Tea Q. 51 (3), 118-126, 1982. Printed in Sri Lanka.

DEVELOPMENT OF A DEFINED DIET TO REAR THE TEA TORTRIX, *HOMONA COFFEARIA* NIETNER, *IN VITRO*

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Wheat starch was the only source of carbohydrate that enabled the successful growth and development of the tea tortrix, *Homona coffearia* Nietner in a defined dietary medium. The ratio of carbohydrates to protein was also found to be critical in this case the optimum ratio was found to be 20g of wheat starch to 5g of casein hydrolysate. Further increase of the protein supplement beyond 5g proved to be detrimental. Reduction in the levels of vitamins did not alter growth but the level of sterol and salt was found to be critical and reduction or increases led to growth delays and affected pupal size.

INTRODUCTION

A simplified meridic diet consisting of only brewer's yeast and the fatty acids linoleic and linolenic acids was successfully developed to rear the tea tortrix, *Homona coffearia* Nietner, *in vitro* (Sivapalan & Gnanapragasam, 1979). Although this is a very simplified diet to prepare, brewer's yeast contains several unknown dietary constituents. Attempts were therefore made to develop a completely defined diet to elucidate in detail the dietary requirements of this insect to successfully rear it to adulthood.

Initial attempts were made by adding those ingredients that are known to be essential for most insects. These included known sources of carbohydrates, proteins, sterols, vitamins and minerals. By a process of supplementation and deletion in a series of experiments, progress was made towards the development of a completely defined diet.

MATERIALS AND METHODS

The different ingredients for the respective diets were mixed thoroughly into a homogenous mixture and stirred into 100 ml of hot water (Tables, 1, 2, 3 & 4). The fatty acids linoleic and linolenic acids (in the form of methyl esters, 99.9% pure-Sigma Chemicals, USA), were delivered along with antifungal agents in 5ml of alcohol and acetone (50% v/v). Ten grammes of agar was finally stirred into the hot medium and about 5g of the diet was dispensed into 150 x 15mm glass tubes and plugged with cotton wool. The diet tubes were then autoclaved at 1.4 kg/cm^2 for 20 min.

A total of 23 diets in 3 sets of experiments consisting of 6, 11 and 6 diets respectively were evaluated. Each diet was replicated 8 times and the tubes were inoculated and maintained in a room under controlled conditions as described by Sivapalan *et al*, 1977.

The state of development of the larvae was observed daily and pupae removed, sexed and weighed individually and transferred to separate petri dishes to observe moth emergence.

Experiment 1: *Evaluation of a suitable source of carbohydrate*

To a diet consisting of the basal ingredients presented in Table 1, various sources of carbohydrates were added individually to assess their respective efficacy. These different carbohydrates included sucrose, and the simple sugars, glucose, mannose and fructose as well as pure starch and wheat starch. A total of 6 diets were thus assessed in this experiment. The simplified meridic diet consisting of only Brewer's yeast and the fatty acids linoleic and linolenic acids was also included as a control.

Experiment 2: *Effect of varying levels and proportions of carbohydrates to proteins.*

Having evaluated the most suitable carbohydrate source for this insect, varying ratios of this carbohydrate and the protein source (casein hydrolysate) were tested to improve diet efficacy so as to be comparable to that of the meridic diet. A total of 11 diets were prepared along with the control meridic diet and the composition of these different diets are presented in Tables 1 and 3.

Experiment 3: *Effect of varying levels of sterol, vitamins and salt mixture on the growth and development of the tea tortrix reared on a starch-based holidic diet.*

In order to ascertain the optimal levels of essential dietary ingredients in the developed holidic diet, a total of 6 diets were prepared by varying the levels of sterol, vitamins and salt mixture. The composition of the different diets are presented in Tables 1 and 4.

RESULTS

Experiment 1: Evaluation of a suitable source of carbohydrate

The results of experiment 1 are presented in Table 2. As seen from Table, it was only when wheat starch was used as a carbohydrate supplement (D 76) that there was a significant improvement in the development of the caterpillars and they succeeded to pupate and emerge as normal healthy adults. However, when compared to the control meridic diet, the number pupating was low, the pupal weight small and the larval duration prolonged.

In the diet containing sucrose as the carbohydrate supplement, the larvae were able to reach only upto the final larval instar, but growth was very slow and they all failed to pupate. At the end of 45-60 days the larvae became quiescent, shrank and the head became bent resembling the pre-pupal behavioural signs during normal development (Gnanapragasam & Sivapalan, 1980) but failed to pupate and died after about 75 days.

Glucose was found to be a very poor carbohydrate since in this diet, growth was very much retarded and the larvae were able to grow only upto the second instar.

In the case of the diet containing fructose, mannose and pure starch as the respective carbohydrate supplement, growth was extremely poor and all larvae died within a few days following inoculation, without undergoing a single moult.

Experiment 2: *Effect of varying levels and proportions of carbohydrates to proteins*

Larval duration: The mean larval duration of caterpillars reared in the different test diets are presented in Table 3. As seen from Table, when supplemented with 5g of wheat starch to both 5 and 7.5g of protein source (D 76 and D 83), the larval duration was significantly prolonged when compared to the rest of the tested diets. Further increases in the starch levels to 10, 15 and 20g (irrespective of the level of casein hydrolysate) significantly reduced the larval duration. In the diets supplemented with 25 and 30g of wheat starch, although the male larval duration was significantly shortened the female larval duration was prolonged. Growth performance was observed to be best in the diet containing 15g of wheat starch and 5g of casein hydrolysate and in this diet the larval duration was not significantly different from that observed in the control meridic diet (D 36).

TABLE 1 — *Composition of basal diets for rearing the tea tortrix* *Homona coffearia* *in a holidic diet*

Ingredients	*Basal Diet I Expt. I*	*Basal Diet II Expt. 2*	*Basal Diet III Expt. 3*
Wheat starch	Nil	Nil	20.0g
Casein hydrolysate[1]	5.0g	Nil	5.0g
Ergosterol	2.0g	2.0g	Nil
Multivitamins[2]	2 caps	2 caps	Nil
Wesson's salt mixture	0.4g	0.4g	Nil
Cellulose	5.0g	5.0g	5.0g
Linoleic acid[3]	0.1g	0.1g	0.1g
Linolenic acid[3]	0.05g	0.05g	0.05g
Agar (fine)	10.0g	10.0g	10.0g
Methyl-p-hydroxy benzoate	0.2g	0.2g	0.2g
Sodium benzoate	0.1g	0.1g	0.1g
Distilled water	100 ml	100 ml	100 ml

[1] Enzymatically hydrolysed, vitamin free (Nutritional Biochemicals, USA).
[2] Multivitamin capsules (Strong-Pfizer, Inc. New York, USA).
[3] Linoleic and linolenic acids in the form of methyl esters, Sigma Chemicals, USA.

TABLE 2 — *Mean larval duration ± S.D., Mean pupal number and Mean pupal weight ± S.D., of Homona coffearia reared on different diets*

Diets	*Supplements to basal diet 1*	*Larval duration days*		*Pupal No.*[1] *per tube*	*Pupal weight (mg)*	
		Males	*Females*	$(\sqrt{n + 1})$	*Males*	*Females*
D 67	5.0g sucrose	Nil*	Nil*	Nil*	Nil*	Nil*
D 72	5.0g Glucose	Nil**	Nil**	Nil**	Nil**	Nil**
D 73	5.0g Mannose	Nil***	Nil***	Nil***	Nil***	Nil***
D 74	5.0g Fructose	Nil***	Nil***	Nil***	Nil***	Nil***
D 75	5.0g Pure starch	Nil***	Nil***	Nil***	Nil***	Nil***
D 76	5.0g Wheat Starch	46.6±3.0	50.0±10.0	1.41 b	29.2±2.4	63.0±1.0
D 36	Meridic diet (control)	22.7±3.0	27.5±2.4	1.75 a	34.7±4.0	76.9±6.9

[1]Mean followed by the same letter not significant ($P=0.05$) - Duncan's Multiple Range Test
*Larva grew upto final instar, showed pre-pupal behavioural signs.
**Larva grew only upto the second instar and died after a few weeks
***Larva died soon after inoculation.

TABLE 3 — *Mean larval duration ± S.D., Mean pupal number and Mean pupal weight ± S.D., of Homona coffearia reared on different diets*

Diet	*Supplement to basal diet II (mg)*		*Larval duration (days)*		*Pupal No.*[1] *per tube*	*Pupal weight (mg)*	
	Wheat starch[2]	*Casein*[3]	*Males*	*Females*	$(\sqrt{n+1})$	*Males*	*Females*
D 76	5.0	5.0	41.5±5.5	47.3±1.5	1.45 ef	23.8±2.0	63.0±14.3
D 83	5.0	7.5	40.7±7.2	40.7±3.1	1.31 f	33.0±5.0	58.0±14.1
D 84	10.0	5.0	30.0±4.8	36.0±5.7	1.65 cd	28.0±4.5	70.0± 7.0
D 85	10.0	7.5	32.0±0.0	0	1.05 g	23.0±0	0
D 86	15.0	5.0	26.9±2.1	28.5±1.8	1.57 de	28.0±5.0	85.8±18.7
D 87	15.0	7.5	26.8±3.5	32.0±2.2	1.57 de	33.6±4.7	81.3±21.2
D 88	20.0	5.0	31.3±5.2	34.6±5.3	1.80 b	28.0±3.5	77.4±18.0
D 89	20.0	7.5	36.1±5.5	34.5±4.4	1.73 bc	31.0±5.3	71.3±13.6
D 90	20.0	10.0	31.6±5.8	36.0±1.0	1.45 cf	34.0±4.1	65.9± 9.5
D 91	25.0	5.0	32.3±5.7	41.0±3.8	1.83 ab	34.4±3.7	76.1±15.5
D 92	30.0	5.0	32.8±5.9	38.8±5.5	1.80 b	31.0±2.6	75.0± 7.1
D 36	Meridic diet (control)		21.2±2.6	25.5±2.9	1.96 a	31.9±4.5	75.5± 9.7

[1]Mean followed by same letter not significant (P=0.05) - Duncan's Multiple Range Test
[2]Pure starch (Nutritional Biochemicals, USA)
[3]Enzymatically hydrolysed, vitamin free, (Nutritional Biochemicals, USA).

TABLE 4—*Effect of varying levels of salt mixture, vitamins & Sterols on the mean larval duration ±S.D, Mean pupal number and mean pupal weight ± S.D., of Homona coffearia reared on different diets.*

Diet	*Supplement to basal diet III*			*Larval duration (days)*		*Pupal No.*[1] *per tube*	*Pupal weight (mg)*	
	Salt mixture[2] *(g)*	*Vitamins*[3]	*Sterol*[4] *(g)*	*Males*	*Females*	$(\sqrt{N+1})$	*Males*	*Females*
D 88 (control)	0.4	2 caps	2.0	31.3±5.2	34.6±5.3	1.83 a	34.7±2.6	80.0±14.4
D 93	1.0	2 caps	2.0	30.5±3.1	37.0±4.2	1.49 b	28.0±9.1	96.3±10.3
D 94	0.4	3 caps	2.0	35.5±4.6	42.6±3.3	1.55 b	31.3±3.0	88.0±12.2
D 95	0.4	1 cap	2.0	29.7±4.6	37.0±6.6	1.87 a	35.1±3.9	83.0±10.2
D 96	0.4	2 caps	1.0	37.2±4.5	42.0±1.6	1.47 b	29.2±2.4	81.3± 7.5
D 97	0.4	2 caps	4.0	35.5±4.2	43.5±5.9	1.69 ab	31.6±12.2	111.7±12.2

[1]Mean followed by same letter not significantly different (P=0.05) - Duncan's Multiple Range Rest
[2]Wesson salt Mixture
[3]Multivitamin capsules (Strong Pfizer, USA)
[4]Ergosterol, 99.9% pure (Sigma Chem, USA).

Pupation: The mean number of pupae harvested and the mean pupal weights are also presented in Table 3. As seen from Table, the largest number of pupae was harvested from the diet supplemented with 25g of wheat starch and 5g of casein hydrolysate (D 91) and this number was almost equal to that harvested from the control meridic diet D 36. The diets supplemented with 20g and 30g of wheat starch (D 88 and D 92) respectively (each receiving 5g of casein hydrolysate) also gave significantly large number of pupae not significantly different from that of the diet receiving 25g of wheat starch. Increasing the level of casein hydrolysate above 5g brought about a significant suppression in pupal numbers, irrespective of the level of carbohydrate.

As seen from Table, varying the levels of starch and casein hydrolysate did not have any significant effect on pupal weights of both male and female pupae harvested from the different test diets. However, the female pupal weights of those harvested from diets supplemented with 5g of wheat starch (D 76 and D 83) were found to be smaller compared to the rest of the diets receiving higher amounts of wheat starch.

Adult emergence: All the pupae succeeded to emerge as normal healthy moths with well formed wings and they all oviposited normally.

Experiment 3: *Effect of varying levels of sterol, vitamins and salt mixture on the growth and development of the tea tortrix reared on a starch-based holidic diet*

Larval duration: The mean larval duration of caterpillars reared in the different diets are presented in Table 4. As seen from Table, no significant difference was observed in the mean larval duration other than in diet D 97, where the level of sterol was doubled. In the latter diet, the females had a significantly longer larval duration compared to all the other tested diets.

Pupation: The mean number of pupae harvested from the different test diets along with the respective mean pupal weights are presented in Table 4. As seen from Table, the number of pupae harvested from the diets supplemented with the higher level of salt mixture (D 93) and vitamins (D 94) as well as from diet supplemented with half the amount of sterol (D 96) were significantly lower than that of the control diet D 88. The number of pupae harvested from the diet D 95, supplemented with only half the amount of vitamins and the diet D 97 supplemented with double the amount of sterol was not significantly different from that of the control diet D 88.

There was no significant difference in pupal weights other than those from the diet D 97 supplemented with double the amount of sterol which yielded significantly bigger pupae.

Adult emergence: There was no significant difference in the number of emerging adults as all the larvae that pupated succeeded to emerge as normal healthy adults.

DISCUSSION

Even though some rare group of insects could grow without a dietary source of carbohydrate, most insects need a considerable amount as a source of energy for growth and development (Trager, 1953). In certain insects like *Musca* (Diptera) carbohydrates could be replaced by fats and proteins. This depends on the ability to convert these compounds into intermediate products suitable for use in the cycles of energy transformation (Chapman, 1971).

The results of the Experiment 1 in the present investigation have shown that it was only when wheat starch was used as a carbohydrate supplement in a completely defined diet, that the larvae were able to grow satisfactorily, pupate and emerge as normal healthy moths. Pure wheat starch is known to contain 28% amylose and 72% amylopectins. (Brown, 1960). Amylose consists of glucose units in straight chains linked through glycosidic linkages whilst amylopectins consists of chains of glucose which are branched and cross-linked. The latter therefore is likely to increase the 'roughage' of the diet and consequently infiuence the digestibility coefficient. Since substitution with pure starch (consisting of only amylose) was not found to be effective in improving the growth, it is apparent that it is the amylopectins that are important. Consequently a source of carbohydrate that is rich in amylopectins is the one that seems to be beneficial.

Carbohydrates are known to have a considerable influence on the feeding behaviour of insects. As attractants, arrestants and incitants they influence orientation. As stimultants, they influence the maintenance of feeding (Chipendale, 1978). Earlier investigations on the tea tortrix have shown that sucrose was phago-inhibitory (Sivapalan & Gnanapragasam, 1979). Larvae in diets lacking sucrose were found to eat voraciously and produced large faecal pellets. The larvae growing in holidic diets supplemented with wheat starcn also eat voraciously and had large faecal pellets. Thus, besides contributing to the 'roughage' of the diet, it is possible that the amylopectins serve as phagostimultants and consequently enable the larvae to continuously feed actively and further improve the digestibility coefficient to the optimum level. It is interesting to note that the tea leaf is also rich in amylopectins (Wickremasinghe, Personnel Communication).

Besides demonstrating the suitability of wheat starch as a good carbohydrate supplement for the development of the tea tortrix, the results of Experiment 2 have further shown that for successful development in such holidic diets, it is essential to have the right ratio of carbohydrates to protein. Increasing the level of wheat starch from 5g to 20g resulted in a significant improvement in development. However, further increase to 25g and 30g was not very satisfactory as such changes prolonged female larval duration. Thus the optimal level of wheat starch needed for the growth of this insect in a holidic diet was 20g/143g of diet.

Five gramme of casein hydrolysate was found to be the optimum level as any further increase led to a significant suppression in pupal numbers. Similar effects were observed in the earlier investigations where levels of casein hydrolysate brought about a suppression in the development (Sivapalan & Gnanapragasam, 1979). This was attributed to the possible toxic effects caused by the accumulation of peptides and also due to the imbalance brought about by the addition of excess protein thereby altering the nutritive quality of the diet and consequently the digestibility coefficient.

From the results of Experiment 3 it appears that the amount of vitamins provided in the control diet (D 88) could be halved as the larval duration, pupal number and pupal weight were not significantly different from that of the control. The amount of salt mixture and sterol provided in the control diet seems to be the optimal level, as further changes suppressed pupal number. Although significantly bigger pupae were harvested from the diets supplemented with double the amount of sterol, the pupal number remained unchanged, whilst the larval duration became prolonged indicating that doubling the amount of sterol was not satisfactory.

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