*Environment & Ecology* 27 (4A) : 1773—1778, 2009
 *ISSN 0970-0420*

# Role of Standardized Feed Additives on Biochemical Profiles in Hemolymph of Mulberry Silkworm Hybrids $CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$ (Jayalakshmi)

S. N. SUDHAKARA, NEELU NANGIA, G. GOVINDA RAJ[1], P. S. JAGADISH[2], N. G. MALLESHI[3], CHIKKALINGAIAH AND K. N. KRISHNAMURTHY

*Department of Sericulture, [1]Department of Entomology, [2]AICSMIP, [3]CFTRI, Mysore, University of Agricultural Sciences, Bangalore 560065, India*

**Abstract**

The silkworm hybrids $CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$ (Jayalakshmi) reared on mulberry shoots supplemented separately with two best feed additives selected along with control for estimation of biochemical profiles in larval hemolymph showed that application of flours of horse gram + grain amaranthus (50 : 50) in rearing schedule recorded significant difference in total protein, total carbohydrates, DNA content, higher protease, amylase, invertase and trehalase activities in hemolymph for daily once and alternate day feeding schedule from fourth instar up to spinning in both hybrids.
**Key words : Silkworm hybrids, Feed additives, Horse gram, Grain amaranthus, Biochemical profiles.**

The most important physiological factor in silkworm growth and silk productivity is nutrition. Insect nutrition primarily concerns biochemical substances that are necessary to activate various metamolic processes resulting in growth and development. Silk is the most beautiful natural fiber applauded as the emperor of textiles and identified as one of the fast growing foreign exchange earner for India. Sericulture demands low investment providing higher returns and generates elaborate employment opportunities especially in economically weaker sections of the society. In improving the biochemical profiles in silkworm, additional nutritional status of feed additives along with mulberry leaves is essential. So the present investigation was conducted to assess the biochemical qualities using two popular silkworm hybrids ($CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$) and two feeding schedules.

## Methods

In the present investigation, popular bivoltine silkworm hybrid $CSR_2 \times CSR_4$ and a new multi × bi-hybrid $ND_7 \times CSR_2$ (Jayalakshmi) were reared with mulberry shoots of $V_1$ variety harvested from irrigated garden, and supplemented individually / combination by dusting separately with flours, simultaneously maintaining unsupplemented control. Bulk rearing of both hybrids was done upto third moult and late age worms were separated to cater to two feeding schedules with feed additive treatments. The flours of 5 g/10 g of each of the feed additives were weighed and placed separately in plastic measuring cups and level was marked in each cup with inerasable ink.

The feed additive application was through marked measuring cups, ensuring that the flours were sieved (150 μ), and dusted on mulberry shoots at 5g / 10 g per kg of shoots based on marked levels of measuring cups to simplify application and fed to silkworm hybrids ($CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$) by dusting with plastic sieves during late age. There were two batches in the schedule of feed additive application. Leaves dusted with the feed additives was provided once daily during fourth instar till spinning. In the second batch, feed additives were provided once every alternate day from fourth instar till spinning. In both batches however, the remaining two feeds/day were normal (unsupplemented). To keep the bed dry and to facilitate easy moulting, feeding was resumed half an hour later after dusting bed disinfectant (Resham Jyothi), when more than 95%

**Table 1.** Influence of feed additives on total protein (μg/ml) content in haemolymph of mulberry silkworm hybrids $CSR_2$ × $CSR_4$ and $ND_7$ × $CSR_2$ (Jayalakshmi). $H_1$ : $CSR_2$ × $CSR_4$, $H_2$ : $ND_7$ × $CSR_2$ (Jayalakshmi), $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $D_2$ | $H_2$ $D_1$ | $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 235.88 | 228.56 | 348.20 | 345.51 | 289.54 |
| $FA_8$ : CFTRI mixture (100%) | 227.20 | 221.52 | 314.97 | 308.42 | 268.03 |
| $FA_{10}$ : Control / unsupplemented | 140.15 | | 302.00 | | 221.08 |
| *F*-Test | | * | | | * |
| SE ± | | 1.07 | | | 0.308 |
| CD at 5% | | 1.80 | | | 0.898 |

of the worms were out of moult. To ascertain the effect of standardized feed additives on rearing parameters, observations on fourth and fifth instar larval weight (initial and terminal), disease incidence, effective rate of rearing, fourth and fifth instar larval duration and total larval duration were recorded during rearing period. The data were analyzed statistically using three way factorial completely randomized design as outlined by Cochron and Cox (2).

The hemolymph profile of two silkworm hybrids, $ND_7$ × $CSR_2$ and $CSR_2$ × $CSR_4$ was studied during the larval stage (fifth day of fifth instar), in both feeding schedules with feed additive treatments and compared with control. In this experiment, feed additives, $FA_4$ : horse gram flour + grain amaranthus flour (50 : 50%), $FA_8$ : CFTRI mixture (100%) and $FA_{10}$ : unsupplemented control (Table 1).

*Collection of Hemolymph.* The hemolymph of ten randomly selected worms was collected separately from two batches of feeding schedules of late age worms (on fifth day of fifth instar) by incising the first pair of proleg with sterilized micro scissor. The oozing hemolymph was drawn into small clean glass test tube and used freshly/refrigerated at 5C for estimation of protein, total carbohydrates, enzymes and DNA contents.

Total protein estimation was conducted according to protein-dye binding method (3) and total sugars in all the samples were estimated by phenol sulfuric acid method (4).

The amylase, invertase and trehalase activities were determined by measuring the amount of the reducing substance released (5, 6). While, protease activity was measured following Eguchi and Iwamoto (7) with slight modification (pH of 11.0) (8). Total DNA estimation was conducted following standard method.

## Results and Discussion

Significant differences in total protein (235.88, 228.56 μg/ml ; 348.20, 345.51 μg/ml, Table 1), total carbohydrates (6.950, 6.903 μg/ml ; 7.870 μg/ml, 7.770 μg/ml, Table 2) and DNA content (2017.00, 2008.33 μg/100 ml ; 2089.33, 2080.66 μg/100 ml, Table 3) in hemolymph was registered in $H_1D_1FA_4$, $H_1D_2FA_4$,

**Table 2.** Influence of feed additives on total carbohydrates (μg/ml) content in hemolymph of mulberry silkworm hybrids $CSR_2$ × $CSR_4$ and $ND_7$ × $CSR_2$ (Jayalakshmi). $H_1$ : $CSR_2$ × $CSR_4$ , $H_2$ : $ND_7$ × $CSR_2$ (Jayalakshmi), $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $D_2$ | $H_2$ $D_1$ | $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 6.950 | 6.903 | 7.870 | 7.770 | 7.373 |
| $FA_8$ : CFTRI mixture (100%) | 6.900 | 6.863 | 7.483 | 7.403 | 7.162 |
| $FA_{10}$ : Control / unsupplemented | 4.820 | | 6.803 | | 5.811 |
| *F*-Test | | * | | | * |
| SE ± | | 0.010 | | | 0.003 |
| CD at 5% | | 0.020 | | | 0.008 |

**Table 3.** Influence of feed additives DNA (μg /100 ml) content in hemolymph of mulberry silkworm hybrids $CSR_2$ × $CSR_4$ and $ND_7$ × $CSR_2$ (Jayalakshmi). $H_1$ : $CSR_2$ × $CSR_4$, $H_2$ : $ND_7$ × $CSR_2$ (Jayalakshmi). $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $H_1$ $D_2$ | $H_2$ $D_1$ | $H_2$ $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 2017.00 | 2008.33 | 2089.33 | 2080.66 | 2048.83 |
| $FA_8$ : CFTRI mixture (100%) | 1821.00 | 1816.33 | 2013.00 | 2006.66 | 1914.25 |
| $FA_{10}$ : Control / unsupplemented | 1777.83 | | 1887.33 | | 1832.58 |
| *F*-Test | | | * | | * |
| SE ± | | | 1.150 | | 0.333 |
| CD at 5% | | | 1.950 | | 0.973 |

$H_2D_1FA_4$ and $H_2D_2FA_4$ with horse gram + grain amaranthus flour (50 : 50) feed additive application daily once and alternate days from fourth instar up to spinning in both hybrids, as compared to control (Tables 1 to 3).

Next best was CFTRI mixture as feed additive in $H_1D_1FA_8$, $H_1D_2FA_8$, $H_2D_1FA_8$ and $H_2D_2FA_8$ in respect of total protein (227.20, 221.52 μg/ml ; 314.97 μg/ml, 308.42 μg/ml, Table 1), total carbohydrates (6.900, 6.863 μg/ml ; 7.483, 7.403 μg/ml, Table 2), DNA content (1821.00, 1816.33 μg/100 ml ; 2013.00, 2006.66 μg/100 ml, Table 3), respectively as compared to control for the traits.

Minimum quantity was recorded in unsupplemented control for hybrid $CSR_2$ × $CSR_4$ ($H_1D_1FA_{10}$ and $H_1D_2FA_{10}$), in respect of total protein (140.15, 302.00 μg/ml, Table 1), total carbohydrates (4.820, 6.803 μg /ml, Table 2) and DNA content (1777.83, 1887.33 μg/100 ml, Table 3), respectively followed by hybrid Jayalakshmi ($H_2D_1FA_{10}$ and $H_2D_2FA_{10}$).

However, higher protease (58.67, 57.29 μg/ml/h ; 51.67, 50.36 μg/ml/h, Table 4), amylase (68.76, 67.37 μg/ml/h ; 64.48, 63.32 μg/ml/h, Table 5), invertase (64.36, 63.27 μg/ml/h ; 60.70, 59.52 μg/ml/h, Table 6) and trehalase activity (72.47, 71.34 μg/ml/h ; 66.80, 65.36 μg/ml/h, Table 7) were noticed in hemolymph in, $H_1D_1FA_4$, $H_1D_2FA_4$, $H_2D_1FA_4$ and $H_2D_2FA_4$, respectively with horse gram + grain amaranthus flour (50 : 50) feed additive application daily once and alternate day application from fourth instar up to spinning in both hybrids.

Succeeding was with CFTRI mixture as feed additive in $H_1D_1FA_8$, $H_1D_2FA_8$, $H_2D_1FA_8$ and $H_2D_2FA_8$ in respect of protease (50.47, 49.44 μg/ml/h ; 48.46, 47.48 μg/ml/h, Table 4), amylase (60.33, 59.39 μg/ml/h ; 56.66, 55.52 μgml/h, Table 5), invertase (58.63, 57.46 μg/ml/h ; 54.78, 53.42 μg/ml/h, Table 6) and trehalase activity (65.45, 64.33 μg/ml/h ; 61.45, 60.36 μg/ml/h, Table 7), respectively. Minimum enzyme activities were recorded in unsupplemented control for hybrid $CSR_2$ × $CSR_4$ ($H_1D_1FA_{10}$ and $H_1D_2FA_{10}$), in respect of protease (43.90, 35.74 μg/ml/h) (Table 4). amylase (54.90, 52.84 μg/ml/h) (Table 5), invertase (51.94, 49.95 μg/ml/h) (Table 6) and trehalase activity (57.86, 54.82 μg/ml/h) (Table 7), respectively

**Table 4.** Influence of feed additives on protease enzyme activity (μg/ml/h) in hemolymph of mulberry silkworm hybrids $CSR_2$ × $CSR_4$ and $ND_7$ × $CSR_2$ (Jayalakshmi). $H_1$ : $CSR_{22}$, $H_2$ : $ND_7$ × $CSR_2$ (Jayalakshmi). $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $H_1$ $D_2$ | $H_2$ $D_1$ | $H_2$ $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour+grain amaranthus flour (50 : 50%) | 58.67 | 57.29 | 51.67 | 50.36 | 54.50 |
| $FA_8$ : CFTRI mixture (100%) | 50.47 | 49.44 | 48.46 | 47.48 | 48.96 |
| $FA_{10}$ : Control / unsupplemented | 43.90 | | 35.74 | | 39.82 |
| *F*-test | * | | | | * |
| SE ± | 0.050 | | | | 0.014 |
| CD at 5% | 0.080 | | | | 0.040 |

**Table 5.** Influence of feed additives on amylase enzyme activity (µg/ml/h) in hemolymph of mulberry silkworm hybrids $CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$ (Jayalakshmi). $H_1$ : $CSR_2 \times CSR_4$, $H_2$ : $ND_7 \times CSR_2$ (Jayalakshmi), $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $H_1$ $D_2$ | $H_2$ $D_1$ | $H_2$ $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 68.76 | 67.37 | 64.48 | 63.32 | 65.98 |
| $FA_8$ : CFTRI mixture (100%) | 60.33 | 59.39 | 56.66 | 55.52 | 57.97 |
| $FA_{10}$ : Control / unsupplemented | 54.90 | | 52.84 | | 53.87 |
| *F*-Test | | * | | | * |
| SE ± | | 0.100 | | | 0.028 |
| CD at 5% | | 0.160 | | | 0.082 |

**Table 6.** Influence of feed additives on invertase enzyme activity (µg/ml/h) in hemolymph of mulberry silkworm hybrids $ND_7 \times CSR_2$ and $CSR_2 \times CSR_4$ (Jayalakshmi). $H_1$ : $CSR_2 \times CSR_4$, $H_2$ : $ND_7 \times CSR_2$ (Jayalakshmi), $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $H_1$ $D_2$ | $H_2$ $D_1$ | $H_2$ $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 64.36 | 63.27 | 60.70 | 59.52 | 61.96 |
| $FA_8$ : CFTRI mixture (100%) | 58.63 | 57.46 | 54.78 | 53.42 | 56.07 |
| $FA_{10}$ : Control /unsupplemented | 51.94 | | 49.95 | | 50.94 |
| *F*-Test | | * | | | * |
| SE ± | | 0.030 | | | 0.007 |
| CD at 5% | | 0.040 | | | 0.021 |

followed by hybrid Jayalakshmi ($H_2D_1FA_{10}$ and $H_2D_2FA_{10}$).

Nutrient status of mulberry leaves improved by value addition through supplementation of horse gram + grain amaranthus flours and CFTRI mixture by one of the two feeding schedules (daily once and alternate day) might have encouraged the larvae to accept the feed additives along with mulberry leaves. The protein/amino acid and carbohydrate content of mulberry leaves ($V_1$ variety) was estimated (22.20%). Horse gram and grain amaranthus, and CFTRI mixture, are a rich source of protein, fat, minerals and carbohydrate.

The variation in biochemical profiles of hemolymph may be due to the nutritional composition of the feed additive flour. CFTRI mixture has higher protein content (35%) compared to other tested flours. The inheritant composition of horse gram + grain amaranthus flours and CFTRI mixture, have increased the nutritive value of food consumed by silk-

**Table 7.** Influence of feed additives on trehalase enzyme activity (µg/ml/h) in hemolymph of mulberry silkworm hybrids $CSR_2 \times CSR_4$ and $ND_7 \times CSR_2$ (Jayalakshmi). $H_1$ : $CSR_2 \times CSR_4$, $H_2$ : $ND_7 \times CSR_2$ (Jayalakshmi), $D_1$ : Daily once, $D_2$ : Alternate day.

| Feed additive treatments | Hybrids $H_1$ $D_1$ | $H_1$ $D_2$ | $H_2$ $D_1$ | $H_2$ $D_2$ | Feed additive mean |
|---|---|---|---|---|---|
| $FA_4$ : Horse gram flour + grain amaranthus flour (50 : 50%) | 72.47 | 71.34 | 66.80 | 65.36 | 68.99 |
| $FA_8$ : CFTRI mixture (100%) | 65.45 | 64.33 | 61.45 | 60.36 | 62.90 |
| $FA_{10}$ : Control / unsupplemented | 57.86 | | 54.82 | | 56.34 |
| *F*-Test | | * | | | * |
| SE ± | | 0.030 | | | 0.007 |
| CD at 5% | | 0.040 | | | 0.021 |

worms in late age. The present findings are in conformity with the findings of Horie and Watanabe (9) who demonstrated the supplementation of soybean protein increased the protein and amino acid content in the larval hemolymph of the silkworm, *B. mori.*

The present findings are in consensus with findings of Krishnan et al. (10) who concluded that 2.00% of hydrolyzed protein supplementation increases the total protein concentration in the larval hemolymph. Similarly, Vanishree et al. (11) reported that the supplementation of P-soyatose (2%) increases the accumulation of storage proteins in the hemolymph. The larvae, having derived a rich source of amino acids from P-soyatose besides mulberry, could have increased the synthesis and subsequent accumulation of storage proteins. The protein concentration increased rapidly after the fourth moult and reached a maximum value at the end of the fifth instar (12).

Shigemastsu (13) reported that the hemolymph proteins mainly originate from fat body. Shamala Devi and Sarangi (14) concluded that the level of carbohydrates was found to be higher in fed, than starved batch compared to other starved batches (i.e., 2, 4 and 6 h). The effect of starvation on the level of carbohydrates was found to be higher in bivoltine compared multivoltine. Also, Neelu Nangia and Meera (15) reported that $NB_4D_2$ had highest reducing sugar content in the hemolymph as compared to PM and its hybrid. Similar trend was also observed by Meera (16) and Neelu Nangia and Meera (17) on glycerol content which was prominent in bivoltine breed, $NB_4D_2$ as compared to the multivoltines. Mahadev Kumar (18) found that the concentration of reducing sugar in larval hemolymph was high in BV followed by CB and MV, which might be due to higher rates of food assimilation and conversion in bivoltine. These observations are in tune with the findings of Nagata and Kobayashi (19) who reported that higher sugar content in the diet increases the storage protein levels in the hemolymph which helps in better growth and development.

The hemolymph present in silkworm may contain more amounts of potassium, calcium and magnesium with lower sodium contents. Therefore, stimulation of protein and DNA synthesis by calcium, magnesium or other metal ions may be possible because of their existence in the hemolymph.

## References

1. Dandin S. B., J. Jayaswal and K. Giridhar. 2005. *Handbook of sericulture technologies.* Central Silk Board, Bangalore, India. 285 pp.
2. Cochron W. G. and G. M. Cox. 2000. *Experimental design procedures for the behavioral sciences.* Cole Publ. Co. 319—380 pp.
3. Bradford M. M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analyt. Biochem.* 72 : 248—254.
4. Dubois M., K. A. Gillies, J. K. Hamilton, P. A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28 : 350—356.
5. Bernfeld P. 1955. Amylases, $\alpha$ & $\beta$. S. P. Colowick and N. D. Kaplan (eds). *In* Pp. 149—158. *Methods of enzymology*, Vol. I. Academic Press, New York, USA.
6. Baker J. E. 1991. Purification and partial characterization of amylase allozymes from the lesser grain borer, *Rhyzopertha dominica. Insect Biochem.* 22 : 303—311.
7. Eguchi M. and S. Iwamoto. 1976. Alkaline protease in the midgut tissue and digestive fluid of silkworm, *Bombyx mori. Insect Biochem.* 6 : 491—496.
8. Sarangi S. K. 1986. Studies on the midgut protease activity during fifth instar development of the silkworm, *Bombyx mori* L. *Indian J. Seric.* 11 : 165—169.
9. Horie Y. and K. Watanabe. 1983. Effect of various kind of dietary protein and supplementation with limiting amino acids on growth, hemolymph components and uric acid excretion in the silkworm, *Bombyx mori* L. *J. Insect Physiol* 29 : 187—200.
10. Krishnan M., K. M. Subburathinum and S. Janarthnan. 1995. Effect of hydrolysed protein (P-soyatose) on hemolymph protein, larval and pupal characters of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae). *Sericologia* 35 : 227—235.
11. Vanishree V., X. Nirmala and M. Krishnan. 1996. Response of five different races of silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae), to hydrolyzed soya protein supplementation. *Sericologia* 36 : 691—698.
12. Banno Y., S. Tochihara, Y. Kawaguchi and H. Doira. 1993. Hemolymph proteins during larval development of *Bombyx mori* L. : Proteins of Young larvae. *J. Seric. Sci. Jpn.* 62 : 187—194.
13. Shigemastsu M. 1958. Synthesis of blood protein by the fat body of the silkworm, *Bombyx mori. Nature* 182 : 880—882.
14. Shamala Devi D. and S. K. Sarangi. 2001. Effect of starvation on the levels of carbohydrates in the hemolymph of V. instar larva of *Bombyx mori* L. *Proc. Nat. Sem. Mulb. Seri. Res.* India, *Nutritional management and quality improvement in sericulture.* 515—521 pp.
15. Neelu Nangia and P. Meera. 2000. Larval hemoly-

mph and fat body profiles of popular breeds of mulberry silkworm, *Bombyx mori* L. *Proc. NSTS-99, Silkworm management in tropics.* Vol. 2. 221—224 pp.

16. Meera P. 1999. *Hemolymph and fat body profiles in pure races and cross breed of Bombyx mori L. M. Sc. (Seri.)* thesis. Univ. Agric. Sci, Bangalore, India. 121 pp.
17. Neelu Nangia and P. Meera. 2000. Profile of some bio-chemical parameters of egg and expression of micro-injected DNA of popular mulberry silkworm breeds. *Proc. NSTS-99, Silkworm management in tropics.* Vol. 2. 73—75 pp.
18. Mahadev Kumar D., Sowri and S. K. Sarangi. 2002. Changes in protein and reducing sugar content of the hemolymph during V instar development of *Bombyx mori* L. *Bull. Ind. Acad. Seri.* 6 : 103—106.
19. Nagata M. and M. Kobayashi. 1990. Quantitative changes in storage proteins during larval development of the silkworm, *Bombyx mori* L. *J. Seric. Sci. Jpn.* 59 : 461—468.