International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 16-03-2026, Accepted: 16-04-2026, Published: 01-05-2026
Volume 11, Issue 2, 2026, Page No. 200-203

Haemolymph lipid and protein alteration during larval development in *Samia ricini* infected with *Nosema*

Alika Borphukan Borah[1*], Dhiramani Bhuyan[2], R K Bhola[3]
[1] Department of Zoology, University of Science and Technology, Meghalaya, Baridua, Ri-Bhoi, Meghalaya, India
[2] Department of Zoology, Jagannath Barooah University, Jorhat, Assam, India
[3] Department of Zoology, Gauhati University, Guwahati, Assam, India
Corresponding Author: Alika Borphukan Borah

Abstract
Aim of the present investigation was to analyse the haemolymphs lipid and protein concentration in the last instar larva of *Samia ricini* inoculated with *Nosema* in three different seasons. It was found that during the development of 5th instar larva of *Samia ricini*, in normal group haemolymphs lipid concentration was found to be comparatively higher on first two days, declining thereafter and again increasing up to highest level on 6th day during all three different broods in the year. On the other hand, infected groups exhibited significant increase of lipid concentration on all days except 1st day of July-August brood. During the month of July to August, it was found that haemolymphs protein concentration decreased significantly compared to control on day 3 post inoculation. The decline in protein concentration continued till the last day (day 6). During September-October and November-December, no significant difference was observed in haemolymphs protein concentration between control and inoculated larva till day 5 post inoculation. On day 6 post inoculation haemolymphs protein concentration was found to be significantly low compared to normal control.

Keywords: *Nosema*, haemolymphs, *Samia ricini,* Pathogen

Introduction
Ericulture along with associated by-products is the sustainable source of livelihood for rural families in Northeast India. There are many factors that affect the success of commercial silk cocoon production, the menace of diseases being the prime one. The pebrine disease, caused by the microsporidian pathogen *Nosema* is one of the most deadly diseases in silkworms. Even though, most of the work has been done on the breeding aspect of silkworms, not much work has been published so far on pathological aspect of Samia *ricini*. Haemolymph is the only extracellular fluid of insects with diverse functions and reservoir for the products required for every physiological activity of the insect body, thus changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues (Pawar & Ramakrishnan,1977) [21]. Pathogens induce several biochemical and physiological alterations in insects (Martignoni,1964) [16]. The progress of infection by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents Rajitha & Savithri,2014 [25]. To date, reports on cellular and biochemical changes after parasitic infection in *Samia ricini* are scanty. Present investigation was carried out to understand the change of haemolymph lipid and protein concentration during the development of *Samia ricini* infected with *Nosema*.

Material and Methods
Some disinfected eggs of *Samia ricini* were collected from Central Silk Production Centre, Azara, Guwahati and it was reared as per the sericulture manual, which was given by the Directorate of Sericulture, Government of Assam. *Nosema* spores were collected from infected larvae and crushed in a glass mortar and pestle with isotonic buffered saline. Crushed suspension was filtered through muslin cloth and stored in deep freezer. Spores were purified by 60% sucrose gradient method.

Inoculation of larvae with *Nosema* spores
Newly hatched healthy 5th instar larva was randomly collected from stock room, starved for 6 hours and divided into 4 batches, each batch with 50 larvae. 3 µl spore suspension containing $8x10^5$ spores/ml was smeared on each castor leaf. Leaves were dried in air and used to inoculate larvae of three batches. Experimental batches were reared in a separate room. Control batch was provided with leaves smeared with distilled water. The inoculation was done during 3 different broods from July to December (July-August, September- October and November-December).

Haemolymph Collection
Haemolymph was collected in a pre-chilled test tube containing a few crystals of thiourea by cutting the first proleg of 5th instar larva. Haemolymph was collected at an interval of 24hours for 6 days from day 0 post-inoculation. All the collected haemolymph was centrifuged at 3,000 rpm for 3 min and supernatant was used for estimation of lipid.

Estimation of Lipid and Protein
Haemolymph lipid was estimated by Sulpho-phosphovanillin technique (Frings and Dunn, 1970) [11], a modified technique of Chabrol and Charonnat (1937) [7]. A Total haemolymph protein was estimated according to Lowry *et al.* (1951) [15] method.

Result and Discussion
Haemolymph lipid concentration was found to be 0.820 mg/ml in control and 0.788 mg/ml in the infected larva on day 1 during summer (Table 1). No significant difference

was observed on day 1 between control and infected larvae. The haemolymph lipid concentration remained almost constant from day 2-5 in the normal larvae and increased significantly on day 6. Infected group exhibited significantly higher lipid concentration compared to respective control from day 2-6.

During the month of September-October the lipid concentration was found to be 0.818 mg/ml in normal larvae on day 1, decreased on day 3 and increased thereafter till day 6 (1.12 mg/ml). No significant difference was observed in between control and infected larvae on day 1. However, it was found to be significantly higher in infected group which increased from 1.20 mg/ml on day 2 to 1.80 mg/ml on day 6.Haemolymph lipid concentration were found fluctuating from 0.92-1.37 mg/ml in normal 5th instar larvae from day 1-6 in November-December, an increasing trend was observed in infected group where it was found to increase from 1.2 mg/ml on day 1 to 2.11 mg/ml on day 6. Although no significant difference in lipid level was observed on day 1 between control and infected group, but it was found to be significantly higher in infected group from day 2-6. In the control group the lipid concentration remained almost static from day 1-6, whereas the infected group exhibited a twofold increase from 1.2 mg/ml on day 1 to 2.11 mg/ml on day 6. All the data were analysed by using the statistical computer application SPSS 16.0 was used. The data generated was average of three independent experiments. All the data were subjected to independent sample t-test and means were compared 0.05 level of significance.This study shows significant increase in the lipid concentration of haemolymph in infected larvae than normal larvae. An increased level of lipid content was recorded by many authors. It is mentioned that lipids are mobilized to the haemolymph in response to immune challenge (Cheon *et al*., 2006, Dettloff *et al*., 2001, Mullen *et al*., 2003) [8, 10, 18]. A study of mosquito-parasite interaction (*Plasmodium falciparum* and *Anopheles gambiae*) confirmed that infected field mosquitoes present an increased transcriptional level of lipophorin proteins (Mendes *et al*., 2008). Aboul *et al.* (1991) [1, 17] found double fold elevation of haemolymph lipid level in *Plodiainter punctata* larvae after treatment with *Bacillus thuringiensis* and suggested that increased lipid level may be due to conversion of some proteins into fat (Raina,1980; Rostom *et al*.,1972) [23, 26] during starvation. Similar observations were reported by Govindan *et al.* (1998) [12] in silkworm larvae infected with NPV. Lipids could be used as an energy source or for membrane biogenesis at the site of infection or in hemocytes (Arrese and Soulages, 2010) [2]. On the other hand a decreased level of haemolymph lipid was reported by Rajasekhar *et al.* (1992) [24], suggesting that pathogen might have utilized the lipid. Sarma *et al.* (1994) and Bennett and Shotwell (1972) [5, 27] explained that infested larvae use lipid for producing enzymes to remove pathogens. During July to August it was found that haemolymph protein concentration decreased significantly compared to control on day 3 post inoculation. The decline in protein concentration continued till the last day (day 6). During the months of September-October and November-December, no significant difference was observed in haemolymph protein concentration between control and inoculated larva till day 5 post inoculation. On day 6 post inoculation haemolymph protein concentration was found to be significantly low compared to normal control (Table 2). Here it was found that during the development of 5th instar larva of *Samia ricini*, there is a rapid increase in the haemolymph protein concentration which attains its peak at the end of larval life irrespective of the season. Similar observations have been recorded where protein concentration was found to be higher at the end of larval stage in the silkworms (Ito and Arai, 1963, Banno *et al*., 1993, Murthy *et al*., 2014) [4, 14, 19]. Nutrition plays an important role in the development and metamorphosis particularly in lepidopteran insects where adult is a non-feeding stage (Srivastava *et al*., 1982) [29]. High concentration of haemolymph proteins have been correlated with high consumption of mulberry leaves and subsequently high rate of conversion and their accumulation in haemolymphs of *Bombyx mori* (Banno *et al*., 1993, Aruga, 1994) [3, 4]. It has been proved that larval fat body is active in the synthesis and secretion of haemolymph proteins during the larval growth. When larva cease to feed these proteins are again selectively removed from the haemolymph and stored as intracellular granules for use at the time of metamorphosis (Chen, 1978) [9]. High protein concentration is an indication of greater metabolic activity. Synthesis and utilization of haemolymph proteins are conditioned by genetic and hormonal control (Hurliman and Chen, 1974) [13]. In the present study, a steady increase in the haemolymph protein concentration during the development of last larval instar in all the three broods indicates the influence of dietary proteins with no effect of season provided dietary status remain the same. Padma Sree & Ramani (2015) also found significant reduction in protein and lipid content of hemolymph compared to control Group after inoculation of fungal pathogen.After this study higher protein concentration was observed in the larva of *Antheraea mylitta* infected with polyhedrosis virus, however, on the last day the protein concentration was found to decrease significantly compared to control (Singh *et al*., 2011) [28]. Increase in the haemolymph's protein concentration was related to increased synthesis and release of fat body proteins, production of antimicrobial substances such as lectins, defensins and attacins. Pombo (1998) [22] also reported production of viral induced protein in silkworm larva infected with baculovirus. Watanbe *et al*. (1971) reported active synthesis of midgut proteins as well as polyhedron proteins induced by polyhedrosis viral infection which continued till the end of larval life.

Table 1: Haemolymph lipid concentration (mg/ml) in 5th instar larvae of *Samia ricini* infected with *Nosema* in three different broods

Season	Haemolymph lipid Concentration (mg/ml)						
	Days after Inoculation						
	Treatment	1	2	3	4	5	6
July-August	Control	0.820±0.178	0.773±0.206	0.773±0.316	0.750±0.404	0.828±0.183	1.180±0.730
	Infected	0.788±0.318	0.838±0.577	1.200±0.163*	1.440±0.743*	1.450±0.491*	1.540±0.568*
September-October	Control	0.818±0.466	0.810±0.209	0.698±0.222	0.715±0.273	0.775±0.403	1.12±0.103
	Infected	0.893±0.318*	1.20±0.105*	1.60±0.098*	1.32±0.135*	1.67±0.543*	1.80±0.183*
November-December	Control	1.00±0.178	0.92±0.147	0.81±0.240	0.72±0.568	1.00±0.16	1.37±0.543
	Infected	1.20±0.105*	1.47±0.674*	1.67±0.543*	1.59±0.960*	1.80±0.183*	2.11±0.338*

*Significantly different $p<0.05$

Table 2: Haemolymph protein concentration (mg/ml) in 5th instar larvae of *Samia ricini* infected with *Nosema* in three different broods.

Season	Haemolymph Protein Concentration (mg/ml)						
	Days after Inoculation						
	Treatment	1	2	3	4	5	6
July-August	Control	36.68±3.32	43.06±2.98	47.11±3.97	52.12±4.31	53.73±4.07	60.00±3.68
	Infected	35.40±3.23	41.86±4.67	33.60±2.28*	30.60±1.94*	28.20±1.39*	23.33±1.63*
September-October	Control	45.38±3.60	46.52±3.09	51.31±1.79	55.11±3.38	54.82±4.35	60.66±4.80
	Infected	43.47±3.61	45.54±2.83	49.66±2.44	53.81±3.36	52.95±4.00	53.29±2.32*
November-December	Control	44.69±5.32	47.25±4.31	52.62±1.90	52.69±1.94	55.18±2.62	61.20±3.43
	Infected	42.47±5.69	46.02±4.26	51.43±2.35	52.00±1.31	52.58±2.74	51.06±5.81*

*Significantly different $p<0.05$

Conclusion:

The Present study has significant alteration in haemolymphs lipid and protein of 5th instar *Samia ricini* larvae infected with *Nosema.* A statistically significant increase in haemolymphs lipid concentration was observed in infected larvae from day 2 post inoculation, indicates mobilization of lipid to meet increased energy demand and immune response during infection caused by *Nosema*. On the other hand decrease in haemolymphs protein level in infected larvae indicates in possible utilization of proteins in defence mechanism and metabolic adjustments. These findings highlight the haemolymphs lipid and protein concentration as the indicators during infection and physiological stress for better understanding and management of disease condition in eri silkworm culture.

Acknowledgment:

All the authors are thankful to the Department of Zoology Gauhati University Assam for providing us the necessary facilities to carry out this research work.

Conflict of Interest: The Authors declare no conflict of interest

References

1. Aboul ER, Kamel MY, Salama HS, Moursy E, Razek A. Changes in the biochemistry of the haemolymph of *Plodia interpunctella* after treatment with *Bacillus thuringensis*. J. Islam. Acad. Sc.,1991:4(1):29-35.
2. Arrese LE, Soulages JL. Insect fat body: energy, metabolism and regulation. Annu. Rev. Entomol, 2010, 55, 207-225. Doi: 10.1146
3. Aruga H. Principles of Sericulture. New Delhi: Oxford IBH Publishing Co., 1994.
4. Banno Y, Tochihara S, Kawaguchi Y, Doira H. Protein of young larva of silkworm, *Bombyx mori* L. J. Sric. Sci. Japan.,1993:62(3):187-194.
5. Bennet GA, Shotwell OL. Hemolymph lipid of healthy and diseased Japanese beetle larvae. J. Insec. Physiol.,1972:18:53-62.
6. Borphukan A, Bhola RK. Haemolymph Protein and Carbohydrate Alteration during Larval Development in *Samia ricini* Infected with Nosema. International Journal of Science and Research,2015:4(5):1824-1827.
7. Chabrol E, Charonnet R. Une novelle reaction pour l'etude des lipides. Presse Med.,1937:45:1713-1714.
8. Cheon HM, Shin SW, Bian G, Park JH, Raikhel AS. Regulation of lipid metabolism genes, lipid carrier protein lipophorin and its receptor during immune challenge in the mosquito *Aedes aegypti*. J. Biol Chem.,2006:281:8426-8435.
9. Chen PS. Protein synthesis in relation to cellular activation and deactivation. In Biobhemistry of Insects (ed. Rockstein N). Academic Press, Newyork, 1978, 145-200.
10. Dettloff M, Wittwer D, Weise C, Wiesner A. Lipophrin of lower density is formed during immune responses in the lepidopteran insect *Gallerie mellonella*. Cell Tissue Res.,2001:306(3):449-458.
11. Frings CS, Dunn RT. A colorimetric method for determination of total serum lipids based on the sulpho-phospho-vanillin reaction. Am J. Clin. Path.,1970:53:89-91.
12. Govindan R, Narayanaswamy TK, Devaiah MC. Text book of Principles of Silkworm pathology. Seri Scientific Publishers, Bangalore, 1998, 239.
13. Hurliman RF, Chen PS. Ontogenetische Veranderungen des enzymmusters in der haemolymphe von *Phormia rigina*. Revue. Swisse. Zoology,1974:81:648-654.
14. Ito T, Arai N. Food values of mulberry leaves for the silkworm *Bombyx mori* L. determined by means of artificial diets. I. Relationship between kinds of mulberry leaves and larval growth. Bull.Seric.Exp.Stn.Japan.,1963:18(4):226-229.
15. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reageant. J.Biol Chem.,1951:193:265-275.
16. Martignoni ME. Mass production of insect pathogens. In: De Bach P Ed. Biological control of insect pest and weeds. Reinhold, New York, 1964, 579-609.
17. Mendes AM, Schlegelmilch T, Cohuet A, Ambene AP, Iorio DM, *et al*. Conserved mosquito/parasite interactions affect development of *Plasmodium falciparum* in Africa. PLoS Pathog.,2008:4(5):e1000069. doi: 10.1371/journal.ppat.1000069. PMID: 18483558; PMCID: PMC2373770
18. Mullen L, Goldworthy G. Changes in lipophorins are related to the activation of phenoloxidase in the hemolymph of *Locusta migratoria* in response to injection of immunogens. Insect Biochem. Mol. Biol.,2003:33:661-670.
19. Murthy VNY, Ramkumar B, Jayaram GN, Lokesh G. Critical biochemical analysis in different body tissues in three commercial silkworm (*Bombyx mori* L.) races. As. J.N. & app. Sci.,2014:3(2):20- 30.
20. Padma Sree Vidya Devi P, Ramani Bai M. Biochemical activity in the haemolymph of silkworm, *Bombyx mori* L. during the infection of fungal pathogen, *Beauveria bassiana* (Bals) Vuill. International Journal of Multidisciplinary Research and Development,2015:2(5):320-322.

21. Pawar VM, Ramakrishnan N. Biochemical changes in larval haemolymph of *Spodoptera litura* (Fabricius) due to nuclear polyhedrosis virus infection. Indian Journal of Experimental Biology,1977:15:755-758.
22. Pombo V, Vellose LM, Ribeiro BM, Bao SN. Structural and ultra structural changes during infection of UFL-AG-286 cells with baculovirus AgMNPV. J. Invert.Pathol.,1998:72:239-245.
23. Raina AK. Protein content of various developmental stages of three strains of the print bollworm *Pectinophora gossypiella*. Experientia,1980:36:57-58.
24. Rajasekhar R, Prasad B, Subramanyam Reddy S, Bhaskar M, Murali K, Govindappa S. Variations in the tissue biochemical composition in the Muscardine infected silk worm larvae *Bombyx Mori* (L). Ind. J. Comp. Anim. Physiol.,1992:10(1):40-44.
25. Rajitha K, Savithri G. Amino acid profiles in the haemolymph of silkworm, *Bombyx mori* L. infected with fungal pathogen, *Beauveria bassiana* (Bals) vuill. International Journal of Applied Biology and Pharmaceutical Technology,2014:5(1):163-166.
26. Rostom ZMF, Algauheri AMI, Fattah AMM. Free amino acids and total protein in the hemolymph of *Pectinophora gossypiella* during induction and termination of diapauses. Proc. Egypt Acad. Sci.,1972:25:71-79.
27. Sarma BJ, Samson MV, Siva Prasad, Balavenkatasubbaiah VM, Dutta RK. Biochemical changes in the hemolymph of the silkworm *Bombyx mori* L virus (BmNPV). Sericologia, 1994, 34, 539-541.
28. Singh PG, Sinha AK, Roy DK, Sahay A, Madhusudan KN, *et al*. Cellular and biochemical changes of *Antheraea mylitta* D. on immunization with attenuated *Antheraea mylitta* cytoplasmic polyhedrosis virus. Int.J.Zoo.Res.,2011:7(3):263-271.
29. Srivastava AD, Mishra SD, Poonia FS. Effect of food deprivation on larval duration, cocoon (shell) weight and fecundity of eri silk moth *Philosamia ricini*. Ind. J. Seric.,1982:21(2):11-15.
30. Watanabe H. Pathophysiology of cytoplasmic polyhedrosis in the silkworm. In: Cytoplasmic polyhedrosis (Ed. by Aruga H and Tanada Y), Univ. Tokyo press, Tokyo, 1971, 151-167.