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# Changes in Free Amino Acids during Embryonic Development of *Trabala vishnou* Lef (Lasiocampidae : Lepidoptera)

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Qualitative and quantitative changes in free amino acids (FAA) during embryonic development of *Trabala vishnou* have been studied. Only 14 FAA were present in freshly laid eggs. Cysteic acid, cystine, γ-aminobutyric acid, lysine, threonine and tryptophan appeared on different days of embryonic development. The total FAA content rose up to day 4 then declined on days 5 and 6 and thereafter increased again during the subsequent two days. Individually, most of the FAA followed this broad pattern of quantitative change. However, proline and aspartic acid declined through 9 days of embryonic development; contrary to this, alanine showed an increasing trend throughout. Increase in the FAA content is apparently associated with the breakdown of yolk reserve and the decrease of different FAA during different days of development may be related to the events of histogenesis and specific protein synthesis during organogeny.

**Key Words:** **Insect, *Trabala vishnou*, Free amino acids (FAA), Egg, Embryo**

## Introduction

The breakdown of the yolk reserves during embryogenesis contributes to the rise in level of amino acids that are utilized for protein synthesis. Studies on the Free Amino Acid (FAA) contents during larval, pupal and adult stages of various insects have been carried out by several workers (reviewed by Chen 1971). But for Drilhon and Busnel (1950), Shaw (1955), Crone-Gloor (1959), Chen and Briegel (1965) Indira (1963), MacFarlane and Hogan (1966), Roberts and Smith (1971) and Shukla and Verma (1978), relatively few investigators have examined the pattern of FAA during embryonic development of insects.

Some of these workers have reported approximate quantitative changes in the egg FAA pool (Shukla & Verma 1978). The present investigations were undertaken with a view to examine the pattern of changes (both qualitative and quantitative) during the embryonic development of *T. vishnou*.

## Materials and Methods

The larvae collected from castor plants in the vicinity of University Campus were reared at 28±2°C in the laboratory according to the method employed by Katiyar et al. (1975). Fresh leaves of Castor were provided as food after every 24 hours until the larvae pupated. The adults were kept in wooden cages (15 × 15 × 15cm) with wire mesh on all sides except the bottom. Only one pair of adult male and female was kept in a cage. Plain parchment paper was used to line the cages at the bottom and the sides. This helped easy collection of eggs. A swab of sterilized cotton soaked in 5% sucrose solution was kept in each cage primarily to maintain humidity and also to serve as a source of nourishment to the adults. After laying, the egg patches were kept in an incubator at 28 ± 2°C (relative humidity 70%) for embryonic development.

*FAA Extraction Procedure*

The eggs at different stages of development were collected, weighed and kept at -10°C in a deep freeze for subsequent analysis. The extraction procedure as described by Pant and Lal (1970) was followed. Eggs were properly homogenized in 80% ethanol. The homogenate was transferred to evaporating dishes and boiled while occasionally stirring on a water bath so as to obtain the volume 5–10 ml. The extract was filtered under vacuum, which was created by a suction pump. The filterate was evaporated to dryness. The dry sample was then washed twice with 3 ml of ether to remove lipids. Thereafter, the sample was dissolved in 20% ethanol so as to make the volume up to 1 ml. The solution was then centrifuged for 15 min at 4000 rpm. The clear supernatant was used for two-dimensional paper chromatographic analysis of FAAs.

*Chromatographic Technique*

Two-dimensional, ascending paper chromatographic technique of Consdon, Gordon and Martin (1944) was followed with a few modifications employing (28×28 cm) Whatman No. I paper. All runs were made in a dark room. This precaution gave better resolution.

The solvent system of Ganti and Shanmugasundram (1963) was used. Phenol saturated with water and ammonia (80 : 20 : 1.5) (v/v) was used for the first run. To prevent certain constituents of the filter paper (e.g. copper salts) from interfering with spreading of the spot, 8 hydroxyquinoline .02% (w/v) was added in the solvent system.

1N butanol, acetic acid and water (4 : 1 : 5) (v/v)-upper layer-was used for second directional run. 0.2% ninhydrin in 95% ethanol was used as the locating reagent which was uniformly sprayed on the dried chromatogram with the help of a glass atomizer. The chromatograms thus sprayed were initially dried at room temperature, then kept in chromatographic oven at 80±2°C for 20 min. Different spots of amino acids on the chromatograms were identified by co-chromatography of known samples.

*Quantitative Estimations*

All quantitative estimations during this study were carried out on Spectronic-20-spectrophotometer (Baush and Lomb). The spots were eluted for 30 min in 5 ml ethanol (50%). Optical density was measured at 540 nm (except for proline which was read at 420 nm). The standard curve of synthetic glycine (BDH Poole, England) was prepared by varying the spot loads so as to obtain the range of glycine concentration from 0.01 ml to 0.06 ml raising in steps of 0.01 ml.

Observations for each stage were made in three replicates.

## Results and Discussion

Qualitatively, the pattern of FAA varied on different days of embryonic development. In all, 14 amino acids-including 5 essential amino acids: arginine, histidine, leucine/isoleucine, methionine and valine were present in the eggs during the first 24 hr (table 1). Lysine and threonine appeared on day–2 and tryptophan appeared on day–5. Late appearance of these three essential amino acids indicated that they are released only gradually as a result of breakdown of the yolk. Of the sulphur bearing FAA, only methionine was found in the freshly laid eggs. However, cysteic acid appeared for only two days (day–5 and day–6), and cystine from day–6 to day–9. γ-Amino butyric acid (GABA) was also detected only on two days (day–6 and day–7). Only two unidentified spots were seen in the freshly laid eggs and until day–5, four such spots were found on day–6; and only one spot was noted on the last day (table 1).

The concentrations of various amino acids during different embryonic stages of *T. vishnou* have been given in table 1. Changes in the total concentration of FAA on different days of embryonic development are shown in figure 1. With the advancement of development the total concentration of FAA increased up to day–4. Thereafter, it declined sharply on days–5 and 6. On day–7 and 8 it rose again to reach almost the same level which was found on day–4. Finally, it declined on day–9, but even so, its level remained higher than that found in the freshly laid eggs. The analysis of variance showed a high significance at 5% level ($F_{(9,20)}=2.39$). Earlier workers have not reported a sharp decline in the total FAA concentration almost mid way during embryonic development (Indira 1963 on *Sphaerodema;* Chen & Briegel 1965 on *Culex pipiens*). The lepidopteran embryos undergo significant morphogenetic changes during this period. The neuroblasts give rise to ganglion cells, the body wall formation begins, the mouth parts are formed and the gut formation is well under way (Srivastava & Shukla 1979). It is thus assumable that the total FAA level considerably reduced due to histogenesis occurring during days 5 and 6 of embryonic development.

In the freshly laid eggs, alanine, aspargine, glutamic acid, histidine, leucine isoleucine, ornithine and serine/glycine ranged from 7.54% to 15% of the total FAA. Also, in the eggs of *Bombyx mori*, *Schistocerca gregaria*, *Sphaeroderma molestum* and *Teleogryllus commodus*, the concentration of alanine and glutamic acid has been especially high (Legay 1960, Colombo et al. 1961, Indira 1963 and MacFarlane & Hogan 1966). Arginine, aspartic acid, cysteic acid, cystine, glutamine, lysine, methionine, proline, threonine and tyrosine were detected in relatively low quantities (between 1.68% to 5% of the total FAA). Only two

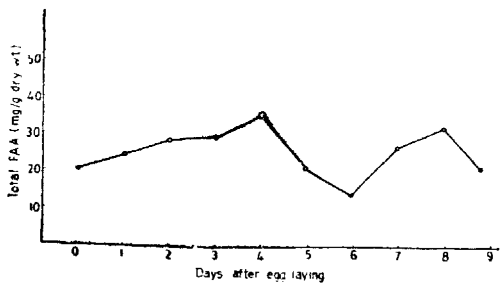


**Figure 1**

**Table 1** *Concentration of individual FAA in different embryonic developmental stages of* T. vishnou *(mg/g dry wt). Standard deviations have been mentioned in parenthesis* (±)

| Name of FAA | 0-day | 1-day | 2-day | 3-day | 4-day | 5-day | 6-day | 7-day | 8-day | 9-day |
|---|---|---|---|---|---|---|---|---|---|---|
| Alanine | 1.9905 (±0.09) | 2.3370 (±0.81) | 2.9222 (±0.82) | 3.2553 (±0.71) | 2.4764 (±0.31) | 2.2293 (±0.01) | 2.2161 (±0.77) | 6.2306 (±0.87) | 6.2547 (±0.25) | 6.0304 (±0.67) |
| Arginine* | 1.0794 (±0.01) | 1.6525 (±0.12) | 2.2888 (±0.16) | 0.4785 (±0.01) | 3.2228 (±0.41) | 1.6143 (±0.21) | 0.7570 (±0.14) | 1.1672 (±0.31) | 2.4253 (±0.09) | 0.2359 (±0.00) |
| Aspargine | 1.5618 (±0.001) | 1.9820 (±0.22) | 2.1412 (±0.67) | 1.6307 (±0.02) | 2.4399 (±0.21) | 0.8328 (±0.14) | 0.8947 (±0.06) | 1.0071 (±0.17) | 1.5813 (±0.09) | 0.8390 (±0.01) |
| Aspartic acid | 0.4363 (±0.02) | 0.6515 (±0.08) | 0.7640 (±0.09) | 0.9127 (±0.01) | 0.7465 (±0.01) | 0.7046 (±0.12) | 0.2411 (±0.08) | 0.2267 (0±.01) | 0.1101 (±0.002) | Trace |
| Cysteic acid | — | — | — | — | — | 0.4484 (±0.01) | 0.0550 (±0.001) | — | — | — |
| Cystine | — | — | — | — | — | — | 0.2340 (±0.08) | 0.6069 (±0.02) | 0.9219 (±0.07) | 0.4719 (±0.01) |
| —Amino butyric acid | — | — | — | — | — | — | 0.0550 (±0.001) | 0.4134 (±0.03) | — | — |
| Glutamic acid | 3.0623 (±0.57) | 3.4151 (±0.73) | 3.8374 (±0.06) | 3.5835 (±0.41) | 3.6594 (±0.45) | 2.1844 (±0.16) | 1.0324 (±0.72) | 3.0682 (±0.22) | 2.5671 (±0.09) | 0.7865 (±0.01) |
| Glutamine | 0.6584 (±0.004) | 0.8417 (±0.01) | 1.2259 (±0.09) | 0.9857 (±0.61) | 1.3201 (±0.21) | 0.6662 (±0.17) | 0.6194 (±0.45) | 0.7337 (±0.02) | 0.7658 (±0.18) | 0.2883 (±0.11) |
| Histidine* | 2.9934 (±0.18) | 2.8907 (±0.27) | 2.5447 (±0.09) | 3.4073 (±0.61) | 3.1682 (±0.21) | 2.8858 (±0.17) | 1.3214 (±0.45) | 2.5412 (±0.02) | 1.8438 (±0.19) | 1,7934 (±0.01) |
| Leucine/Isoleucine* | 1.73.02 (±0.09) | 1.8305 (±0.17) | 1.9481 (±0.09) | 2.5190 (±0.09) | 2.4490 (±0.51) | 1.8546 (±0.21) | 2.0371 (±0.17) | 2.6679 (±0.02) | 3.3614 (±0.19) | 2.4122 (±0.20) |
| Lysine* | — | — | 0.6361 (±0.01) | 0.9735 (±0.01) | 1.7844 (±0.11) | 0.4612 (±0.01) | 0.1101 (±0.01) | 0.6069 (±0.04) | 0.9573 (±0.02) | 2.5957 (±0.22) |
| Methionine* | 0.6584 (±0.01) | 0.6507 (±0.06) | 0.6048 (±0.01) | 0.8031 (±0.01) | 0.9104 (±0.01) | 0.5765 (±0.01) | 0.0825 (±0.01) | 0.3801 (±0.02) | 0.6098 (±0.01) | 0.4090 (±0.01) |
| Ornithine | 1.8757 (±0.76) | 1.8750 (±0.45) | 1.2596 (±0.02) | 0.9917 (±0.01) | 2.5673 (±0.09) | 0.8968 (±0.02) | 0.5643 (±0.02) | 1.3206 (±0.07) | 1.9076 (±0.08) | 0.8914 (±0.06) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Proline | 0.9187 (±0.02) | 0.9185 (±0.09) | 0.8480 (±0.02) | 0-8475 (±0.02) | 0.8193 (±0.01) | 0.6125 (±0.01) | Trace | 0.4135 (±0.03) | 0.4125 (±0.02) | 0.3932 (00) |
| Serine/Glycine | 1.6460 (±0.63) | 1.9517 (±0.75) | 2.1905 (±0.71) | 2.1906 (±0.08) | 2.6402 (±0.11) | 2.8315 (±0.61) | 1.5554 (±0.41) | 1.8676 (±0.23) | 1.9005 (±0.09) | 1.8616 (±0.19) |
| Threonine* | — | — | 0.9656 (±0.01) | 0.8883 (±0.02) | 2.0029 (±0.21) | 0.5765 (±0.02) | 0.0550 (±0.01) | 0.7403 (±0.03) | 1.0140 (±0.13) | 0.1573 (±0.16) |
| Tryptophan* | — | — | — | — | — | 0.2178 (±0.01) | 0.2340 (±0.02) | 0.6069 (±0.02) | 1.0637 (±0.19) | 0.4195 (±0.13) |
| Tyrosine | 0.3674 (±0.06) | 0.2550 (±0 12) | 06.396 (±0.01) | 1.5638 (±0.31) | 1.2381 (±0.11) | 0.5509 (±0.03) | 0.3280 (±0.01) | 0.6670 (±0.01) | 0.5602 (±0.03) | 0.2095 (±0.01) |
| Valine* | 0.6021 (±0.01) | 0.9550 (±0.09) | 1.3015 (±0.02) | 1.9470 (±0.21) | 1.9574 (±0.11) | 0.7366 (±0.05) | 0.4267 (±0.01) | 1.1205 (±0.09) | 1.4254 (±0.12) | 1.4683 (±0.10) |
| US-1 | — | — | — | — | — | — | — | — | 0.9219 (±0.09) | — |
| US-2 | — | — | — | — | — | — | 0.2340 (±0.02) | — | 0.9219 (±0.09) | — |
| US-3 | 0.6890 (±0.51) | 0.8908 (±0.62) | 0.9666 (±0.62) | 1.0466 (±0.21) | 0.8740 (±0.20) | 0.4356 (±0.24) | 0.3028 (±0.09) | 0.3468 (±0.22) | 1.0637 (±0.33) | 0.2097 (±0.22) |
| US-4 | 0.4364 (±0.34) | 0.9210 (±0.14) | 1.0076 (±0.31) | 0.9127 (±0.30) | 1.2904 (±0.12) | 0.6406 (±0.22) | 0.3028 (±0.12) | 0.5458 (±0.01) | — | Trace |
| US-5 | — | — | — | — | — | — | 0.3028 (±0.03) | — | — | — |
| Total | 20.7060 (±0.89) | 24.0187 (±0.85) | 28.2928 (±0.69) | 28.9375 (±0.45) | 35.5627 (±0.55) | 21.8569 (±0.57) | 13.9616 (∓0.51) | 27.0799 (±0.35) | 32.5899 (±0.72) | 21.4729 (±0.43) |

*Note :* Essential amino acids have been marked with astersik

*US* unidentified spot

Analyses of variance was worked out for different FAA as well as total FAA for value of $(F_{(9, 30)} = 2.39)$ and significance worked out at 5% level amino acids namely GABA and tryptophan were found to have the initial concentration of less than 1% (table 2). These observations differ from those of the previous workers (Crone-Gloor 1959 on *Drosophila;* Roberts & Smith 1971 on *Melanoplus)* who reported considerably high levels of aspartic acid and glutamic acid. The fact that alanine, leucines and lysine constituted a fairly high titre at the time of hatching, being 28.08, 12.08 and 11.23 percent of the total FAA, may be taken to indicate the significant role of these amino acids during the larval stages. Similarly, histidine and valine increased considerably through embryogenesis. Since most of these are essential amino acids, their importance for subsequent stages of development cannot be over emphasized. The analyses of variance of different essential FAA was noted to be significant, however, it was highly significant in the case of lysine and threonine at 5% ($F_{(9,20)} = 2.39$). The ANOVA of non-essential FAA was also significant except in the case of arginine, aspargine, aspartic acid and GABA, where it was not significant.

When considered individually, all amino acids showed a marked reduction in quantity on day–5 and day–6 (table 1). Most of the amino acid pool appears to be mopped up during this period as it could well be coincidental with the active phase of differentiation accompanying histogenesis (Srivastava & Shukla 1979).

Subsequent increase may be as a result of the degradation of the remaining quantities of yolk reserves.

An increase in alanine at the expense of aspartic acid during embryogenesis is similar as in the eggs of *Drosophila* and *Melanoplus* (Crone-Gloor 1959 and Roberts & Smith 1971). Likewise, cysteic acid appeared for the first time on day–5 and declined sharply on day–6. Cystine appeared on day–6 and rose quickly until day–8 to nearly four times its initial level and subsequently declined on day–9. The decline in the levels of methionine and cystic acid is correlated with the rise in the level of cystine, indicating the inter conversion of these sulphur containing amino acids. Fu (1957) has demonstrated in eggs of *Melonoplus* that the sulphur containing amino acids are constant through embryogenesis. Our observations confirm this constancy on days 5, 8 and 9.

Roberts and Smith (1971) have observed a continuous decrease in proline in the eggs of *Melanoplus*. Similar trend was also observed in this study. Possible reason for the decrease of proline could be its incorporation in the cuticular protein during sclerotization during differentiation (Hackman 1959).

Tyrosine content decreased within 24 hr of development. This may be due to sclerotization. It increased up to day–4 and then generally decreased up to the time of hatching. Chen and Briegel (1965) demonstrated a rise in the level of tyrosine in the eggs of *Culex pipiens*. They ascribed its decrease during development due to pigmentation and sclerotization.

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**Table 2** *Percentage of different FAA in the freshly-laid eggs and in the embryos just before hatching*

| Name of FAA | Alanine | Arginine* | Aspargine | Aspartic acid | Cystine | Glutamic acid | Glutamine | Histidine* | Leucine/ Isoleucine* | Lysine* | Methionine* | Ornithine | Proline | Serine/ glycine | Threonine* | Tryptophan* | Tyrosine | Valine* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Freshly laid eggs | 9.61 | 5.21 | 7.54 | 2.11 | — | 14.79 | 3.18 | 14.45 | 8.36 | — | 3.18 | 9.06 | 4.44 | 7.95 | — | — | 1.77 | 2.91 |
| Embryos just before hatching | 28.08 | 1.10 | 3.91 | Trace | 2.20 | 3.66 | 1.34 | 8.35 | 1.23 | 12.09 | 1.90 | 4.15 | 1.83 | 8.67 | 0.73 | 1.95 | 0.98 | 6.84 |

*Note :* GABA and cysteic acid were absent in freshly laid eggs and on day-9
Essential FAA have been marked with asterisk

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