Rearing *Chrysoperla carnea* (Neuroptera: Chrysopidae) on Chemically Defined Diets

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Larvae of lacewings developed into adults on four chemically defined diets of different amino acid composition. The diets were composed of 23 amino acids, sucrose, trehalose, 5 organic acids, 6 fatty acids, cholesterol, 11 mineral salts, and 17 vitamins. The adults on the best diet produced more than 1,000 eggs over 2 months.

INTRODUCTION

Powdered drone honeybee (DP) offers appreciable nutrition for some aphidophagous insects including coccinellids (MATSUKA and NIIJIMA, 1985) and chrysopids (OKADA et al., 1974). Since a substitute diet was successfully used for *Chrysopa nipponensis* (HASEGAWA et al., 1983), we started developing chemically defined diets for the lacewing. FERRAN et al. (1981) reported that powdered female honeybee is also good for rearing *Chrysoperla carnea*, which is now known to be synonymous with the former (TSUKAGUCHI, 1985).

HAGEN (1987) claimed that *C. carnea* is the most intensively studied chrysopid and that the nutritional requirements of such predaceous insects have been largely determined only for *C. carnea* (see also CANARD et al., 1984). VANDERZANT (1969, 1973) reported on artificial diets composed of protein hydrolyzate, fructose, vitaminous substances, minerals, and lipids (cholesterol, soybean lecithin and oil). Since mass rearing of *C. carnea* was found to be possible using lepidopteran eggs (CANARD et al., 1984; HAGEN, 1987), until the present study, nutritional studies have not advanced.

As discussed later, previous artificial diets were insufficient, so we formulated several totally synthetic diets based on the composition of artificial diets for the coccinellid *Harmonia axyridis* (NIIJIMA et al., 1977), other predaceous insects (SINGH, 1977), and analyses of drone powder and aphids. Our diets were sufficient to rear lacewing larvae to adults that produced a good number of eggs.

MATERIALS AND METHODS

Chemically defined diets. The compositions of the four different diets (CDH 1–4) are listed in Tables 1 and 2. A preliminary diet, which was composed of 23 amino acids, sucrose, 4 fatty acids, cholesterol, lecithin, 6 mineral salts, and 15 vitamins (composition not shown), seemed insufficient. Subsequently, the amino acid and mineral composition was modified based on the analytical data from several aphids (HARPER and KALDY,

Table 1. Composition of chemically defined diets CDH 1-4

	A	B		
Amino acids ^a	43.5	34.8	Nicotinic acid	0.05
Sugars	(43.5)	(52.2)	Pyridoxine (B ₆)	0.02
Sucrose	34.8	41.76	Ca-Pantothenate	0.05
Trehalose	8.7	10.44	Biotin	0.005
			Folic acid	0.005
Fatty acids	(3.2)		Cobalamine (B ₁₂)	0.0025
Myristic acid	0.2		L-Ascorbic acid (C)	0.3
Palmitic acid	1.0		p-Aminobenzoic acid	0.0025
Stearic acid	0.2		Carnitine (B _T)	0.0025
Oleic acid	1.0		Vitamin P	0.0025
Linoleic acid	0.3		β-Carotene (A)	0.01
Linolenic acid	0.5		Ergosterol (D)	0.005
			α-Tocopherol (E)	0.005
Cholesterol	0.5			
			Minerals	(6.9)
Organic acids	(1.3)		K ₂ HPO ₄	4.20
Malic acid	0.5		MgSO ₄ · 7H ₂ O	1.91
α-Ketoglutaric acid	0.3		NaCl	0.43
Succinic acid	0.3		CaCO ₃	0.20
Fumaric acid	0.1		FeCl ₃ · 6H ₂ O	0.070
Citric acid	0.1		ZnSO ₄ · 7H ₂ O	0.060
			CuCl ₂ · 2H ₂ O	0.015
Vitamins	(1.10)		MnCl ₂ · 4H ₂ O	0.010
Choline chloride	0.3		KI	0.004
Inositol	0.3		CoCl ₂	0.0005
Thiamin (B ₁)	0.02		Na ₂ MoO ₄ · 2H ₂ O	0.0005
Riboflavin (B ₂)	0.02			
			Total	100.0

^a See Table 2 for amino acid composition.

1978; KALDY and HARPER, 1979; NIJIMA et al., 1986) and other insects (SINGH, 1977; MATSUYAMA et al., 1974). Two series of each diet, which differed from each other in the ratio of sugars and amino acids (expressed as C: N): A (1: 1) and B (1: 1.5), were prepared (Table 1). Each component was mixed with distilled water (ca. 10 vol.); fat-soluble materials in ethyl ether were added and then the mixture was lyophilized to obtain the powdery diet. Some subsequent modifications and their results will be described in the text.

Insects and rearing. The adult lacewings (G₀) were collected during May to October, 1983 in Machida City, Tokyo. The first generation (G₁) was reared on a drone powder diet (MATSUKA and NIJIMA, 1985) in petri dishes (9 cm diameter) in an incubator at 25±1°C, 14L-10D. To get eggs, a male and a female adult were confined in a petri dish 6 days after emergence. Newly hatched larvae (G₂) were used as experimental material. Rearing containers for individual larvae were 6 cm diameter petri dishes. The diet, and water in a sponge, were put on separate glass pieces in the petri dish. The developmental period, cocoon weight (at spinning), adult weight at emergence, survival, number of eggs laid and hatching were recorded.

Table 2. Relative composition of amino acids in CDH 1-4

	CDH-1	CDH-2	CDH-3	CDH-4
Glycine	5.5	4.5	3.0	3.5
Alanine	7.0	5.0	3.0	5.5
Valine	6.0	5.5	18.0	5.0
Leucine	8.5	7.5	6.5	6.0
Isoleucine	4.0	5.5	4.5	4.0
Serine	4.0	5.0	7.0	4.5
Threonine	2.5	4.0	5.5	3.5
Aspartic acid	5.0	4.5	3.5	6.0
Glutamic acid	10.0	6.5	4.5	9.5
Asparagine	5.0	4.0	3.0	6.0
Glutamine	10.0	6.5	4.5	9.5
Lysine	6.5	4.5	10.0	7.5
Arginine	3.0	2.5	7.0	5.0
Cysteine	1.0	1.0	1.0	1.0
Cystine	0.5	0.5	0.5	0.5
Methionine	1.0	2.5	2.0	2.0
Phenylalanine	2.5	5.0	3.5	4.0
Tyrosine	3.5	13.0	4.0	5.5
Tryptophan	1.5	1.5	1.5	1.5
Histidine	1.0	2.5	1.5	2.5
Proline	9.0	5.5	3.0	4.5
β -Alanine	2.0	2.0	2.0	2.0
γ -Aminobutyric acid	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0

RESULTS

All the modified diets (CDH 1-4) supplied sufficient nutrients for larval development although some developmental retardation was observed when compared with control larvae reared on drone powder (Table 3). The body weights of adults reared on the test diets were also slightly lower than these of the controls. However, in both cases, no physical abnormality was found at emergence, which is often observed when a nutritionally insufficient diet is fed. The 1st and 2nd instar larvae seemed to be sensitive to the quality of diets. CDH-1 and CDH-3 were inferior to CDH-2 and CDH-4 in both developmental period and adult weight.

Since CDH-4 seemed to be the best diet, adults reared on this diet were further reared on it to get another generation. In the next generation (G_3), 13 normal adults were obtained from 29 larvae (Table 3). The survival ratio (44.8%) and body weights were slightly inferior to those of the previous generation. Further rearing was not tried because the insects are very sensitive to inbreeding (unpublished).

The ratio of sugars to amino acids in CDH-4 was changed and its effect on the larval development was examined (Fig. 1). A C:N ratio between 3:7 and 8:2 sustained larval development, but a ratio of less than 2:8 or more than 9:1 did not. The body weight of adults thus obtained was around 4 mg. Lacewings do not seem to require a specific ratio of carbohydrate and amino acids.

The effect of further modification to the diet CDH-4A was examined. CDH-4A

Table 3. Development of *Chrysoperla carnea* on CDH 1-4

Diet	n	1st instar	2nd instar	3rd instar		Pupa	1st instar to adult	Adult weight (mg)
				(free living)	(in cocoon)			
Control ^a	14	4.4±0.5 100	3.9±0.5 100	3.6±0.6 100	3.4±0.5 100	6.1±0.6 71.4	21.3±0.8 71.4	5.2±0.5
CDH-1A	15	8.6±1.0 80.0	7.6±0.5 66.7	6.8±0.8 66.7	3.1±0.3 66.7	6.0±0.5 53.3	32.3±1.4 53.3	4.1±0.6
CDH-1B	14	8.8±0.8 85.7	8.9±1.0 78.6	8.0±1.2 78.6	3.0±0 78.6	6.2±0.4 64.3	34.6±1.7 64.3	3.9±0.5
CDH-2A	15	6.3±0.5 80.0	5.5±0.7 66.7	5.4±0.5 53.3	3.3±0.5 53.3	5.2±0.8 33.3	24.8±0.8 33.3	4.4±0.5
CDH-2B	15	7.0±0.7 80.0	7.2±2.1 73.3	5.9±1.4 66.7	3.2±0.4 60.0	5.8±0.4 40.0	27.8±0.8 40.0	4.8±0.8
CDH-3A	15	8.7±1.7 100	7.5±0.9 100	7.0±0.7 100	2.9±0.3 93.3	5.9±0.3 80.0	32.0±2.0 80.0	4.5±0.5
CDH-3B	15	8.6±0.9 80.0	7.7±0.5 80.0	6.4±0.7 80.0	2.8±0.4 80.0	6.2±0.4 66.7	31.9±1.2 66.7	4.4±0.5
CDH-4A	15	6.3±0.5 86.7	5.2±0.6 80.0	5.3±0.5 80.0	2.6±0.5 80.0	5.4±0.5 66.7	24.8±0.8 66.7	4.8±0.7
CDH-4A ^b	29	6.6±1.6 93.1	5.8±1.5 72.4	6.5±1.9 69.0	3.1±0.3 55.2	6.0±0 44.8	26.0±1.1 44.8	4.6±0.5
CDH-4B	14	6.4±0.5 85.7	5.8±0.8 85.7	5.4±0.9 85.7	2.6±0.5 85.7	6.0±0.5 64.3	26.0±1.0 64.3	5.0±0.6

^a Control diet is drone powder. Each data composed of the duration of the stage (days±SD, upper) and survival ratio (%), lower).

^b Next (G₃) generation. Eggs were obtained from adults which developed on the same diet, CDH-4A.

contains 5 organic acids, 3 fatty acids and 3 fat-soluble vitamins. Larvae reached adulthood even when each group of these substances was omitted (Table 4) although exclusion of the fatty acids reduced the survival percentage; development was also inferior without the 3 vitamins. Changing mineral contents in the diet (70-130% of the standard diet) did not show any apparent effect.

The nutritional value of CDH-4 for adults was also examined. The same diet was fed by the same method. A female oviposited more than 1,000 eggs in about 2 months and the hatching rate was high except during the last 2 weeks (Fig. 2). These results were rather better than for the DP controls. An adult in the following generation also produced normal hatchable eggs.

DISCUSSION

Fortification of a preliminary recipe with several organic acids, mineral salts, vitamins and other nutrients improved the development of lacewings. Some data (Table 4) suggest trimming can be done, and VANDERZANT (1973) omitted ascorbic acid from her recipe without impairment. She replaced casein and soy hydrolyzates with an amino acid mixture to investigate dietary requirement. Although she found that 10 amino acids were indispensable for rearing *C. carnea*, even the complete mixture

Fig. 1. Development of *Chrysoperla carnea* fed on CDH-4 at various C:N ratios.Table 4. Development of *Chrysoperla carnea* on modified CDH-4A

Diet	n	1st instar	2nd instar	3rd instar		Pupa	1st instar to adult	Adult weight (mg)
				(free living)	(in cocoon)			
CDH-4A ^a	17	6.3±0.8 100	5.8±1.6 94.1	6.2±1.0 94.1	2.8±0.4 94.1	5.9±0.5 82.4	27.0±2.9 82.4	4.3±0.5
—organic acids	18	6.7±0.8 100	5.9±0.8 100	6.2±0.6 100	3.4±0.5 100	6.2±0.7 83.3	28.1±1.1 83.3	4.5±0.8
—fatty acids	18	6.9±0.7 94.4	6.5±1.7 83.3	7.1±1.9 77.8	3.4±0.5 77.8	6.5±0.5 33.3	29.3±1.9 33.3	4.3±0.5
fat-soluble vitamins ^b	18	7.1±1.2 94.4	9.2±3.9 72.2	7.7±2.8 55.6	3.5±0.5 55.6	6.2±0.6 55.6	32.0±4.5 55.6	4.2±0.7
2/3 minerals	18	6.6±0.9 94.4	5.8±0.6 88.9	5.9±0.6 88.9	3.0±0 88.9	6.2±0.6 72.2	27.5±1.5 72.2	4.2±0.8
4/3 minerals	18	7.2±0.8 94.4	6.3±1.0 88.9	6.8±1.8 88.9	3.2±0.4 88.9	6.1±0.3 77.8	29.1±1.2 77.8	4.2±0.5

^a Each data composed of the duration of the stage (days±SD, upper) and survival ratio (%), lower).

^b β-Carotene (A), ergosterol (D), α-tocopherol (E).

gave only 5% emergence. All our modified diets sustained larval growth and 30–80% of the 1st instar larvae reached adulthood, although differences were observed among the diets. Since the compositional differences were only amino acids, further improvement might be possible if their combination were examined more carefully.

Fig. 2. Oviposition and hatching of *Chrysoperla carnea* fed on drone powder (DP) and CDH-4A. I and II represent different individuals.

Growth retardation of the 1st and 2nd instar may be due to physical feeding problems. Lacewing larvae usually suck hemolymph from their prey. HAGEN and TASSAN (1965) prepared paraffin-covered droplets to simulate this feeding and VANDERSANT (1969) used a liquid diet with cellulose sponge. Our artificial diets were powdery and hygroscopic so they absorbed water and changed their physical state. Control of the fluidity may also affect growth. This factor was neglected in the present study because it did not concern the chemical and nutritional basis of the study. However lacewing larvae do develop normally when powdered aphid is fed.

When we started this investigation we supposed that *Chrysopa nipponensis* is a true aphidophagous species because drone powder was an effective diet. Adult *Chrysoperla carnea*, however, is known to feed on honeydew (HAGEN, 1950) and pollen (SHELDON and MACLEOD, 1971). Nutritional studies showed that such a feeding habit favored easier rearing than an obligatory aphidophage. Symbiotes may also complicate the nutritional physiology (see HAGEN, 1987). BIGLER et al. (1976) reared larvae of *C. carnea* and *Chrysopa perla* on artificial diets. The former species developed easily on two diets but only one larva of the latter species, which is an aphid feeder during adulthood, reached adulthood from 80 larvae fed on the same diets. The present taxonomy separates these two species into separate genera, and our experimental results might reflect the taxonomy. On the other hand, our recipes have been successfully applied to *Chrysopa septempunctata* which is a strict carnivore (unpublished data). Thus, the totally synthetic and successful diets developed in this study could promote nutritional studies of aphidophagous insects such as coccinellids and syrphids, which have not been as well studied as phytophagous insects.

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