,7-dimethyl-2,4-nonadienoate) was procured from Dr. L. Streinz, Institute of Organic Chemistry and Biochemistry, Czech Republic. This was one of the compounds short-listed for large scale use in Indian sericulture for improved cocoon/silk production. R394 emerged the strong candidate for this study due to the varied responses it elicited according to the time of application. Bivoltine x bivoltine (KA x NB4D2) silkworm hybrids were reared on fresh mulberry leaves (S36 variety) in the laboratory at 25±1°C temperature, 75±5% RH under 12:12 (L:D) photoperiod following standard procedure. On resumption to 5th instar, 100 larvae were transferred to ventilated plastic trays (56 x 36cm) for treating them with R394 followed by biochemical estimations. The juvenoid was prepared at a concentration of 0.31 nl/ml in the form of an emulsion using 1-2 ml of acetone and 1-2 drops of the surfactant, tween-20 in water. The emulsion was applied topically to silkworm at the rate of 12.5 ml/100 larvae as a single dose so that the dose becomes 0.039 nl/larva. This dose of R394 was determined after a series of dose response studies conducted on silkworm using wide range of concentrations (Nair et al. 1999). After leaving for 30 minutes, the larvae were fed with fresh mulberry leaves *ad libitum*. Different such batches were treated at 24, 48, 72 and 96 h of 5th instar. Untreated control was maintained in parallel for comparison of the results. Five male and five female larvae were collected from each batch including the control on the day when spinning was about to commence and dissected in insect ringer. After collecting the haemolymph, two tissues *viz.*, posterior silk gland (PSG) and fat body were isolated from the larvae and pooled treatment-wise. Before isolating the PSG, the whole silk gland weight was recorded. The tissues were stored at –20°C until the estimations were carried out. On reaching maximum growth, the larval weight was recorded and on maturation the larvae were left for cocoon formation. Cocoons were harvested on the 6th day and from each treatment, 10 male and 10 female cocoons were cut open and weight of cocoon and cocoon shell were recorded. Further, shell percentage was calculated.

Total protein was estimated following the method of Lowry et al. (1951) using crystalline bovine serum albumin as standard. The total carbohydrate content was estimated following the method of Carroll et al. (1956) using glucose standard and the total lipid content by the method of Folch et al. (1957). The observations were computed and the data were statistically analyzed employing one way analysis of variance (ANOVA) to ascertain the statistical significance between the control and the treated silkworm. The variables of economic traits and silk gland weight were triplicate where as the biochemical variables were replicated 5 times.

Results

Influence of juvenoid on larva and silk gland weight and cocoon characters

The response of larva and silk gland weight to the treatment of juvenoid was dependent on the time of application.

Maximum improvement was noticed in the treatment at 48 and 72h. The improvement was to the tune of about 7.5% in larval weight and above 20% in the silk gland weight when compared to the control. The treatment at 24 showed an increase of 12% in the silk gland weight (Table 1).

R394 application had a substantial positive influence on the cocoon weight and shell weight. The pattern of increase was prominent with a maximum change of 14.27% in the 72h treatment followed by 9.5 % increase in the 24 and 48 h treated batches. Similar was the trend in cocoon shell weight. The maximum improvement was noticed in the 72h treated batches. This was followed by the 48h and 24h treated batches. The maximum improvement was 15.73%. There was no significant difference in the shell percentage.

TABLE 1. Influence of the juvenoid, R394 on the mature larva and silk gland weight and the cocoon traits of *B. mori* (Hybrid: KA x NB4D2). Each value is the mean of 10 male and 10 female larvae. Values in the parentheses are percentage difference from the control.

Treatment hour in 5th instar	Larval weight (g)	Silk gland weight (g)	Cocoon weight (g)	Cocoon shell weight (g)	Shell percentage
24	4.425	1.679*	2.076*	0.417*	20.09
	(3.02)	*(12.53)*	(9.56)	(8.37)	(-1.09)
48	4.627*	1.798*	2.074*	0.423*	20.42
	(7.72)	*(20.51)*	(9.44)	(10.01)	(0.52)
72	4.620*	1.810*	2.165*	0.445*	20.57
	(7.55)	*(21.31)*	(14.27)	(15.73)	(1.28)
96	4.457	1.536	1.938	0.395	20.36
	(3.76)	*(2.96)*	(2.26)	(2.52)	(0.25)
Control	4.296	1.492	1.895	0.385	20.31
SE ±	0.045	0.036	0.023	0.004	0.189
CD at 5 %	0.178	0.118	0.065	0.011	0.538

* Significant ($P < 0.05$)

Influence on biochemical constituents

The influence of the juvenoid treatment on the biochemical constituents of the three selected tissues was prominent. The total protein content of the haemolymph of the treated silkworm varied significantly among themselves and also when compared to the control. The highest haemolymph protein content was observed in the silkworms treated at 72h which was 33 % more than that of the control followed by 48 h (19.60%) and 24h (17%). Interestingly, there was no much difference between total protein content of 96h treated silkworm and that of the control (Table 2). The sequence was reversed in the fat body. In this case, though the protein content ranged from about 67 to 83mg/g tissue among the treated silkworms, the lowest level of protein was observed in the silkworm treated at 72h. This was 22.29% less than that of the control. In the PSG, there was a marked positive and significant difference in total protein content in the treated silkworm compared to the

control. The maximum protein content was noticed in the silkworm treated at 72 h with a percentage change of 37.88% followed by that treated at 48h with 25.25 % (Table 2).

The concentration of total carbohydrate also followed varied pattern in the different tissues studied. In the haemolymph, the total carbohydrate was maximum in the control (Table 3). The lowest content was available in the 72h treated silkworms, which was 22.69% less than that of the control. The 48h treated silkworms followed this with 20.09% reduction. These changes were statistically significant. The total carbohydrate content in the fat body followed a different pattern. The maximum content was in the control silkworm but unlike in the haemolymph, the minimum was in the 24h treated silkworm. Regarding the silk gland the highest carbohydrate concentration was found in the silkworm treated at 24h and the lowest in the 96h treated batch. However, the differences were statistically insignificant.

The juvenoid application did not exert any significant effect on the total lipid content in haemolymph and fat body. But in the silk gland the treatment at 24, 48 and 72h induced a notable decline. The lowest lipid content in the haemolymph was noticed in the silkworm treated at 72h, which was 8.71% less than the control. The other treated batches were almost near to the control. In the fat body, the total lipid content varied from 80 to 85mg/g tissue. The lowest content was found in the larvae treated at 72h which was 5.88 % less than that of the control. In the silk gland, the highest lipid content was noticed in the control the lowest being in the 48 h treated silkworms. The variation in the total lipid content of the silkworm treated at 24, 48 and 72 h found to be significant compared to the control. The silk gland of the 96 h treated silkworm had total lipid content almost at par with the control (Table 4).

TABLE 2. Changes in the total protein content in the tissues of *B. mori* on administration of the juvenoid, R394. Each value is the mean ± SD of 5 separate observations. Tissues of 10 larvae (5 males and 5 females) were pooled for each sample.

Treatment hour in 5th instar	*Tissues*		
	Haemolymph mg/ml	*Fat body mg/g wet tissue*	*Silk gland mg/g wet tissue*
24	45.360 ± 2.353* (17.01)	74.275 ± 3.434* (-13.45)	112.112 ± 2.916* (11.79)
48	46.366 ± 2.055* (19.60)	72.447 ± 4.094* (-15.58)	125.605 ± 6.320* (25.25)
72	51.617 ± 1.752* (33.15)	66.695 ± 2.807* (-22.29)	138.280 ± 5.543* (37.88)
96	39.114 ± 1.432 (0.89)	82.702 ± 2.586 (-3.63)	105.496 ± 2.581 (5.19)
Control	38.767 ± 1.815	85.380 ± 2.282	100.287 ± 3.155
SE ±	1.200	1.491	1.999
CD at 1 %	5.697	7.073	9.485

Values in the parentheses are percentage difference from the control.
* Significant ($P < 0.05$)

TABLE 3. Changes in the total carbohydrate content in the tissues of *B. mori* on administration of the juvenoid, R394. Each value is the mean ± SD of 5 separate observations. Tissues of 10 larvae (5 males and 5 females) were pooled for each sample.

Treatment hour in 5th instar	*Tissues*		
	Haemolymph mg/ml	*Fat body mg/g wet tissue*	*Silk gland mg/g wet tissue*
24	15.229 ± 1.012* (-12.89)	46.970 ± 1.275* (-43.83)	12.167 ± 1.576 (7.35)
48	14.035 ± 0.807* (-20.09)	60.916 ± 2.044* (-27.15)	10.467 ± 0.613 (-7.67)
72	13.578 ± 0.818* (-22.69)	55.192 ± 1.708* (-33.99)	10.937 ± 0.358 (-3.53)
96	15.898 ± 0.287* (-9.48)	57.933 ± 2.179* (-29.92)	10.062 ± 0.396 (-8.60)
Control	17.563 ± 0.746	83.620 ± 2.358	11.337 ± 0.636
SE ±	0.466	1.178	NS
CD 5 %	1.519	3.845	

Values in the parentheses are percentage difference from the control.
* Significant ($P < 0.05$)
NS: Non-significant

TABLE 4. Changes in the total lipids content in the tissues of *B. mori* on administration of the juvenoid, R394. Each value is the mean ± SD of 5 separate observations. Tissues of 10 larvae (5 males and 5 females) were pooled for each sample.

Treatment hour in 5th instar	*Tissues*		
	Haemolymph mg/ml	*Fat body mg/g wet tissue*	*Silk gland mg/g wet tissue*
24	32.369 ± 1.751 (-5.59)	82.437 ± 3.607 (-3.77)	42.421 ± 1.868 (-11.06)*
48	32.697 ± 2.768 (-4.63)	81.578 ± 3.769 (-4.77)	43.278 ± 2.588 (-9.23)*
72	32.287 ± 2.612 (-8.74)	80.635 ± 3.963 (-5.88)	42.826 ± 2.001 (-10.21)*
96	33.678 ± 2.822 (-1.77)	84.637 ± 3.068 (-1.20)	46.232 ± 1.456 (-3.07)
Control	34.285 ± 2.368	85.668 ± 3.581	47.695 ± 1.902
SE ±	NS	NS	1.252
CD 5 %			4.084

Values in parentheses are percentage difference from the control.
* Significant ($P < 0.05$)
NS: Non-significant

Discussion

In the silkworm, *B. mori*, the last larval instar is the most active feeding period during which the larvae accumulate large quantity of bimolecular reserves in various tissues and are endowed with unique biochemical adaptation to conserve nutritional resources for cocoon spinning, metamorphosis and reproduction (Hugar and Kaliwal 1998). In the present study an effort has been made to determine whether the alterations in the major biochemical constituents are anyway related to the enhanced productivity. As seen in the results, the exogenous juvenoid had a clear positive impact on the traits such as larval weight, silk gland weight and cocoon and shell weight. As the cocoon and shell weight changed almost correspondingly, there was no much change in the shell percentage. Quite a few earlier reports suggest similar increase of 10-25 % in the cocoon traits on using different juvenoid compounds (Akai et al. 1985, Chowdhary et al. 1990, Muroga et al. 1975). Silk gland weight and larval weight were also reported to remarkably increase on JHA or vertebrate steroid administration to 5th instar silkworm (Reddy et al. 1992).

The three tissues viz., haemolymph, fat body and silk gland selected to study the effect of juvenoid on the biochemical changes assume a lot of significance when the role played by these tissues in silk synthesis is considered. At the stage of cocoon spinning, the cells in the silk glands, especially posterior silk gland synthesize large amount of fibroin, the main protein in the silk filament. The synthetic activity of this protein implies coordinated functioning of all elements of the cell machinery devoted to fibroin assembling and maturation which would primarily depend on the availability of resources. The changes in the haemolymph pool of nutrients certainly affect silk gland development and link it to food digestion and reserve mobilization (Sehnal and Akai, 1990). Since haemolymph is the immediate environment of the organs in the silkworm, the metabolic activity and the development are affected by the haemolymph (Nakayama et al. 1990). Fat body plays a very vital role in the storage of biomolecules and responds to the fluctuation of the metabolites in the haemolymph fairly quickly (Tojo et al. 1981).

Proteins are the chief organic constituents of the cell. These macromolecules are concerned with the regulation of all biochemical events in the organism (Harper et al. 1993). Accumulation of proteins in haemolymph and fat body during last instar development has been established by Chen (1985). Apart from this, in silk gland, accumulation of protein is a well-established fact. Our data show that the juvenoid treated mature larvae had a high content of haemolymph protein. But in the fat body, the total protein content was comparatively low in the treated larvae. Regarding the silk gland there was a significant positive difference in the total protein content especially in silkworms treated at 72h. These observations correspond to the changes noticed in the cocoon and cocoon shell weight as well as in the silk gland weight and larval weight. The decreased protein content in the fat body might be because some of the proteins were channelised to haemolymph through which it was transported to silk gland. Such high protein content seen in the haemolymph suggests that the protein synthesis and accumulation is mediated by the juvenoid to an extent. The report of Thomas and Nation (1966) indicates that withdrawal of JH inhibits protein synthesis may be correlated to this. The role of haemolymph and fat body in synthesis and storage of proteins in *B. mori* towards silk spinning have been documented earlier (Tojo et al. 1981). It was also indicated that such synthesis and release

is hormonally mediated and JH and ecdysterone play important roles in this (Riddiford and Truman 1978). The high protein content in the haemolymph and silk gland towards spinning indicates a hormonally mediated preparation of these tissues mainly silk gland for attaining competence for cocoon spinning. Reddy et al. (1992) reported a notable elevation in the protein content in haemolymph, fat body and silk gland on the administration of exogenous hormones or their analogues. The present observation is in conformity with the changes found except that in the fat body.

It is vivid from the present study that the total carbohydrate has declined in the treated silkworm in all the three tissues investigated. But the extent of reduction is less in silk gland compared to fat body and haemolymph. This indicates an increased mobilization of carbohydrate reserves both from fat body and haemolymph mainly towards the cocoon spinning process. Interestingly, such prominent changes were not visible in the silk gland, which implies that silk gland is independent of the exogenous juvenoid in respect of its carbohydrate content. Reports on the changes of carbohydrate content due to JHA applications are scant. But there is evidence that the carbohydrate content in the select tissues are bound to change notably on physiological changes such as BmNPV infection (Gururaj et al. 1999) or under the influence of insecticides (Nath et al. 1997).

Lipids constitute not only an essential and integral component of cell membranes but also act as an important source of energy for various metabolic activities of which reproduction and flight are important (Gilbert 1967). In domesticated silkworm, since flight is not an important function and is limited to a short precopulatory phase, its physiological role for reproduction may assume much more importance. Nonetheless, the role of lipids as an energy source for cocoon spinning and metamorphic activity cannot be overemphasized. In the present study, however, the haemolymph lipid content neither changed significantly among the treated batches nor was the difference particularly prominent between the treated and control. In the fat body lipid content, larvae that were treated at 72h showed the maximum negative difference from the control. Similarly, in the silk glands larvae that were treated at 24, 48 and 72h, declined to a significant level compared to the control. The literature reveals that removal of active CA resulted in an accumulation of fat body triacylglycerol and such hypertrophy can be reversed through implantation of CA to the allatectomized insects. As per Gilbert (1967) inclusion of CA with incubated fat body tissue suppresses the incorporation of fatty acid into triacylglycerol. These are indications that JH plays a crucial role in lipid metabolism. But in this study such prominent changes were not noticed in the fat body lipids.

This study makes it clear that the major tissues such as silk gland, fat body and haemolymph of silkworm respond to juvenoid treatment in ways specific to these tissues which culminates in increased silk production. This underlies the fact that juvenoids are bioactive compounds on silkworm and the enhanced yield on its exogenous administration is substantiated by the biochemical changes.

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KEY WORDS: *Bombyx mori,* cocoon parameters, juvenoid R394, larval weight, silk gland weight.

Μεταβολές στην περιεκτικότητα επιλεγμένων ιστών του μεταξοσκώληκα *Bombyx mori* L. σε ολικές πρωτεΐνες, υδατάνθρακες και λιπίδια, υπό την επίδραση της νεανικής ορμόνης R394

K. SASHINDRAN NAIR[1], JULA S. NAIR[1] KAI V.A. VIJAYAN[2]

[1]*Central Sericultural Research & Training Institute, Mysore –570008, Karnataka, INDIA*
[2]*Department of Studies in Zoology, Manasagangotri, Mysore- 570006, Karnataka, INDIA*

ΠΕΡΙΛΗΨΗ

Η νεανική ορμόνη R394 (ethyl 9-cyclohexyl-3,7-dimethyl-2,4-nonadienoate) εφαρμόστηκε τοπικά σε προνύμφες 5ου σταδίου του μεταξοσκώληκα *Bombyx mori* L. (υβρίδιο: KA x NB4D2) σε δόση 0,039nl/προνύμφη για 24, 48, 72 και 96 ώρες, για την βελτίωση της παραγωγής μεταξιού. Επιλέχθηκαν τρεις κύριοι ιστοί, ο οπίσθιος μεταξογόνος αδένας (PSG), η αιμολέμφος και το λιπώδες σώμα, οι οποίοι συλλέχθηκαν από πλήρως αναπτυγμένες προνύμφες και υπολογίστηκε η περιεκτικότητά τους σε ολικές πρωτεΐνες, ολικούς υδατάνθρακες και ολικά λιπίδια. Το αποτέλεσμα έδειξε ότι η περιεκτικότητα των κύριων μεταβολιτών διέφερε σημαντικά στους παραπάνω ιστούς, ανάλογα με τον χρόνο εφαρμογής της νεανικής ορμόνης. Η υψηλότερη περιεκτικότητα σε πρωτεΐνες παρατηρήθηκε στην αιμολέμφο και το μεταξογόνο αδένα των προνυμφών που δέχτηκαν την επέμβαση για 72 ώρες, ενώ η περιεκτικότητα σε πρωτεΐνες του λιπώδους σώματος ήταν η μικρότερη στην ίδια μεταχείριση. Η χαμηλότερη ολική περιεκτικότητα σε υδατάνθρακες καταγράφηκε στις προνύμφες που δέχτηκαν την επέμβαση για 72 ώρες, ενώ η υψηλότερη στον μάρτυρα τόσο στην αιμολέμφο όσο και στο λιπώδες σώμα, χωρίς να παρατηρείται σημαντική μεταβολή στον μεταξογόνο αδένα. Η ολική περιεκτικότητα σε λιπίδια δεν εμφάνισε αξιοσημείωτη παραλλακτικότητα κατά την εφαρμογή της νεανικής ορμόνης, με εξαίρεση τον μεταξογόνο αδένα σε προνύμφες που δέχτηκαν την επέμβαση για μέχρι 72 ώρες, όπου παρατηρήθηκε μείωση. Το αποτέλεσμα υποδηλώνει ότι η νεανική ορμόνη προκαλεί εξειδικευμένες για κάθε ιστό αντιδράσεις από άποψη μεταβολής της περιεκτικότητας σε κύριους μεταβολίτες, η οποία είναι ανάλογη με τις αντίστοιχες μεταβολές που παρατηρήθηκαν στο βάρος του βομβυκίου και το βάρος του κελύφους του βομβυκίου.