International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 18-12-2021, Accepted: 03-01-2022, Published: 19-01-2022
Volume 7, Issue 1, 2022, Page No. 59-61

Changes in free amino acids and hydrogen-ion-concentration between the healthy and diseased *Antheraea proylei* larva

O K Monoroma[1], M Suresh Singh[2], O Dwijamani Singh[3]
[1] Department of Zoology, Manipur College, Imphal, Manipur, India
[2] Department of Zoology, G.P. Women's College, Imphal, Manipur, India

Abstract
Certain changes in the free amino acids composition and hydrogen-ion-concentration between the healthy and diseased larval gut were studied in *Antheraea proylei*. The healthy larval gut wall and gut contents has been found to contain thirteen and twelve free amino acids respectively in different concentration whereas the diseased larval gut wall and gut contents showed only five and four amino acids respectively. This has revealed that the pathogenic organisms affect the amino acids composition adversely besides changing the hydrogen-ion-concentrations of the digestive tract to a great extent.

Keywords: *Antheraea proylei*, hydrogen-ion-concentration, gut wall and gut contents

Introduction
The oak tasar silkworm *Antheraea proylei* is a sericigenous insect of economic importance. It is attacked by a number of micro (Virus, Bacteria, Fungi etc.) and macro (Hymenopteran and Dipteran parasites) organisms in nature causing severe damage to this economically important insect. The rearing and biochemical aspects of the mulberry and non-mulberry silkworm have been worked out by several workers like Agarwal and Jolly (1981) [7], Devi and Prasad (1987) [2] and Rajak Jitendra (2008), but no works seems to have been done on the damage caused by micro and macro-organisms to this insect. Keeping this in view, the present piece of work has been taken up to know the changing pattern of certain physiological parameters like free amino acids and hydrogen-ion-concentration in the digestive system of this insect with special emphasis during the diseased conditions.

Material and Methods
The experimental insect(5^{th} instar healthy and diseased larvae) of Oak Tasar Silkworm, *Antheraea proylei* brought from the regional tasar research station, Mantripukhri, Imphal, were kept inside the cage in the laboratory. They were starved for 24 hours before they were dissected when still alive. The extracts of the required liquid were made as quickly as possible.
The silkworms were washed in distilled water and dissected to clear the alimentary canal. The debris of muscles, trachea, Malpighian tubules and other adhering tissues were removed from the gut. The entire digestive tract after a thorough wash in distilled water was kept on a separate sterilized slide. The adhering liquid was removed by absorbent paper. All the gut contents were separated out leaving only the gut wall by means of a soft brush. The gut wall was then washed two to three times with distilled water and kept on a clear filter paper to absorb all the water contents. The gut contents and gut wall were crushed and homogenised separately with a little volume of 80% ethanol and were centrifuged for 15 minutes at 15,000 R.P.M. in order to get a clear supernatant liquid. Two dimensional paper chromatographic procedures adopted by Micks and Ellis (1952) [6] and Devi and Prasad (1987) [2] for the qualitative estimation of free amino acids were employed. The identification of the free amino acids was done by the method adopted by Consden *et. al.* (1944) [6] by comparing their R_f values with those of the corresponding reference amino acids. Universal indicator method (David, 1927) [5] and paper indicator method (Waterhouse, 1949) [4] were adopted for the estimation of hydrogen-ion-concentration. In case of the universal indicator method the known buffer solutions of different pH ranges were kept in the crucible. A drop of universal indicator is added in each of the cavity and mixed properly. Simultaneously a drop of the fluid to be tested was taken and mixed with the universal indicator. The change in the colour was compared with the colour resulted by the known buffer solution. In case of the paper indicator technique, small pieces of indicator paper were cut and touched to the fluid to be tested and the colour change immediately after touch was noted and compared with the colour range in the indicator paper. Thus, the pH of the gut in both healthy and diseased larvae were determined.

Results and Discussion
The qualitative estimation of free amino acid (Table-1) in this silkworm reveals that the gut contents of healthy fifth instar larvae contain 12 free amino acids. The amino acids namely Dihydroxyphenylalanine, isoleucine, leucine, amino-butyric acid, phenylalanine, proline, hydroxyproline and tyrosine are entirely absent from the gut contents and gut wall. Glutamic acid, glycine, ornithine, threonine, serine and methionine were detected in higher concentration. The extract of the gut content of the diseased larvae has revealed a marked reduction in the free amino acids composition. The absence of such a large number of free amino acids indicates that most of the amino acids are utilized by the pathogenic organisms. The presence of some of these amino acids is in conformity with the findings in infected larvae of *A. mylitta* (Agarwal *et. al.*, 1974). The amino acids like

cysteine and ornithine have been detected in higher concentration in the gut content of the diseased larvae. All other amino acids are entirely lacking. Similarly the gut-wall extract of the healthy larvae has shown the presence of a fairly good number (13) free amino acids. Almost similar number of free acids were also detected in *A. mylitta*. The detection of fairly good number of these amino acids might be due to the fact that this instar is the voracious feeder and hence the amino acids increase as the larvae grow.

Table 1: Free amino acids in the larval gut of *Antheraea proylei* during healthy and diseased conditions.

Name of the amino acids	Gut-wall		Gut-contents	
	Healthy	Diseased	Healthy	Diseased
Alanine	-	-	+	-
Amino-Butyric-acid	-	-	-	-
Arginine	++	-	+	-
Aspartic Acid	+++	-	++	+
Cysteine	++	-	++	+++
Cystine	+	-	-	-
Dihydroxyphenylalanine	-	-	-	-
Glutamic acid	+++	-	+++	-
Glycine	+++	+	+++	-
Histidine	-	-	+	-
Hydroxyproline	-	-	-	-
Iso-leucine	-	-	-	-
Nor-leucine	+	-	-	-
Leucine	-	-	-	-
Lysine	+++	+	+	-
Methionine	+++	+++	+++	-
Ornithine	+++	+++	+++	+++
Phenylalanine	-	-	-	-
Proline	-	-	-	-
Serine	+++	-	+++	-
Threonine	-	-	+++	+
Tryptophan	++	-	-	++
Tyrosine	-	-	-	-
Valine	+++	-	-	-

(+++) = Higher concentration
(++) = Moderate concentration
(+) = Traces
(-) = Absence

Table 2: Hydrogen-ion-concentration of the digestive tract of healthy and diseased 5th instar larvae of *Antheraea proylei*

Hydrogen-ion-concentration (pH)					
Gut-contents of healthy larvae			Gut-contents of diseased larvae		
Stomodaeum	Mesenteron	Proctodaeum	Stomodaeum	Mesenteron	Proctodaeum
8.0	9.0	6.0	6.5	7.5	6.5
8.5	9.0	6.5	6.0	7.5	6.0
8.0	9.5	6.0	6.0	7.5	6.5
8.0	9.5	6.5	6.0	7.5	6.0
8.5	9.0	6.5	6.5	8.0	6.5
9.0	9.5	6.5	6.5	8.0	6.0
9.0	9.5	6.5	6.0	7.5	6.5
9.0	9.0	6.5	6.5	8.0	6.5
9.0	9.0	6.5	6.5	8.0	6.0
9.0	9.0	6.0	6.5	8.0	6.5
Range -8.0-9.0	9.0-9.5	6.0-6.5	6.0-6.5	7.5-8.0	6.0-6.5
Mean 8.6	9.2	6.35	6.3	7.75	6.3

The gut-wall extract of the diseased larvae has also indicated marked reduction in the free amino acids like that of the gut contents. Very few amino acids have been detected in the gut-wall of the diseased larvae. Only four free amino acids namely glycine, lysine, methionine and ornithine are detected in the fifth instar larvae out of which the later two amino acids are present in higher concentration. Ornithine has been detected in the same concentration in both the gut wall and the gut contents of the diseased larvae. It seems that the pathogen does not affect the ornithine at all. The observation on the hydrogen-ion-concentration of the gut contents of the healthy and diseased larvae (Table 2) has revealed that the pH ranges from 8.0 - 9.0 in the foregut, 9.0-9.5 in the mid gut and 6.0 - 6.5 in the hind gut in the healthy larvae whereas in the diseased larvae has been found to range from 6.0 - 6.5 in the foregut, 7.5 - 8.0 in the midgut and 6.0 - 6.5 in the hind gut. It is evident that the pH also has been found to be changed in the diseased larvae. The highly alkaline pH becomes acidic during the diseased conditions. This drastic change in the pH of the gut seems to have been brought about by the pathogenic organism present in the body of the diseased

larvae.

Conclusion

The study has thus revealed that the entire physiological process of the tasar worm is adversely affected by the microbial pathogens. Marked reduction in the free amino acids compositions has been brought about by the pathogens and the hydrogen-ion-concentration is changed from highly alkaline medium to the acidic medium. The cumulative effects of all these physiological parameters ultimately result in the immature death of the larvae and affect the cocoon production and the tasar silk yield.

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