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Hemocyte and Biochemical Changes of *Antheraea mylitta* D. Infected with *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (*AmCPV*)

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Abstract: ***The hemocyte and biochemical changes in Antheraea mylitta after per oral inoculation of Cytoplasmic Polyhedrosis Virus (AmCPV) was observed. The total haemocyte count (THC) increased gradually from day 1 (13650/mm^3) to 8 (16625/mm^3) in uninoculated silkworms kept as control. In AmCPV treated silkworm larvae the THC increased up to day 2 (12700 - 14480/mm^3) of inoculation. Thereafter, a decrease was noticed from day 3 - 8 (14350 - 3812/mm^3). The differential haemocyte count (DHC) was different in inoculated silkworms then the control lot of silkworms. In the haemolymph of control larvae the prohaemocyte, plasmatocytes and granulocytes were more in number whereas oenocytoids were less in number. The number of degenerated cells increased among treated lot up to 8th days of post inoculation. The total protein content in the haemolymph of treated larvae during the first and second day was similar to that of control. On third day, the protein content increased among treated larvae. But from fourth day onwards, the protein content decreased (20.25 mg/ml) against control (33.73 mg/ml). The total carbohydrate content gradually increased during fifth instar in control lot larvae, whereas, it steadily increased up to 4th day and then showed a down ward trend from 5th day treated larvae. The total lipid content in the haemolymph decreased among treated larvae day 2, whereas in control larvae, an increasing trend was noticed.***

Keywords: *Antheraea mylitta*, *AmCPV*, Hemocyte and Biochemical

1. Introduction

Immune system includes certain types of blood cells. It also includes chemicals, carbohydrates, lipids and proteins. In insects, several types of haemocytes are observed in the haemolymph (Butt & Shields, 1996). Saran *et al*. (2002) classified the blood cells in the silkworm, *B. mori* L. and *A. mylitta* in to six types *viz*. prohaemocytes, plasmatocytes, granulocytes, spherulocytes, imaginal spherulocytes and oenocytes. Proteins are the derivatives of high molecular weight polypeptides. They play a vital role in the formation of structures in organisms. Like carbohydrates and fats proteins also can be utilized for energy purpose. In silkworm, blood glucose level can be correlated to their level of metabolism, and is comparable with mammalian blood glucose. Lipids play an important role in the biochemical processes delaying growth and development of insects.

To date, reports on hemocyte and biochemical response in *AmCPV* infected silkworm, *A. mylitta* are scanty. Hence, present investigation was conducted to study hemocyte and biochemical changes of *Antheraea mylitta* D. infected with *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (*AmCPV*).

2. Materials and Methods

Cytoplasmic Polyhedrosis Virus (*AmCPV*) Inoculum

Cytoplasmic polyhedrosis virus was purified from diseased silkworm as suggested by Aizawa, (1971).

Inoculation of *AmCPV*

AmCPV (1 x 10^5 PIB/ml.) was smeared on to the *Terminalia tomentosa* (assan) leaves and fed to 4th instar of Daba B.V eco race after 24 hr of moult. The treated and controlled batches were reared in indoor rearing condition.

Samples collection

Every day from 0 to 8th day 6 larvae/day were collected from each replication, the haemolymph from all the 6 larvae was collected in to three eppendoff tubes (2 larvae haemolymph/tube) on ice and stored at 4°C. A total of 6 tubes represented 3 replication collections.

Estimation of haemocytes count

Every day total haemocyte count (THC) estimation in the haemolymph of treated and control batches was determined following the method described by Tauber and Yeager (1935) using haemocytometer. The THC per mm^3 of haemolymph was estimated according to the formula suggested by Jonesh (1962). Different haemocytes were identified based on the morphological features as described by Nittono (1960). The observations were made on THC and DHC counts.

Estimation of biochemicals

The total protein, total carbohydrate and total lipid content in hemolymph was estimated by the method of Lowry *et al*. (1951), phenol-sulphuric acid method - Dubois *et al*., 1956 and Folch *et al*., 1951, respectively.

Annalysis of data

Data recorded for THC and DHC counts, total protein, total carbohydrate and total lipid content were statistically analyzed using Completely Randomized Design (Snedecor and Cockron, 1971).

3. Results and Discussion

Total Haemocyte Counts (THC)

The total hemocyte count in the control silkworm increased from 1st day to 6th day and decreased on 7th and 8th day. In the control, total hemocyte counts was 13650/mm^3 and

increased to 17702/mm^3 by 6th day (Table 1). On 7th day total hemocyte count was 16878/mm^3 and 8th day the count was decreased to 16625/mm^3. While in *AmCPV* treated silkworm the count was increased after inoculation up to 2nd day of infection and then there was a decrease for a period ranging from 3-8 days.

The present observations are in agreement with the earlier investigation that the number of haemocytes may increase (Balavenkatasubaiah *et al*, 2001) and decrease (Gillium and Shimanuki, 1967) to counter foreign body when infected. The cellular responses to infection have been worked out in many insect by earlier workers (Horohove and Dunn, 1983). On the basis of the above findings of the earlier workers it is evident that CPV induce the defense response through multiplication of haemocytes as is indicated by the increase in total haemocyte counts of the hemolymph of the worms.

Table 1. Total haemocyte count in AmCPV treated and healthy silkworm, *A. mylitta*

Treatment	Days post inoculation							
	1	2	3	4	5	6	7	8
AmCPV treated	12700	14480	14350	12671	9851	6203	5009	3812
control	13650	12530	12982	14455	16670	17702	16878	16625
S.E. ±	131.26	141.51	126.07	105.82	155.98	1823.12	191.76	191.23
C. D. at 5%	137.18	152.26	177.43	124.28	256.75	308.45	289.45	331.87

Differential Haemocyte Counts (DHC)

In control hemocytes showed a gradual increasing trend during developmental period. The number of prohemocyte, plasmatocytes, granulocytes and spherulocytes *viz.* 22-35, 32- 38, 35-38 and 17-28, respectively in control (Table 2). While during progressive infection, the gradual decrease in number of prohemocyte, spherulocytes and oenocytoids *viz.* 28-12, 24-9 and 13-3 respectively was noticed in *AmCPV* treated batch. The plasmatocytes and granulocytes increased up to 3rd day and decresing trend was observed from 5th day onwards. The number of oenocytoid was less in both the treatments and ranged from 3 to 15. Few vermiform cells, synonyms of plasmatocyte were recorded in both treatment and control. The number of degenerated cells was comparatively less in control than the treated batch.

The number of prohemocyte decreased due to the conversion of prohemocyte to other types of haemocyte during course of infection and number of plasmatocytes and granulocytes increased as both are involved in defense mechanism against entry of pathogens.

Table 2. Differential haemocyte count in AmCPV treated and healthy silkworm, A. mylitta

Treatment	Haemocyte	Days post inoculation							
		1	2	3	4	5	6	7	8
AmCPV treated	PR	28	25	24	23	18	17	15	12
	PL	33	39	44	42	38	35	32	25
	GR	35	45	48	45	44	41	32	27
	SP	24	20	18	17	15	14	12	9
	OE	13	11	10	8	8	5	4	3
	VER	1	-	-	-	-	-	-	-
	DEG	22	24	25	26	30	37	39	42
Control	PR	22	25	27	29	30	33	34	35
	PL	32	33	35	37	39	41	37	38
	GR	35	36	38	39	42	40	38	38
	SP	17	18	20	22	25	26	27	28
	OE	5	6	8	10	10	12	13	15
	VER	-	-	-	-	4	1	-	-
	DEG	10	12	13	14	16	9	7	8

PR = Prohaemocyte GR = Granulocyte OE = Oenocytoid DEG = Degenerated cell
PL = Plasmatocyte SP = Spherulocyte VER = Vermiform cell

Biochemical changes in *AmCPV* treated and control batches

Total protein content

The hemolymth protein in control silkworm increased gradually from 16.31 mg/ml on 1st day to 33.73 mg/ml on day 8th (Table 3). In treated silkworm, the total hemolymph protein have shown increasing trend from 1st (16.26 mg/ml) to 3rd day (24.22 mg/ml) and decreasing trend from 4th day onwards and reached 20.25 mg/ml by 8th day from the inoculation.

The results indicated that changes occured in the hemolymph protein, during the course of *AmCPV* infection. The difference in hemolymph protein healthy silkworm and treated silkworm becames more pronounced as the diseases progresses. This would probably indicate that during infection the synthesis and release of proteins from fat bodies are greatly increased. There are reports of production of antimicrobial substances such as lectin, defensin and attacin with the entry of foreign bodies (Wago, 1995).

Table 3: **Total protein content in AmCPV treated and healthy silkworm, *A. mylitta***

Treatment	Total protein content mg/ml							
	Days post inoculation							
	1	2	3	4	5	6	7	8
AmCPV treated	16.26	20.14	24.22	23.58	22.16	21.59	20.98	20.25
Control	16.31	20.19	23.54	26.74	28.53	30.34	32.83	33.73
S.E. ±	0.21	0.28	0.19	1.37	0.48	0.52	0.41	0.56
C. D. at 5%	1.92	1.27	1.21	1.52	1.68	1.93	2.64	2.15

Total carbohydrate and total lipid contents

The hemolymph carbohydrate in control silkworm increased gradually from 6.75mg/ml on 1st day to 11.12 mg/ml on day 8th (Table 4). In inoculated silkworm, the total hemolymph carbohydrate have shown increasing trend from 1st (6.73 mg/ml) to 6th day (8.05 mg/ml) and decreasing trend from 7th day onwards and reached 7.56 mg/ml by 8th day from the treatement.

Table 4: **Total carbohydrate content in AmCPVtreated and healthy silkworm, *A. mylitta***

Treatment	Total carbohydrate content mg/ml							
	Days post inoculation							
	1	2	3	4	5	6	7	8
AmCPV treated	6.73	6.98	7.83	8.31	8.2	8.05	7.93	7.56
Control	6.75	7.02	7.89	8.42	9.86	10.51	10.69	11.12
S.E. ±	0.19	0.27	0.19	1.36	0.46	0.51	0.42	0.57
C. D. at 5%	1.91	1.26	1.21	1.51	1.64	1.93	2.61	2.14

The hemolymph lipid in control silkworm increased gradually from 10.22 mg/ml on 1st day to 12.85 mg/ml on day 8th (Table 5). In inoculated silkworm, the total hemolymph lipid have shown increasing trend from 1st (10.18 mg/ml) to 4th day (10.12 mg/ml) and decreasing trend from 5th day onwards and reached 9.20 mg/ml by 8th day from the treatment.

Table 5: **Total lipid content in AmCPV treated and healthy silkworm, *A. mylitta***

Treatment	Total lipid content mg/ml							
	Days post inoculation							
	1	2	3	4	5	6	7	8
AmCPV treated	10.18	10.59	10.25	10.12	9.88	9.73	9.51	9.20
Control	10.22	10.63	11.15	11.84	12.38	12.65	12.72	12.85
S.E. ±	0.21	0.27	0.19	1.36	0.49	0.52	0.42	0.57
C. D. at 5%	1.93	1.27	1.21	1.52	1.71	1.94	2.62	2.13

The total carbohydrate and lipid contents increased to certain level and decreased steadily as the disease developed and it is reasonable to assume that as the disease progresses the number of pathogens increases and carbohydrates and lipids were utilized as a source of energy required for the growth and development of *AmCPV*.

4. Inference

The total haemocyte count (THC) increases gradually in healthy silkworm where as in *AmCPV* infected silkworm larvae in initial stages THC increases there after decreases. In the healthy larvae the prohaemocyte, plasmatocytes and granulocytes will be more in number whereas oenocytoids were less in number. The number of degenerated cells increases as the intensity of the disease increases. In initial infection of *AmCPV*, total protein, carbohydrate and lipid contents will be improved as like healthy silkworm but, when disease enhances it shows down ward trend.

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