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Improving the artificial diet for adult of seven spotted ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) with orthogonal design

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Abstract

In this study, an orthogonal array design with 16 factors at two levels (2^{16}) was performed to develop an artificial diet rearing the adults of seven spotted ladybird beetle *Coccinella septempunctata*. The parameters of weight gain and survival rate of adults, preoviposition period, fecundity and hatching rate of diet-fed adults were monitored. The 16 factors were included: pork liver, infant formula, sucrose, olive oil, yolk, corn oil, yeast powder, cholesterol, casein, casein hydrolysate, vitamin powder, 65% juvenile hormone III, protein powder, vitamin E, honey and pumpkin. Results indicated that pork liver, sucrose, yolk, yeast powder, juvenile hormone, pumpkin and honey were the main ingredients of the artificial diet contributing to weight gain and survival rate of adults, preoviposition period, fecundity and hatching rate. A follow-up fed with a selection of improved formulas confirmed the validity of the optimization as predicted by the orthogonal array analysis, indicating the usefulness of this method for selecting artificial diets for *C. septempunctata*. The weight gain and fecundity of adults reared on the improved artificial diet were 87.46 and 62.70% of those reared on *Aphis craccivora*; the survival rate and hatch rate were similar between the diet-fed and aphid-fed, while the preoviposition period was significantly shorter for *C. septempunctata* fed on the diet than on *A. craccivora*. The latter formula was superior to any formerly developed formulas and may thus have potential for the improved artificial diet mass rearing of *C. septempunctata*.

Keywords: *Coccinella septempunctata*, artificial diet, orthogonal design, key factor, diet improvement

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Introduction

The ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is considered to be an important biocontrol agent for soft-bodied insects such as aphids, white flies, jassids and small lepidopterous larvae (Victor, 1997; Zhu & Park, 2005; Sarwar & Saqib, 2010; Lucas *et al.*, 2013; Mahyoub *et al.*, 2013; Ugine & Losey, 2014; Skouras *et al.*, 2015). Deligeorgidis *et al.* (2005) also reported in his findings that *C. septempunctata* is a good biocontrol source for thrips and whiteflies in greenhouse crops. Both *C. septempunctata* adults and larvae are known primarily as predators of aphid but they also prey many other pests such as soft scales, mealybugs, spiders and mites (Rizvi *et al.*, 1994). The beetle has been increasingly used to control soft-bodied insects. However, due to the impact of climate and farming operations, it is often

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difficult to maintain sufficient level of natural supply of the beetles to control pests effectively (Sun & Wan, 1999; Zhang *et al.*, 2014). Therefore, it is necessary to artificially rear them as supplement predator for effective field control of pests. Currently, lady beetles are reared mainly using aphids as food. This method is costly and time-consuming for the mass production of the insect (Ashraf *et al.*, 2010; Bonte *et al.*, 2010). Furthermore, a natural prey diet is unable to support the beetles' population to sustain year-round because environmental conditions may limit the prey supply and the need to rear prey. Therefore, successful and continuous mass rearing of ladybug at a commercial level is important for this purpose, which requires the availability of artificial diets.

Studies on artificial diets for lady beetles were initiated in the 1950s; Smirnoff (1958) reported a prey powder-based artificial diet that successfully reared a variety of predatory ladybugs. Later on, non-prey compositions and non-insect source components were studied for the diet for various species of lady beetles (Sighinolfi *et al.*, 2008; Zhang *et al.*, 2008; Ashraf *et al.*, 2010; Sarwar & Saqib, 2010; Yazdani & Zarabi, 2011; Tan *et al.*, 2015). However, compared with prey powder-based artificial diets, these diets are inferior with regard to the weight gain, pupation rate and eclosion rate. In particular, low fecundity has been a bottleneck limiting the use of artificial diets for lady beetle (Sun & Wan, 1999).

Orthogonal design, a fractional factorial design approach, is often used to design experiments with multiple-level factors (Oles, 1993). Orthogonal design involves the selection of representative combinations of factors and levels for laboratory experiments and can also be used to aid the design of an improved artificial diet for an insect based on the combination of ingredients (Wu & Leung, 2011*a*). We tested 15 components identified from the most successful lady beetle artificial diets (Venkatesan *et al.*, 1998; Guo & Wan, 2001; Wang *et al.*, 2008; Ashraf *et al.*, 2010; Sarwar & Saqib, 2010; Lü *et al.*, 2015) and a new (pumpkin) component using an orthogonal design. The aim of this study was to identify key diet factors affecting reproduction of the lady beetle adult and develop an improved diet formulation for mass production of eggs, increasing hatching rate and adults of survival rate.

Materials and methods

Experimental insects

The *C. septempunctata* adults used in experiments were reared from eggs obtained from a collection of 300 adults from the experimental fields of Guizhou Provincial Academy of Agricultural Sciences, Guiyang, China. The adults were fed on the *Aphis craccivora* in six cages (49 × 35 × 30 cm) to produce egg masses on shrinking paper. The egg masses were collected three times a day and transferred onto filter paper moistened with distilled water in a 9 cm diameter Petri dish. Distilled water was added to the filter paper once every day. Newly hatched larvae were transferred into cages (49 × 35 × 30 cm) to feed on *A. craccivora* till pupation, and newly emerged (<24 h old) *C. septempunctata* adults were used in all tests. *A. craccivora* was maintained on horsebean plants in the laboratory.

Optimization of artificial diet by using an orthogonal design

The optimization experiments were performed using 16 ingredients. The ingredients were pork liver (A), infant formula (B), sucrose (C), olive oil (D), yolk (E), corn oil (F), yeast powder (G), cholesterol (H), casein (I), casein hydrolysate (J), vitamin powder (K), 65% juvenile hormone III (L), protein powder (M), vitamin E (N), honey (O) and pumpkin (P). The infant formula (B) is a kind of milks for new born baby, contains protein, 16.40 g; fat, 19.00 g; carbohydrates 56.20 g; nicotinamide, 4.00 mg; vitamin A, 0.52 mg; vitamin B1, 0.55 mg; vitamin B2, 0.80 mg; vitamin B5, 2.80 mg; vitamin B6, 0.38 mg; vitamin C, 42.00 mg; calcium, 525.00 mg; magnesium, 30.00 mg; iron, 6.08 mg; zinc, 2.80 mg; and sodium, 120.00 mg per 100 g^1. Vitamin powder (K) contains vitamin A, 1.03 g; vitamin B1, 3.13 g; vitamin B2, 3.13 g; vitamin B6, 0.31 g; vitamin C, 31.25 g; vitamin E, 6.25 g; nicotinamide, 9.38 g; inositol, 31.25 g; calcium pantothenate, 3.13 g; and lysine, 15.63 g per kg. Protein powder (M) contains protein, 75.00 g; fat, 4.50 g; carbohydrate, 15.50 g; sodium, 0.19 g per 100 g. The pumpkin is rich in carbohydrates, vitamins, protein and amino acids, especially, in many microelements, such as potassium, magnesium, calcium, zinc and iron. These natural contents are easier to uptake and utilize by ladybird beetles than the chemicals (Kim *et al.*, 2012; Xiong *et al.*, 2013).

An L_{16} (2^{16}) orthogonal array method was used for the improved composition of artificial diet of *C. septempunctata*. The orthogonal design was employed to assign the 16 factors (ingredients, A–P) at two levels (concentrations, 1–2) (table 1), with 16 tests carried out according to the matrix given in table 2. Fifteen ingredients were selected by referring the previous reports. The pumpkin was used as a new ingredient. Each row of the orthogonal array is a specific set of factor levels to be tested. The level 1 was based on the averaged level reported previously, while the level 2 was a 50% increase of level 1.

Preparation of artificial diets

Pork liver and pumpkin were purchased from the local markets. The pork liver was grinded into pasta with a tissue triturator and the peeled pumpkin was steamed and then grinded as well. The ingredients were weighted and mixed, and then added to 600 ml sterile water containing 12 g molten agar at 40–50 °C, and blended for 3 min to generate a homogeneous and semi-solid diet mixture. The mixture was cooled to room temperature and stored in refrigerator at a 4 °C.

Feeding with artificial diet and aphis

The newly emerged adults were sexed and paired, 50 pairs for a treatment were obtained within a single day. In both the orthogonal array design trial and the follow-up validation experiment comparing the optimized diet against an aphid diet, each pair was placed in a 500 ml plastic jar where the semi-solid of artificial diet (2 × 5 × 5 mm) was supplied on a small piece of plastic strip at the bottom. The pair was provided daily with fresh artificial diet, absorbent cotton with distilled water and shrinking paper. Egg masses laid on paper were collected three times a day and transferred onto filter paper moistened with distilled water in a 9 cm diameter Petri dish. For feeding with *A. craccivora*, freshly collected aphids (roughly 500 individuals) were added to each jar daily through the nylon netting, which served as the lid of the containers.

Experimental conditions

The experiment was conducted in a climate chamber at a controlled temperature of 25 ± 1 °C and relative humidity of 70 ± 5% with a 14 : 10 light : dark regime.

Table 1. Factors (i.e., ingredients) and levels (i.e., concentrations) in an orthogonal design for optimizing of an artificial diet for *Coccinella septempunctata* (1 litre).

Level	A (g)	B (g)	C (g)	D (ml)	E (g)	F (ml)	G (g)	H (g)	I (g)	J (g)	K (g)	L (µl)	M (g)	N (ml)	O (g)	P (g)
1	140	20	60	4	20	4	10	1	10	10	2	6	6	1	10	20
2	210	30	90	6	30	6	15	1.5	15	15	3	9	9	1.5	15	30

Table 2. Orthogonal design for two levels of 16 factors used for artificial diets optimization (1 litre).

Treatment	A (g)	B (g)	C (g)	D (ml)	E (g)	F (ml)	G (g)	H (g)	I (g)	J (g)	K (g)	L (µl)	M (g)	N (ml)	O (g)	P (g)
T1	140	20	90	4	20	4	15	1.5	10	10	2	9	9	1.5	15	30
T2	140	20	90	4	20	4	15	1	15	15	3	6	6	1	10	20
T3	140	20	90	6	30	6	10	1.5	10	10	2	6	6	1	10	20
T4	140	20	90	6	30	6	10	1	15	15	3	9	9	1.5	15	30
T5	140	30	60	4	20	6	10	1.5	10	15	3	9	9	1	10	30
T6	140	30	60	4	20	6	10	1	15	10	2	6	6	1.5	15	20
T7	140	30	60	6	30	4	15	1.5	10	15	3	6	6	1.5	15	30
T8	140	30	60	6	30	4	15	1	15	10	2	9	9	1	10	30
T9	210	20	60	4	30	4	10	1.5	15	10	3	9	6	1.5	10	20
T10	210	20	60	4	30	4	10	1	10	15	2	6	9	1	15	30
T11	210	20	60	6	20	6	15	1.5	15	10	3	6	9	1	15	20
T12	210	20	60	6	20	6	15	1	10	15	2	9	6	1.5	10	20
T13	210	30	90	4	30	6	15	1.5	15	15	2	9	6	1	15	30
T14	210	30	90	4	30	6	15	1	10	10	3	6	9	1.5	10	30
T15	210	30	90	6	20	4	10	1.5	15	15	2	6	9	1.5	10	20
T16	210	30	90	6	20	4	10	1	10	10	3	9	6	1	15	20

T1–T16 denote 16 treatments of the orthogonal design.

Biological parameters assessed

Female adults, both upon newly emerging and after feeding on a treatment for 10 days, were starved for 24 h before being anesthetized with CO_2 and weighted using an electronic balance (Sartorius BSA124S, Sartorius Group, Beijing, China). The survival rates of the females were recorded on the 50th day after eclosion. The preoviposition period and number of oviposited eggs were determined by daily checking the oviposited eggs. Egg hatching rate was determined by counting the number of first instar larvae hatched 4 days after oviposition in a subset of egg masses.

Statistical analysis

Range analysis was used to indicate the effect of each factor and determine the theoretically optimal level of the various factors. Range (R_j) is the difference between the highest and the lowest mean values of examinational index for different factors (Deng *et al*., 2012). R_j is the absolute value of the difference between the mean values of Y_{jk} (e.g., weight gain) for levels 1 and 2, with means calculated across treatments with the same level k (=1, 2) for factor j (=A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P). The optimal level for each factor is indicated by the largest value of Y_{jk}, and a larger R_j value indicates greater biological significance of the factor (Cui *et al*., 2009; Wu & Leung, 2011*b*).

Ranges (R) was calculated using Microsoft Excel. We analyzed the differences in the weight, survival, preoviposition period, fecundity and hatch rate in the orthogonal experiments to confirm the optimal diet ingredients and their related proportions using variance and range analysis. The differences in each biological character were compared using a one-way analysis of variance (ANOVA) among of *C. septempunctata* fed with the improved artificial diet and aphis (SAS version 8.0). The ANOVA was run with the improved diet and aphis as independent factors, to compare the differences in weight, survival, preoviposition period, fecundity and hatch rate.

Results

Feeding outcomes of orthogonal array design

Results of the artificial diet feeding test are shown in table 3. Ten days after feeding, the treatment T16 gave the greatest weight gain, where the insects gained 11.83 mg per female adult, T12 generated the lowest weight gain with 2.47 mg per insect. The 50-day survival rate was the highest (81.67%) in the treatment T13 and lowest (41.63%) with T4. Eggs were produced in all 16 treatments. However, the treatment T6 had the shortest preoviposition period (6.4 days) and T5 had the longest (13.1 days). The treatment T9 generated the highest amount of eggs per female (284.4 eggs), while T2 was the lowest (117.2 eggs). The T9 gave the highest hatching rate (81.36%), while the T2 was the lowest (58.38%).

Analysis for key factors

The mean values of Y_{jk} and R_j for the range analysis are shown in table 4. As larger R_j values indicate a stronger impact of the considered factor at different levels (Wu & Leung, 2011*a*), the importance of each factor in decreasing order was shown in table 4. The diet theoretically yielding the highest weight gain, survival rate, preoviposition, total fecundity, hatching rate based on the Y_{jk} values of each factor were constituted in table 5. Based on the above results, the improved diet formulas best composition for *C. septempunctata* was (A2B2C1D2E2F1G1H1I1J1K2L2M1N2O2P1) 210 g pork liver,

Table 3. Feeding results of *Coccinella septempunctata* in 16 treatments of the orthogonal design.

Treatment	10-day weight gain of female after eclosion (mg)	Preoviposition (days)	50-day female survival rate (%)	Total fecundity[1]	*n*	Hatching rate (%)
T1	4.95	10.29	58	175.42	4514	64.21
T2	3.72	7.33	62	117.16	3985	58.38
T3	2.68	8.14	66	128.51	4657	67.59
T4	9.8	9.46	42	230.17	4871	76.43
T5	5.73	13.07	54	174.13	4149	69.12
T6	9.04	6.42	44	179.15	4356	71.91
T7	6.58	8.41	48	224.20	4248	78.23
T8	4.46	10.30	56	217.45	3964	74.64
T9	5.24	10.27	60	284.44	3859	81.36
T10	8.91	10.38	66	232.23	4428	75.77
T11	2.97	7.52	60	187.29	3983	66.56
T12	2.47	8.21	64	250.12	4705	62.43
T13	2.91	12.19	82	139.50	4431	68.78
T14	3.52	11.25	70	180.74	3720	71.35
T15	9.05	8.53	76	185.51	4654	74.28
T16	11.83	8.80	68	262.08	3672	78.64
Ta	5.87	9.41	61	198.01	----	71.23

T1–T16 denote 16 treatments of the orthogonal design
Ta is the average of the 16 treatments tested.
n means the amount of eggs with hatching rate.
[1]Average number eggs per fertile female in the 50 days.

30 g infant formula, 60 g sucrose, 6 ml olive oil, 30 g yolk, 4 ml corn oil, 10 g yeast powder, 1 g cholesterol, 10 g casein, 10 g casein hydrolysate, 3 g vitamin powder, 9 µl 65% juvenile hormone III, 6 g protein powder, 1.5 ml vitamin E, 15 g honey and 20 g pumpkin l^{-1}.

Validation of the improved formulas

To validate the improved formulas, the development and reproduction of *C. septempunctata* adults were compared by feeding them with the diet and aphis (table 6). The 10-day weight gain of adults reared on the diet was 1.41 mg lower than on aphis; there was a significant difference ($F = 7.3010$, $P = 0.0077$). Fifty-day survival rate was lower on the diet than on aphis; however, there was no significant difference ($F = 0.3330$, $P = 0.2830$). The preoviposition period was significantly shorter for *C. septempunctata* fed on the diet than on aphids ($F = 0.3110$, $P = 0.0100$). The total fecundity of aphis-fed *C. septempunctata* was higher than that of the diet-fed *C. septempunctata* ($F = 0.0070$, $P = 0.0001$), while their hatch rate was similar ($F = 9.1170$, $P = 0.5149$). Nevertheless, the improved formulas was a remarkable improvement over the formulas in table 3, particularly for fecundity, which had a nearly 60% increase compared with the treatment T9 from unmodified formulas listed in table 3.

Discussion

The five parameters: weight gain, survival rate, preoviposition, fecundity and hatch rate of artificial improved diet fed beetles were 167.46, 114.75, 96.71, 228.93 and 102.78% compared with the averaged values (Ta) of 16 treatments (table 3). The survival rate and hatch rate of improved diet-fed beetles were similar to aphid-fed ones. The adult weight gain over 10 days period and fecundity of improved diet-fed beetles were 87.46% (11.24 mg) and 62.70% (453 eggs) of aphid-fed ones. In addition, the preoviposition period of diet-fed beetles was shorter than these aphid-fed ones (table 6). However, Gong *et al*. (1980) fed adult of *C. septempunctata* with diet could be survived for 2 months, but they were unable to lay eggs. Sarwar & Saqib, (2010) fed adult of *C. septempunctata* with diet laid 18 eggs in life. The *Harmonia axyridis* could be reared from the first instar larvae to fully reproductive adults on a pork liver-based artificial diet; however, adult laid 47 eggs for 10 days only, and the preoviposition for 13.50 days (Sighinolfi *et al*., 2008).

Components and their contents in diet are critical to meet the nutrition demand of insect. Therefore, diet components need to be investigated at different contents for their effect on the growth and development of *C. septempunctata* to develop an improved diet composition. The outcomes of this study suggested that the orthogonal array design is an efficiency method to develop a good artificial diet for feeding *C. septempunctata*. Our study revealed that (1) yeast powder and honey were the main ingredients in the diet affecting weight gain; (2) pork liver and sucrose were the primary ingredients affecting survival rate; (3) juvenile hormone and pumpkin were the major ingredients affecting preoviposition period; (4) juvenile hormone, sucrose and pork liver were the important ingredients that impact on the fecundity; and (5) yeast powder and yolk were the main ingredients affecting hatching rate of adult *C. septempunctata*.

The addition of yeast powder in diet is mainly to increase the content of free amino acids. Chen *et al*. (1989) found that yeast powder added to basal diet reduced the growth period of larvae, increased the larval pupation rate and adult body weight. However, if yeast powder was doubled in amount, feeding effect decreased, suggesting that excessive amino acids in artificial diets may disrupt the balance with nitrogenous compounds and other nutrients. Our study showed that 50% (level 2) increase in diet at level 1 reduced the weight gain, egg production and hatching rate.

In our study, the weight gain and fecundity of *C. septempunctata* were significantly increased when honey content was increased in the diet (level 2). Fu & Chen (1982) showed that adding honey and sucrose to liver-based diet promoted

Table 4. Range analysis of artificial diet for *Coccinella septempunctata* tested in the orthogonal design experiment.

Indicator		Factors																Order of significance of factors
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
a	Y_j1	5.87	5.09	5.68	5.50	6.22	6.84	7.79	6.72	5.83	5.59	5.56	5.81	5.56	5.40	4.61	5.86	G > O > F > H > B > N > D > E > K > M > J > C > L > I > A > P
	Y_j2	5.86	6.64	6.06	6.23	5.51	4.89	3.95	5.01	5.90	6.15	6.18	5.93	6.17	6.33	7.13	5.87	
	R_j	0.01	1.55	0.38	0.73	0.71	1.95	3.83	1.71	0.07	0.56	0.62	0.12	0.62	0.93	2.52	0.01	
b	Y_j1	53.63	59.55	56.41	61.68	60.50	61.67	59.11	58.75	61.60	60.42	63.69	61.23	61.55	64.13	63.23	62.25	A > C > N > K > O > H > G > P > B > F > D > I > M > J > L > E
	Y_j2	68.07	62.15	65.29	60.01	61.19	60.01	62.58	62.94	60.10	61.28	58.00	60.46	60.15	57.56	58.47	59.44	
	R_j	14.44	2.60	8.88	1.67	0.69	1.68	3.47	4.19	1.50	0.86	5.69	0.77	1.40	6.57	4.76	2.81	
c	Y_j1	9.14	8.91	9.33	10.11	8.71	9.26	9.37	9.00	9.82	9.13	9.31	8.47	8.68	9.68	9.61	8.24	P > L > D > M > E > E > B > I > H > N > J > A > O > K > C > G
	Y_j2	9.64	9.87	9.45	8.68	10.07	9.52	9.41	9.78	8.96	9.65	9.48	10.32	10.10	9.10	9.18	10.54	
	R_j	0.50	0.96	0.12	1.43	1.36	0.26	0.04	0.78	0.86	0.52	0.17	1.85	1.42	0.58	0.43	2.31	
d	Y_j1	180.8	200.6	218.6	185.3	191.3	212.3	209.5	208.6	203.4	201.9	188.5	179.3	198.1	182.3	192.2	199.0	C > L > A > N > F > D > H > G > K > E > O > I > J > B > P > M
	Y_j2	215.2	195.4	177.4	210.7	204.7	183.7	186.5	187.4	192.6	194.1	207.5	216.6	197.9	213.7	203.8	197.0	
	R_j	34.4	5.29	41.2	25.3	13.3	28.6	23.0	21.2	10.8	7.8	19.1	37.3	0.2	31.4	11.5	2.0	
e	Y_j1	70.06	69.09	72.50	70.11	68.19	73.19	74.39	71.19	70.92	72.03	69.95	70.51	70.92	69.94	69.89	70.09	G > E > B > F > O > N > K > C > A > P > D > J > L > M > I > H
	Y_j2	72.40	73.37	69.96	72.35	74.27	69.27	68.07	71.27	71.54	70.43	72.51	71.95	71.55	72.53	72.57	72.37	
	R_j	2.34	4.28	2.54	2.24	6.08	3.92	6.32	0.08	0.62	1.60	2.56	1.44	0.63	2.59	2.68	2.29	

a, 10-day weight gain of females after eclosion (mg); b, 50-day survival rate (%); c, preoviposition (day); d, total fecundity (no. egg/female); e, hatching rate (%).

Table 5. Improved levels of diet components in the artificial diet for *Coccinella septempunctata* (1 litre).

Indicator	Improved levels of diets
Weight gain	A1B2C2D2E1F1G1H1I2J2K2L2M2N2O2P1
Survival rate	A2B2C2D1E2F1G2H2I1J2K1L1M1N1O1P1
Preoviposition period	A1B1C1D2E1F1G1H2I2J1K1L1M1N2O2P1
Egg production	A2B2C1D2E2F1G2H2I2J1K2L2M2N2O2P2
Hatching rate	A2B2C2D2E2F1G1H1I1J1K2L2M1N2O2P1
Over development and reproduction	A2B2C1D2E2F1G1H1I1J1K2L2M1N2O2P1

feeding, shorten the preoviposition and increased the weight gain. It is likely because that honey contains sucrose that promotes feeding and specific nutritional amino acids.

Pork liver is the main component of the artificial diet. It is rich in protein, cholesterol and minerals. In our study, the pig liver had an important role in improving the survival rate and oviposition rate, indicating that the nutrient components in the liver are easy to be digested and absorbed by the insect. However, casein hydrolysate, protein powder, casein and cholesterol were added in the formula that did not impact the biological parameters of the insect, which may be due to poor absorption and overdosed.

The juvenile hormone plays an important role in the preoviposition and egg production. Vitellogenesis in the lady beetle, *C. septempunctata* is controlled by juvenile hormone, when immature females were reared on an artificial diet, they were characterized by hypertrophy of the fat bodies and slow development of the ovaries, vitellogenin synthesis in the fat body remained at a very low level throughout adult development (Zhai *et al.*, 1987). Chen *et al.* (1984) and Gong *et al.* (1980) added juvenile hormone to the pork liver diet and found that the component improved the egg production and weight, and reduced preoviposition period. The increased contents of juvenile hormone also raised the fecundity of *C. septempunctata* in this study.

Egg yolk was functionally important for the preoviposition, fecundity and hatching rate, while pumpkin shortened the preoviposition stage of the insect, although further work is needed to confirm these results. Earlier studies have shown that addition of vitamin E to pig liver- and sucrose-based diets could promote the reproduction of the ladybird beetle. Lipids could improve food utilization efficiency and conversion rate, leading to shorter preoviposition period and increased egg production (Gong *et al.*, 1980; Fu & Chen, 1982; Chen *et al.*, 1984). The olive oil in the artificial diet seemed to have a phago-stimulating effect on the adult beetles, and the rate of food conversion was the highest (Chen *et al.*, 1984). In addition, the addition of milk powder had certain influence on the biological parameters of the insect, probably due to supplementary nutrition from trace elements in milk powder. Vitamin C powder had a certain effect on survival rate and fecundity.

These findings indicate that the insect has different requirements for nutrients and content for different biological parameters. Over 16 treatments of range settings, the contents of 16 factors were increased by 50% over the averaged values of previous reports. The increased contents of milk and honey could result in increasing the body weight; while corn oil, yeast powder and cholesterol result in decreasing the body weight. The liver and sugar could increase the adult survival

Table 6. Comparison of development and reproduction of *Coccinella septempunctata* fed with the improved artificial diet and aphis.

Feed	10-day weight gain of female after eclosion (mg)	50-day female survival rate (%)	Preoviposition (days)	Total fecundity[1]	*n*	Hatching rate (%)
Artificial diet	9.83 ± 0.47 b	70 a	9.1 ± 0.53 b	453.3 ± 19.82 b	4867	73.21 ± 4.18 a
Aphis	11.24 ± 0.59 a	72 a	10.8 ± 0.68 a	723.1 ± 20.19 a	5136	75.27 ± 4.05 a

Values are mean ± SE. Figures labeled with the same letters in the columns are not significantly different at $P = 0.05$ by one-way ANOVA and Fisher' s Least Significant Difference (LSD) test.
Percentages of egg hatch rate were transformed using arcsine transformation before analysis.
[1]Average number eggs per fertile female in the 50 days. *n* means the amount of eggs with hatching rate.

rate; while vitamins C and E could reduce the adult survival rate. The juvenile hormone, liver, olive oil and vitamin E could increase the fecundity; while the sucrose could reduce the fecundity. The milk and chicken egg could increase the hatching rate; while the yeast could reduce the hatching rate. These results demonstrated that the contents of milk, honey, olive oil, liver, juvenile hormone, vitamin E and chicken egg should be increased; while the contents of corn, oil, yeast powder, cholesterol, vitamin C, pumpkin and sucrose should not be increased. In addition, the contents of casein, casein hydrolysate and protein powder did not affect the biological performances significantly; we suspected that *C. septempunctata* had a low uptake rate of these two ingredients.

In conclusion, the diet we developed here is suitable for rearing *C. septempunctata*. The diet-fed *C. septempunctata* adults had the improved survival rate, fecundity and shortened preoviposition period compared with the previous reports (Sun & Wan, 1999; Sighinolfi *et al*., 2008; Ashraf *et al*., 2010; Yazdani & Zarabi, 2011; Lü *et al*., 2015). The reported diets contained the natural preys, such as aphids, pupae of wasp or eggs of rice moth (*Corcyra cephalonica* molitor) (Sun & Wan, 1999; Wang *et al*., 2008; Rojas *et al*., 2016). The diet we developed is free of such preys, which can save a lot of cost for maintaining these prey(s). Furthermore, the cooking process of the diet is relatively simple and quick. However, the diet-fed adults showed a reduced fecundity, survival rate and egg hatching rate compared with the aphid-fed ones. This diet is not doing well for the *C. septempunctata* larvae. The survival rate and pupation rate of diet-fed larvae were largely lower than these aphid-fed larvae. Thus, we are working on the improving adult diet and developing larval diet.

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