STUDIES ON THE NUTRITION OF THE TEA TORTRIX, HOMONA COFFEARIA NIETNER (LEPIDOPTERA:TORTRICIDAE) WITH PARTICULAR EMPHASIS ON THE REQUIREMENTS OF CARBOHYDRATES, AMINO ACIDS, FATTY ACIDS AND STEROLS.

By

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ABSTRACT

The present investigation was undertaken to further improve the artificial diets developed earlier to rear the tea tortrix, <u>Homona coffearia</u> Nietner <u>in vitro</u> (M.Phil Thesis, Gnanapragasam, 1979) and perfect a completely defined one (holidic diet) to study in detail, the optimal requirements of various essential dietary ingredients (a) carbohydrates, (b) amino acids, (c) fatty acids and (d) sterols, needed for normal growth and development of this insect through successive generations.

Amongst the various tested carbohydrates, wheat starch proved to be the only suitable one as it was only when this source of carbohydrate was used in a completely defined diet that the larvae were able to grow satisfactorily, pupate and emerge as normal healthy adults. Besides demonstrating the suitability of wheat starch as a good carbohydrate supplement, it was evident from the present investigation that, for successful development in defined holidic diets, it is essential to have the right proportions of carbohydrates to proteins. The optimal ratio was 20 g of wheat starch to 5 g of casein hydrolysate. Further increase of the protein supplement beyond 5 g proved to be detrimental. The optimal levels of vitamins, salt mixture and sterols were also ascertained for the above holidic diet. On the basis of diet deletion and supplementation studies it was evident that, for the normal healthy growth of this insect in a completely defined holidic diet, the latter need to be supplemented with only nine amino acids (nine of the 10 amino acids known to be essential dietary supplements for rats). Deletion of L-leucine from a diet containing the full complement of the 10 essential amino acids was not found to affect the development and growth of this insect.

On the other hand, a diet supplemented with all 19 amino acids present in the protein supplement, casein hydrolysate, was found to suppress growth. The poor growth observed in the above diet is attributed to the fact that amino acids linked in peptide chains are supposedly more nutritive than simple mixtures of free amino acids, which may sometimes even prove to be toxic. It was evident that the further additional supplementation of a diet containing the full complement of the 10 essential amino acids with either tyrosine or cystine was detrimental to this insect, as growth was retarded in such diets.

The essential amino acids arginine, isoleusine, phenylalanine, threonine, tryptophane and valine seemed to act as phagostimulants, as the deletion of any one of the above acids suppressed feeding activity. Amongst the ii

essential amino acids, threonine seemed to be the most critical one, as in its absence, none of the larvae developed beyond the second instar. In the absence of any one of the remaining essential amino acids, including arginine, isoleusine, phenylalanine, lysine, histidine, tryptophane, methionine and valine, although growth was severely retarded, at least a few larvae succeeded to reach adult-hood.

The results of fatty acid investigation have shown that either linoleic or linolenic acid could adequately satisfy the polyunsaturated fatty acid requirement of this insect. Further, the results of diet deletion studies in which both linoleic and linolenic acids were deleted and instead supplemented with various other fatty acids, showed that the saturated fatty acids, lauric or stearic acid could adequately substitute the critically needed polyunsaturated fatty acids. This insect seems to possess the mechanism to metabolize and desaturate the long-chain saturated fatty acids, lauric and stearic acids, to linoleic and/or linolenic acid. This is one of the very rare instance amongst the lepidopterous and orthopterous species, as most investigated insects belonging to these orders have to be provided with either one of the essential polyunsaturated fatty acids.

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None of the other fatty acids tested in the present investigation seemed to be having a sparing effect on the polyunsaturated fatty acid requirement, as diets supplemented with capric, caprylic, caproic, myristic, palmitic, oleic and arachidonic acids yielded either partially emerged moths or deformed moths, with naked crumpled wings showing the typical fatty acid deficiency symptoms. In addition to being not able to substitute for linoleic and linolenic acids, the above tested acids (apart from palmitic acid) were also found to be toxic to this insect, as in their presence larval growth was suppressed.

The results of metabolic studies undertaken in this investigation showed that diets devoid of any fatty acids but supplemented with only sodium acetate were able to yield large numbers of normal adults with well formed wings. On the other hand, diets devoid of both fatty acids as well as sodium acetate, failed to produce any adults. This seems to convincingly demonstrate the ability of this insect to metabolize such polyunsaturated fatty acids from simple acetates.

The results of detailed fatty acid analysis by gas-liquid chromatography of of the tissues of insects reared on diets supplemented with (a) both essential fatty acids, linoleic and linolenic acids; (b) devoid of fatty acids but supplemented with sodium acetate; (c) devoid of both fatty

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acids and sodium acetate, further confirms the fact that this insect is capable of synthesising its requirements of essential long-chain polyunsaturated fatty acids from simple acetates. This is indeed one of the rare instances of an insect belonging to the order lepidoptera having such a capacity, as the few earlier reported instances of insects not needing an exogenous source of essential fatty acids were those in which there was a possibility of them deriving their dietary requirements from their intestinal microflora.

The fact that this insect failed to emerge as normal adults from diets lacking both fatty acids as well as a supplemented source of sodium acetate, and the complete absence of linolenic acid from the body tissue of caterpillars reared in such diets, seem to convincingly demonstrate the critical importance of linolenic acid for successful adult emergence. Even though the chemical analysis of larval tissue reared in such diets revealed the presence of linoleic acid, the latter seem to be present in inadequate amounts to help metabolize adequate and detectable amounts of this critical linolenic acid.

The results of sterol investigations have shown that this insect is able to utilize a wide spectrum of sterols, including cholesterol, cholesterol acetate, ergosterol, 7-dehydrocholesterol, sitosterol and stigmasterol. All of

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these above sterols differ from each other both in the degree of ring saturation as well as in the extent of branching of the side chain; <u>eg</u>: presence of a double bond in the side chain (ergosterol, stigmasterol); a second double bond in the cyclic portion (ergosterol, 7-dehydrocholesterol); branching of the side-chain (b-sitosterol and stigmasterol).

The normal development observed in diets supplemented with the phytosterols, b-sitosterol and stigmasterol, demonstrates the ability of this insect to dealkylate C_{28} and C_{29} plant sterols to cholesterol, which is a mechanism found prevalent in all other phytophagous insects belonging to the orders coleoptera, hemiptera, lepidoptera and orthoptera.

The very limited growth observed in diets lacking sterols, as well as in those supplemented with only sodium acetate, showed that, in common with all other insects, this insect too was unable to synthesize sterols. Furthermore, this insect is unable to utilize sterol-related compounds like calciferol (in which the tetracyclic arrangement of the C atom is modified and hence not a true sterol) and lanosterol (a precursor of cholesterol in vertebrates), as growth was very poor in diets supplemented with either of these latter compounds.

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Such detailed nutritional investigations help us to elucidate certain critical metabolic pathways that could be blocked by certain compounds known to be physiological inhibitors (semio chemicals) and thus achieve control at the physiological level. Some of these semio compounds have been already evaluated in the previous investigation and the present study has now enabled us to more effectively assess the activity of additional semio compounds and thereby work towards a cheap and practical method of containing outbreaks of this pest in tea fields.

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