

Regular Article

## Seasonal Variation in the Free Amino Acids in Gut Contents during Developmental Stages of *Antheraea proylei* Jolly (Saturniidae: Lepidoptera)

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**ABSTRACT:** The qualitative analysis of free amino acids has been done in the gut contents of *Antheraea proylei* during its developmental stages. The collection of gut contents from the first instar larvae was not possible as the physiological experiment could not be done. The second instar larvae showed the presence of 8, 7 and 5 amino acids in spring, summer and autumn season respectively. The third instar larvae revealed the presence of 9, 8 and 5 ninhydrin positive spots in spring, summer and autumn seasons. The fourth instar larvae showed the presence of 10, 9 and 7 amino acids in spring, summer and autumn seasons while the fifth instar larvae exhibit 11, 9 and 8 amino acids in spring, summer and autumn season respectively.

**Key words:** Amino acids, *A. proylei*, Instar Larva, Season

### Introduction

Amino acids are molecules that constitute the most important part of biological structure and body chemistry. They can be identified by the presence of the amino group, (-NH<sub>2</sub>) and acid carboxyl group (-COOH) and the remainder of the molecule being variable. Extensive work on the fundamental rearing and biochemical problems of mulberry and non-mulberry silkworms has been carried out by several workers (Ambartsumova, 1968; Prasad and Shukla, 1975; Agarwal and Jolly, 1981; Prasad and Devi, 1987) and several others. But there is scarcity of literature on study of amino acids presence in the gut contents during the different developmental stages of *Antheraea proylei*. Therefore, an attempt was made to estimate the presence of different amino acids in the gut content extract of *Antheraea proylei* during its development stages at the different seasons of a year on account of its physiological role in the growth and development of the worm. The results of the present study are given in this communication.

### Materials and Methods

The developmental stages (2nd, 3rd, 4th and 5th instars) of *Antheraea proylei* worms were chloroformed and carefully dissected. The debris of muscles, trachea malpighian tubules and other adhering structures were removed from the gut. The entire digestive tract after thorough wash in distilled water was kept on a sterilized slide. The adhering liquid was removed by absorbent paper. The gut contents were obtained by syringe. All the gut contents were taken

out leaving only the gut wall. The gut contents were homogenized separately in a small hand homogenizer with a little volume of 80% ethanol solution. This makes the solution to mix uniformly. All the samples were centrifuged separately for 15 mins. at 15,000 rpm. The supernatant was separated out and then mixed with 3 parts by volume of chloroform for removing lipids. For complete separation of the solution, they were kept in dark place for about 12 hrs. in the separating funnels. The layers were separated and the major parts of the amino acids remained in the aqueous phase. The samples were then used for spotting by fine glass capillary tube for thin layer chromatogram.

### Results and Discussions

The qualitative analysis of free amino acids has been done in the gut contents of *Antheraea proylei* during its different developmental stages. As the collection of gut contents from the first instar larvae was not possible, the physiological experiments could not be carried out in this stage. The extract gut content of the second instar larvae showed the presence of 8, 7 and 5 amino acids in spring, summer and autumn seasons respectively. In the spring season, the amino acids detected were Arginine, Cystine, Glutamic acid, Histidine, Leucine, Lysine, Methionine and Threonine in the summer season Arginine, Cystine, Glutamic acid, Leucine and Lysine respectively in autumn. The gut content extract of the third instar larvae revealed the presence of 9, 8 and 5 ninhydrin positive spots in spring, summer and autumn respectively. During spring season, the amino acids detected from the extract of third instar larvae were Arginine, Cystine, Glutamic acid, Histidine, Leucine, Lysine, Methionine, Serine and Threonine. While the amino acids like arginine, cystine, glutamic acids, histidine, leucine, lysine, methionine, Threonine and Arginine, Cystine, Glutamic acid, Leucine and Lysine were detected in the summer and the autumn season respectively. The gut homogenate of the fourth instar larvae showed the presence of 10, 9 and 7 amino acids in spring, summer and autumn seasons respectively. In spring season, the amino acids detected were Amino-butyric acid, Arginine, Aspartic acid, Cystine, Glutamic acid, Lysine, Ornithine, Serine, Tryptophan and Valine while during the summer season the amino acids like Arginine, Aspartic acid, Cystine, Glutamic acid, lysine, Ornithine, Serine, Tryptophan and Valine were recorded but Arginine, Aspartic acid, Cystine, Glutamic acid, Lysine, Tryptophan and Valine were recorded only in the autumn season.

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Table 1: Seasonal variations in the free amino acids in the gut content of the II<sup>nd</sup> and III<sup>rd</sup> instar larvae of *Antheraea proylei* J.**Second instar larvae:**

Spring	Calculated R <sub>f</sub> value		Summer	Calculated R <sub>f</sub> value		Autumn	Calculated R <sub>f</sub> value	
	Chl. Methanol solvent	Phenol solvent		Chl. Methanol solvent	Phenol solvent		Chl. Methanol solvent	Phenol solvent
Arg	0.17	0.68	Arg	0.17	0.68	Arg	0.17	0.68
Cyst	0.18	0.19	Cyst	0.18	0.19	Cyst	0.18	0.19
Glu	0.27	0.30	Glu	0.27	0.30	Glu	0.27	0.30
Hist	0.20	0.87	Hist	0.20	0.87	Leu	0.79	0.83
Leu	0.79	0.83	Leu	0.79	0.83	Lysine	0.25	0.51
Lysine	0.25	0.51	Lysine	0.25	0.51			
Meth	0.48	0.80	Thre	0.32	0.53			
Thre	0.32	0.53						
<b>Third instar larvae</b>								
Arg	0.17	0.68	Arg	0.17	0.68	Arg	0.17	0.68
Cyst	0.18	0.19	Cyst	0.18	0.19	Cyst	0.18	0.19
Glu	0.27	0.30	Glu	0.27	0.30	Glu	0.27	0.30
Hist	0.20	0.87	Hist	0.20	0.87	Leu	0.79	0.83
Leu	0.79	0.83	Leu	0.79	0.83	Lysine	0.25	0.51
Lysine	0.25	0.51	Lysine	0.25	0.51			
Meth	0.48	0.80	Meth	0.48	0.80			
Serine	0.25	0.41	Thre	0.32	0.53			
Thre	0.32	0.53						

Table 2: Seasonal variations in the free amino acids in the gut content of the IV<sup>th</sup> and V<sup>th</sup> instar larvae of *Antheraea proylei* J.

Spring	Calculated R <sub>f</sub> value		Summer	Calculated R <sub>f</sub> value		Autumn	Calculated R <sub>f</sub> value	
	Chl. Methanol solvent	Phenol solvent		Chl. Methanol solvent	Phenol solvent		Chl. Methanol solvent	Phenol solvent
A-Bu	0.38	0.73	Arg	0.17	0.68	Arg	0.17	0.68
Arg	0.17	0.68	Asp	0.31	0.27	Asp	0.31	0.27
Asp	0.31	0.27	Cyst	0.18	0.19	Cyst	0.18	0.19
Cyst	0.18	0.19	Glu	0.27	0.30	Glu	0.27	0.30
Glu	0.27	0.30	Lysine	0.25	0.51	Lys	0.25	0.51
Lysine	0.25	0.51	Omi	0.17	0.27	Tryp	0.63	0.81
Omi	0.17	0.27	Serine	0.25	0.41	Valine	0.49	0.74
Serine	0.25	0.41	Tryp	0.63	0.81			
Tryp	0.63	0.81	Valine	0.49	0.74			
Valine	0.49	0.74						
<b>Fifth instar larvae</b>								
Ala	0.37	0.62	Ala	0.37	0.62	Ala	0.37	0.62
Arg	0.17	0.68	Arg	0.17	0.68	Asp	0.31	0.27
Asp	0.31	0.27	Asp	0.31	0.27	Glu	0.27	0.30
Cyst	0.18	0.19	Cyst	0.18	0.19	Gly	0.26	0.53
Glu	0.27	0.30	Glu	0.27	0.30	Hist	0.20	0.87
Gly	0.26	0.53	Gly	0.26	0.53	Lys	0.25	0.51
Hist	0.20	0.87	Hist	0.20	0.87	Omi	0.17	0.25
Lys	0.25	0.51	Lys	0.25	0.51	Serine	0.25	0.41
Meth	0.48	0.80	Meth	0.48	0.80			
Omi	0.17	0.25						
Serine	0.25	0.41						

Fig. 1: Seasonal variations in the free amino acids in the gut content of the II<sup>nd</sup> instar larvae of *Antheraea proylei* J. (a) Spring, (b) Summer and (c) Autumn

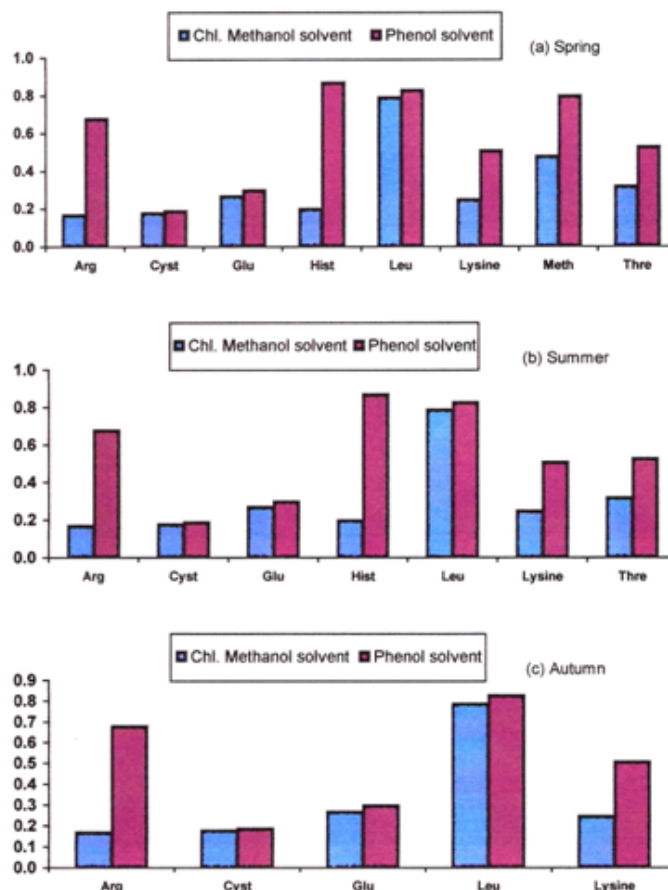


Fig. 2: Seasonal variations in the free amino acids in the gut content of the III<sup>rd</sup> instar larvae of *Antheraea proylei* J. (a) Spring, (b) Summer and (c) Autumn

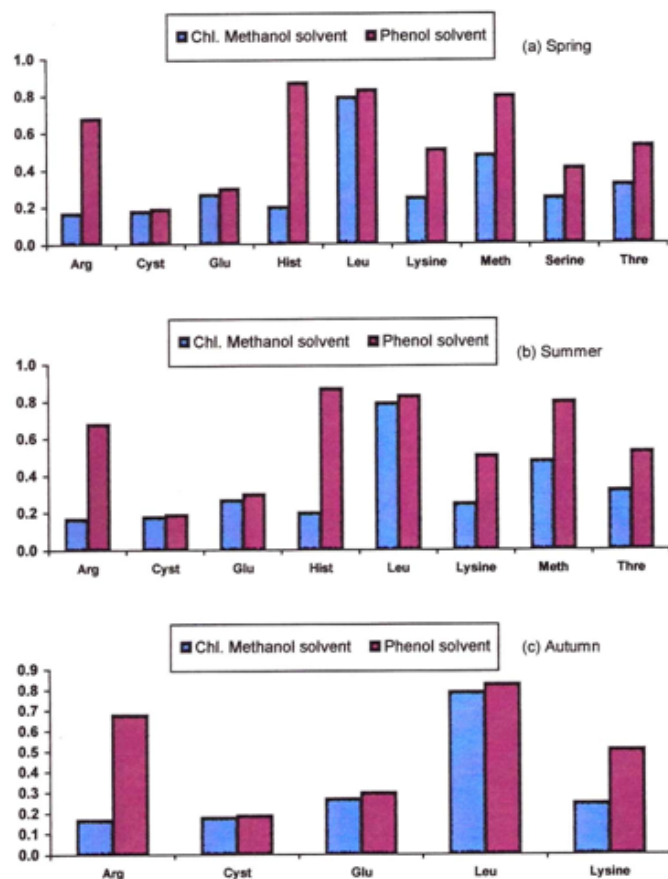


Fig. 3: Seasonal variations in the free amino acids in the gut content of the IV<sup>th</sup> instar larvae of *Antheraea proylei* J. (a) Spring, (b) Summer and (c) Autumn

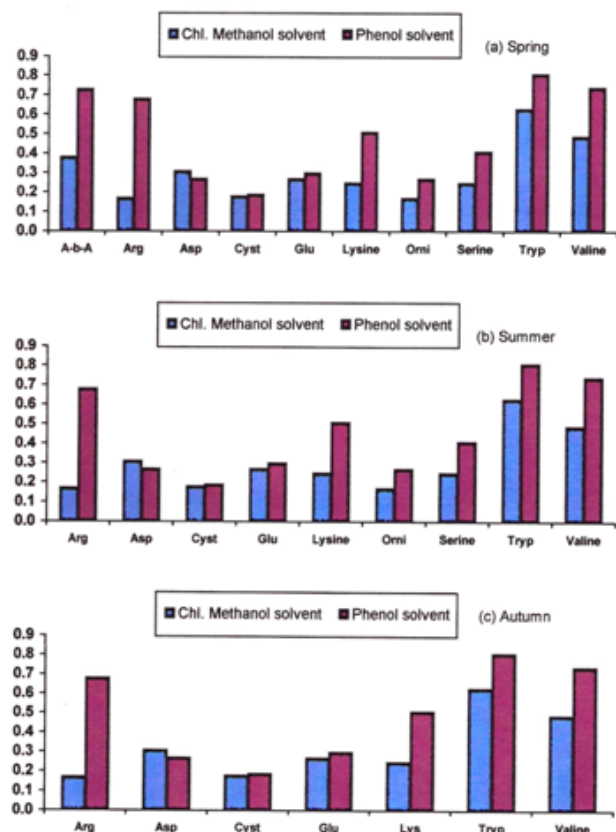
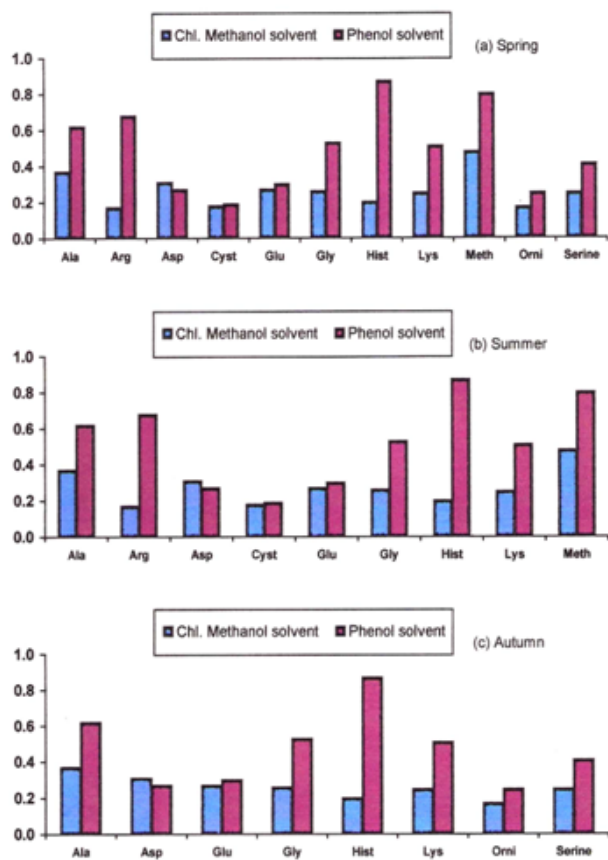


Fig. 4: Seasonal variations in the free amino acids in the gut content of the V<sup>th</sup> instar larvae of *Antheraea proylei* J. (a) Spring, (b) Summer and (c) Autumn



The extract gut content of fifth instar larvae exhibits 11, 9 and 8 amino acids in spring, summer and autumn season respectively. The amino acids detected are Alanine, Arginine, Aspartic acid, Cystine, Glutamic acid, Glycine, Histidine, Methionine, Ornithine and Serine in spring; Alanine, Aspartic acid, Cystine, Glutamic acid, Glycine, Histidine, Lysine and Methionine in summer and Alanine, Aspartic acid, Glutamic acid, Glycine, Histidine, Lysine, Ornithine and Serine in autumn season respectively.

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