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DIETARY AMINO ACID REQUIREMENTS OF THE TEA TORTRIX, *HOMONA COFFEARIA* NIETNER (LEPIDOPTERA. TORTRICIDAE) REARED *IN VITRO*

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The amino acid requirements of the tea tortrix, *Homona coffearia* was studied employing a holidic diet that had been perfected to rear this insect under defined laboratory conditions *in vitro* Only nine of the 10 essential amino acids were found to be essential for the healthy growth of the tea tortrix as deletion of the essential amino acid L-leucine from the diet containing the full complement of 10 essential amino acids was not found to affect the development of this insect Amongst these, threonine was found to be the most critical amino acid, as in its absence none of the larvae developed beyond the second instar The essential amino acids, arginine, isoleucine, phenylalanine, threonine, tryptophan and valine were found to act as phagostimulants Further supplementation of a diet containing the full complement of the 10 essential amino acids with their tyrosine or cystine was detrimental to this insect, as growth was retarded Supplementation with all 19 amino acids present in the protein supplement casein hydrolysate, was also found to suppress growth

INTRODUCTION

In general, although insects like higher animals require the well known 10 essential amino acids, some exceptions have been reported (Michaelbacher, Hoskins and Hermes, 1932 , Lafon, 1939 , House 1947 , Goldberg and de Meillon 1948 , Hinton, Noyes and Ellis, 1951 , Hodgson, Cheildon and Newburgh, 1956 , McGinnis, Newburgh and Cheidelin, 1956 , Cheidelin and Newburgh, 1959 , Strong and Sakomoto, 1963 , Chapman, 1971)

Various studies on amino acid requirements have shown that in order to support optimal growth, the diet should provide the essential and non-essential amino acids in the appropriate quantity in which these are required for the respective insects (Gordan, 1959) Although the amino acid requirements vary with (*a*) age (Beck, 1956 , Rock and King, 1966) , (*b*) nutritional state of the parent (Gordan, 1959) , (*c*) quantitative relationship between other nutrients (House, 1966), it is possible to determine a ratio representing the relative requirements of individual amino acids under a given set of conditions

One of the important limiting factors for the study of the amino acid requirements of insects has been the lack of a defined artificial (holidic) diet Such a defined holidic diet has been perfected to rear the tea tortrix (Gnanapragasam and Sivapalan, 1980) The present investigation was undertaken to study the critical amino acid requirements of the tea tortrix employing the developed holidic diet

MATERIALS AND METHODS

For the purpose of this study, diets were prepared by replacing the protein source (casein hydrolysate) in the starch-based synthetic diet with varying mixtures of known quantities of the respective amino acids Most of the amino acids, with the exception of L-tyrosine and L-cystine, were prepared in solution and the appropriate amounts added to the diets as needed L-cystine and L-tyrosine were dissolved in conc HCl and 2M KOH respectively, as described by Vanderzant (1957) All amino acids were of 99 9% purity (Sigma Chemicals, USA) The rest of the ingredients (other than the fatty acids) for the respective diets were mixed thoroughly into a homogenous mixture and stirred into 100 ml of hot water (Tables 1, 2 and 3) The fatty acids, linoleic and linolenic acids were provided as methyl esters (99 9% pure-Sigma Chemicals, USA) and delivered along with the antifungal agents, in 5 ml of alcohol and acetone (50% v/v) Approximately 5 g of the diet was dispensed into each of 150 X 15 mm glass tubes, plugged with cotton wool and autoclaved at 1 4 kg cm^2 for 20 min

A total of 22 diets were evaluated in two sets of experiments consisting of 12 and 10 diets respectively Each diet was replicated 8 times and the tubes were inoculated with tortrix larvae (4 larvae/diet) and maintained in a room under controlled conditions as described by Gnanapragasam and Sivapalan (1980) The state of development of larvae was observed and pupae removed, sexed and weighed individually and transferred to separate petri dishes to observe moth emergence

Experiment 1 To study the effect of dietary supplementation with non-essential amino acids on the development of the tea tortrix

A total of 12 diets were evaluated in this experiment the starch-based holidic diet containing casein hydrolysate as the protein source (D 88) was included as a control The composition of the different test diets are presented in Tables 2 and 3

Experiment 2 To study the effect of dietary deletion of essential amino acids on the development of the tea tortrix

In order to ascertain the minimal requirements of amino acids for the normal development of the tea tortrix, diet-deletion studies were carried out by deleting one of the 10 essential amino acids at a time from the defined diet containing the full complement of the 10 essential amino acids

A total of 10 such diets were evaluated in this experiment The diet containing all 10 essential amino acids was also included as a control The composition of the different diets are presented in Tables 2 and 4

TABLE 1—*Composition of amino acids in casein hydrolysate and wheat starch-based holidic diet.*

Amino acids	*Casein hydrolysate**	*Holidic diet*
Essential amino acids		
L—arginine	0.232 g	0.300 g
L—histidine	0.094 g	0.100 g
L—isoleucine	0.301 g	0.300 g
L—leucine	0.553 g	0.550 g
L—lysine HCL	0.346 g	0.340 g
L—methionine	0.188 g	0.180 g
L—phenylalanine	0.276 g	0.300 g
L—threonine	0.188 g	0.180 g
L—tryptophan	0.094 g	0.100 g
L—valine	0.326 g	0.320 g
Non essential amino acids		
L—alanine	0.252 g	0.250 g
L—aspartic	0.314 g	0.310 g
L—glutamic	0.538 g	0.530 g
Glycine	0.074 g	0.150 g
L—proline	0.489 g	0.480 g
L—serine	0.365 g	0.360 g
L—cystine	0.037 g	0.050 g
L—cysteine	0.012 g	0.012 g
L—tyrosine	0.321 g	0.321 g
	5.000 g	5.131 g

* Rock and King (1967)

TABLE 2—*Composition of basal diet to rear the tea tortrix, Homona coffearia.*

Ingredients	*Amounts*
Wheat starch[1]	20.0 g
Wesson's salt mixture	0.4 g
Vitamins[2]	1 cap
Ergosterol[3]	2.0 g
Linoleic acid[4]	0.1 g
Linolenic acid[4]	0.05 g
Cellulose	6.0 g
Agar (fine)	12.0 g
Methyl-p-hydroxy benzoate	0.2 g
Sodium benzoate	0.1 g
Distilled water	100 ml

[1] Pure (Nutritional Biochemicals, USA)

[2] Multivitamin capsules (Strong)—Pfizer, Inc. New York, USA (One capsule = 0.8g)

[3] Ergosterol - 99.9% pure (Sigma Chemicals. USA).

[4] Linoleic and linolenic acid furnished as methyl esters (Sigma Chemicals, USA).

TABLE 3—*Effect of dietary supplementation with non-essential amino acids in holidic diets on mean larval duration ± S. D; mean pupal number and mean pupal weight ± S. D. of Homona coffearia*

Diet no.	*Supplement to basal diet 1*				*larval duration (days)*		*pupal No.*[1] *per tube*	*Pupal weight (mg)*	
	Cas[2]	*N*[3]	*E. A.A.*[4]	*N. E. A. A.*[5]	*Males*	*Females*	($\sqrt{n+1}$)	*Males*	*Females*
D 88	+	—	—	—	26.0 ± 1.6	30.3 ± 2.4	1.64 bc	29.0 ± 5.0	76.3 ± 15.8
D 98	—	—	+	—	28.5 ± 3.7	36.3 ± 6.5	1.60 cd	33.0 ± 4.0	73.0 ± 9.3
D 99	—	+	+	—	27.8 ± 3.8	31.8 ± 4.0	1.64 bc	33.6 ± 4.7	73.0 ± 10.7
D 100	—	—	+	L-alanine	29.3 ± 6.7	35.2 ± 4.3	1.67 abc	28.0 ± 5.0	82.0 ± 16.8
D 101	—	—	+	L-aspartic	34.1 ± 4.5	38.7 ± 4.1	1.67 abc	28.6 ± 6.8	78.0 ± 16.9
D 102	—	—	+	L-glutamic	29.3 ± 2.1	36.6 ± 3.6	1.52 cd	31.0 ± 5.6	90.5 ± 3.2
D 103	—	—	+	Glycine	33.5 ± 4.7	37.3 ± 5.8	1.83 ab	29.2 ± 5.8	71.2 ± 26.8
D 104	—	—	+	L-proline	31.8 ± 3.0	35.0 ± 2.0	1.53 cd	32.3 ± 1.4	81.7 ± 27.5
D 105	—	—	+	L-serine	30.8 ± 7.0	36.7 ± 3.6	1.66 abc	32.0 ± 2.2	74.1 ± 12.4
D 106	—	—	+	L-cystine	33.3 ± 2.1	36.0 ± 1.4	1.35 d	33.7 ± 1.0	52.0 ± 0
D 107	—	—	+	L-cysteine	33.7 ± 3.2	37.8 ± 7.1	1.89 a	29.9 ± 2.4	80.0 ± 11.1
D 108	—	—	+	L-tyrosine	Nil	Nil	Nil e	Nil	Nil
D 109	—	—	19	amino acids	Nil	57.0 ± 0	1.05 e	Nil	26.0 ± 0

1. Values followed by same letter is not significant ($P=0.05$)—Duncan's Multiple Range Test.
2. Casein hydrolysate (5 g) ; 3. Tri ammonium citrate (0.1 g) ; 4. Full complement of essential amino acids as per amounts in Table 1.
5. Supplementation with individual non-essential amino acids as per amounts in Table 1.

Note : pH of all diets have been adjusted to 6.0.

TABLE 4—*Effects of dietary deletion of essential amino acids in holidic diets on the mean larval duration ± S. D., mean pupal number and mean pupal weight ± S. D. of Homona coffearia.*

Diet	*Deleted amino acid*	*Larval duration (days)*		*Pupal No.*[1] *per tube*	*Pupal weight (mg)*	
		Males	*Females*	$(\sqrt{n+1})$	*Males*	*Females*
D 98	Nil (control)	33.0 ± 5.3	36.9 ± 4.3	1.68 a	33.0 ± 5.1	84.4 ± 4.6
D 110	L-arginine	45.7 ± 7.1	48.0 ± 8.7	1.49 b	31.3 ± 5.1	59.7 ± 14.1
D 111	L-histidine	41.8 ± 3.2	44.6 ± 6.1	1.57 ab	25.5 ± 2.6	57.0 ± 6.4
D 112	L-isoleucine	33.5 ± 3.5	47.8 ± 6.8	1.49 b	26.3 ± 5.9	50.0 ± 18.0
D 113	L-leucine	37.2 ± 2.2	36.3 ± 5.8	1.68 a	26.0 ± 8.4	80.2 ± 13.9
D 114	L-lysine	37.8 ± 4.9	40.2 ± 4.2	1.65 ab	26.3 ± 6.8	68.8 ± 13.2
D 115	L-methionine	42.0 ± 8.0	43.6 ± 8.2	1.65 ab	26.1 ± 2.6	69.0 ± 17.1
D 116	L-phenylalanine	44.2 ± 5.2	49.8 ± 6.8	1.53 ab	25.5 ± 2.6	68.0 ± 13.2
D 117	L-threonine	Nil	Nil	Nil d	Nil	Nil
D 118	L-tryptophan	43.5 ± 8.9	49.0 ± 12.5	1.40 c	20.5 ± 5.1	38.0 ± 8.7
D 119	L-valine	34.7 ± 4.9	42.0 ± 7.4	1.40 c	28.5 ± 2.8	58.0 ± 25.1

Mean followed by the same letter not significant ($P=0.05$)—Duncan's Multiple Range Test

Note : pH of all diets have been adjusted to 6.0

RESULTS

Experiment 1

Larval duration The mean larval duration in the different diets are presented in Table 3 The larval duration of both male and female caterpillars harvested from the diets supplemented with the 10 essential amino acids, with or without the extra source of nitrogen (D 98 and D 99), was not significantly different from that of the control diet (D 88) The larval duration of males from diets further supplemented with L-alanine (D 100), L-glutamic (D 102) and L-serine (D 105) (in addition to the 10 essential amino acids) were also not significantly different from that of the control diet

The larval duration in all the other diets was significantly prolonged, the longest being in the diet D 109, supplemented with all the 19 amino acids (10 essential + 9 non-essential) and this period was significantly longer than the larval duration in all the above diets

When L-tyrosine was supplemented along with the 10 essential amino acids (D 108), no larvae developed beyond the second instar

Pupation The mean number of pupae harvested along with the mean pupal weights are also presented in Table 3 The largest number of pupae was harvested from the diet supplemented with L-cysteine along with the 10 essential amino acids (D 107) Although this number was significantly higher than that obtained from the control diet, it was not significantly different from those obtained from diets containing the 10 essential amino acids and further supplemented with either L-alanine (D 100), L-aspartic (D 101), glycine (D 103) or L-serine (D 105)

The pupal number from the diet containing only the 10 essential amino acids, with or without the extra source of nitrogen (D 98 and D 99), was also not significantly different from that of the control diet

Larvae failed to pupate in the diet D 108 supplemented with L-tyrosine in addition to the 10 essential amino acids Suppression of pupal numbers was also evident in the diet D 106 supplemented with cystine together with the 10 essential amino acids, as well as in the diet D 109 supplemented with all 19 amino acids The yield in the latter diet was significantly lower than those from the rest of the tested diets

As seen from Table 3 since the range of male pupal weights was very narrow the dietary effects were better reflected in the female pupal weights

A significant suppression of pupal weights was observed in the diet D 109 supplemented with all 19 amino acids

There was no significant difference in the size of pupae harvested from the rest of the tested diets except in the case of the diet D 102, supplemented with L-glutamic acid which yielded significantly larger pupae compared to those obtained from the rest of the tested diets

Adult emergence Most of the larvae that succeeded to pupate emerged as normal healthy moths and this therefore followed the trend observed for pupation

Experiment 2

Daily observations indicated a significant difference in the rate of feeding in the different test diets The larvae ate voraciously and produced large faecal pellets (similar to that observed in the starch-based holidic diet) in the diet containing all the 10 essential amino acids as well as in those diets lacking either L-histidine (D 111), L- leucine (D 113), L-lysine (D 114) or L-methionine (D 115) There was hardly any feeding in the diet where L-threonine was deleted (D 117) Deletion of L-arginine (D 110), L-isoleucine (D 112), L-phenylalanine (D 116), L-tryptophan (D 118) and L-valine was also found to be phago-inhibitory

Larval duration The larval duration in the different test diets are presented in Table 4 Deletion of either L-arginine (D 110), L-histidine (D 111), L-methionine (D 115), L-phenylalanine (D 116) or L-tryptophan (D 118) was found to significantly prolong the larval duration of both male and female caterpillars When isoleucine was deleted (D 112), the female larval duration was significantly prolonged though the male larval duration remained unchanged There was no significant difference in the larval duration of caterpillars reared in the rest of the diets

Pupation The mean number of pupae harvested from the different diets as well as the mean pupal weights are also presented in Table 4

No larvae developed beyond the second larval instar in the diet D 117, where L-threonine was deleted Larvae remained alive in these diets for about 40-50 days and died thereafter

There was a significant suppression in the number pupating from the diets lacking either L-tryptophan (D 118) or L-valine (D 119)

Although the largest number of pupae were harvested from the diet containing all the essential amino acids (D 98) as well as from the diets lacking L-leucine (D 113), this number was, however, not significantly different from those harvested from diets lacking either L-histidine (D 111), L-lysine (D 114), L-methionine (D 115) or L-phenylalanine (D 116)

Again, since the range of male pupal weights was narrow, dietary effects were better reflected in the female pupal weights Except the diet where L-leucine was deleted (D 113), the pupae harvested from all the other tested diets were significantly smaller in size compared to the control i e the diet containing all 10 essential amino acids

Adult emergence: All the larvae that succeeded to pupate in the different diets emerged as normal healthy moths and oviposited normally

DISCUSSION

For growth and development, all insects require amino acids which help in the production of body tissues and enzymes Usually present as proteins, they form a major part of an insect's food (Chapman, 1971) The nutritive value of a particular protein depends mainly on the essential amino acid content, although the pattern of the non-essential amino acids is not altogether without any significance (Rock, 1972) Experiments with chemical diets have demonstrated that free amino acids could be absorbed and utilized by most insects (Friend, 1958) The fat body in the insect is an active site for intermediary metabolism of amino acids and in general, most insects require the 10 essential amino acids needed for rats (Friend, 1958 , House, 1961, 1974 , Rees, 1977) However, exceptions have been reported Certain insects require in addition some of the non-essential amino acids as well (House, 1965, 1974) while others are even able to make do with less than 10 essential amino acids (Dadd and Kreiger, 1968 , Baker, 1979) This has been attributed to the presence of symbionts in the insect

The results of the diet deletion studies showed that the tea tortrix falls under the group of insects requiring less than the 10 essential amino acids for its normal growth and development Only nine of the 10 essential amino acids were found to be indispensable to the tea tortrix, as deletion of leucine did not affect larval duration, pupal number, pupal weight as well as the emergence of normal healthy adults

Generally, those amino acids not needed in the diet, unless provided by intestinal symbionts or by micro-organisms contaminating diet, are readily synthesized by the insect (Ishii and Hirano, 1955 , Kasting and Mc Ginnis, 1962) This seems to be the case with the tea tortrix as well, since no microbial symbionts were found to be present in the gut or the body tissues of this insect (Gnanapragasam and Sivapalan, unpublished data) and also the dietary ingredients used in these investigations were all pure and the larvae were grown under aseptic conditions

The results of Experiment 1 showed that the diet supplemented with all 19 amino acids present in casein hydrolysate performed very poorly (Table 3) Although, several investigators have successfully developed synthetic diets based on different protein sources, in most of them, growth was poor Naylor (1964) reported that amino acid mixtures supposedly equivalent to a given protein do not perform as well as the natural protein itself, since amino acids linked in peptide chains were nutritively more usable than simple mixtures of free amino acids On the other hand, failure to grow on a diet containing 19 amino acids may also be due to too high a concentration of osmotically active material in the diet, as compared to a diet with equivalent whole or partly hydrolysed protein (Dadd, personal communication) The poor performance in some of the diets used in the present study may also be due to an imbalance in the ratio of the respective amino acids, especially the non-essential ones, since the diet containing only the 10 essential amino acids performed equally well as the control diet containing casein hydrolysate as the protein source. Nutritional studies with rats have shown an imbalance amongst the dispensable amino acids proved to be detrimental for normal growth (Abernathy and Miller, 1965). Elvehjem (1956) reviewed the amino acid imbalance in animal nutrition and revealed the existence of toxicity and antagonism of natural amino acids thus complicating the evaluation of the essential requirements. Several investigators of insect nutrition have reported the importance of amino acid balance (Vanderzant, 1958 ; Gordan, 1959 ; Sang, 1959 ; Geer, 1966 ; Dadd and Kreiger, 1968 ; House, 1969; Chapman, 1971).

The present investigation has also shown that supplementation of tyrosine and cystine (in amounts more than what is required by the insect) was detrimental for normal development. An excess of tyrosine has been shown to be detrimental for the growth and development of the silkworm, *Bombyx mori* (Toshio Ito and Narihiko Arari, 1967). In most insects, an exogenous supply of tyrosine is not required as it is readily synthesized from phenylalanine (House, 1974) as demonstrated in *Chilo suppressalis* (Ishii and Hirano, 1958). The growth suppression observed with an exogenous supply of cystine may be either because it caused an imbalance of dietary ingredients or due to the requirement of sulphur-containing amino acid for this insect being adequately satisfied by the availability of methionine.

The presence of cysteine, however, improved the yield of pupae, although the larval duration was prolonged. This improvement may be due to the fact that although nine of the essential amino acids were adequate for normal growth and development of this insect, some may be in sub-optimal amounts and the cysteine may act as a precursor to supplement such deficiency or some growth stimulation may have resulted by its addition. Further, its addition may have also improved the amino acid balance of this mixture, resulting in improved yields.

The deletion studies (Experiment 2) showed the importance of threonine in the diet, as in its absence, none of the larvae were able to grow beyond the second instar, apparently because this insect lacked the capacity to synthesize the above amino acid (Table 4). On the other hand, since there was hardly any signs of feeding in the diet where threonine was deleted, it may be required as a phago-stimulant.

When ascertaining the nutrient status of a particular dietary ingredient through diet-deletion studies, one has to ensure that the deleted ingredient does not serve as a phago-stimulant. In the event such an ingredient serves as a phago-stimulant, the observed poor growth in its absence may not necessarily be due to a specific nutritional requirement for the deleted substance but simply due to under-feeding (Dadd and Kreiger, 1968). Amino acids like L-alanine, L-serine and L-proline have been found to act as phago-stimulants in the diet of Colarado potato beetle, *Leptinotarsa decemlineata* (Hsiao and Fraenkel, 1968) and for Praire grain wireworm, *Ctenecera destructor* (Davis, 1965).

The observed poor feeding activity in the absence of either arginine, isoleusine, phenylalanine, tryptophan or valine and the consequent poor growth performance could be due either to the fact that these were needed to sustain feeding activity or in addition to the lack of adequate synthetic potential of these amino acids.

Since feeding was normal in the diets where either lysine, histidine or methionine was omitted, the observed retarded growth in their absence may be due to a slow or inadequate synthesis of these respective amino acids. This is especially so since at least a small proportion of the larvae in such diets were able to reach the adult stage.

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