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Performance of *Arma chinensis* reared on an artificial diet formulated using transcriptomic methods

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Abstract

An artificial diet formulated for continuous rearing of the predator *Arma chinensis* was inferior to natural prey when evaluated using life history parameters. A transcriptome analysis identified differentially expressed genes in diet-fed and prey-fed *A. chinensis* that were suggestive of molecular mechanisms underlying the nutritive impact of the artificial diet. Changes in the diet formulation were made based on the transcriptome analysis and tested using life history parameters. The quantity of pig liver, chicken egg, tuna fish, biotin, nicotinamide, vitamin B6, thiamine, riboflavin, vitamin C, L-glutamine, and sucrose was reduced, and wheat germ oil, calcium pantothenate and folic acid were increased. Ecuadorian shrimp was added as a partial substitute for tuna fish. Several parameters improved over six generations, including increased egg viability, and decreased egg and adult cannibalism. Additionally, several parameters declined, including longer developmental times for 2nd–5th instars, and decreased nymphal weights. The improvements in life history parameters support the use of transcriptome analyses to help direct formulation improvements. However, the decline in some parameters suggests that additional information, e.g., proteomic data, may be useful as well to maximize diet formulations.

Keywords: *Arma chinensis*, reformulated diet, transcriptome, biological characteristics

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Introduction

The use of natural enemies as biocontrol agents is an important alternative to the environment, health and resistance issues associated with the use of chemical insecticides (Greany & Carpenter, 1998). However, this requires the production of large numbers of beneficial insects at low costs for augmentative and inoculative releases. The development of artificial diets could considerably reduce the costs of mass

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propagation compared with conventional rearing methods (Glenister, 1998; Glenister & Hoffmann, 1998; Ruberson & Coll, 1998; Thompson, 1999; Wittmeyer & Coudron, 2001).

Improvement of artificial diets can be convoluted, tedious and often underappreciated in mass rearing of beneficial insects. The main method for optimizing artificial diet is to measure a few preselected biochemical and (or) physiological parameters and to test the effect of changes in diet formulation on insect performance (Adams, 2000; Wittmeyer & Coudron, 2001; Coudron *et al*., 2002; Coudron & Kim, 2004). Typically, diet components are changed one at a time and insect performance is tested after each change. This endeavor is time-consuming, taking years to decades to optimize a diet, with many attempts ending in failure.

Another approach, using *n*-dimensional mixture designs (Lapointe *et al*., 2008), identified a set of response-optimized meridic diets that contain fewer ingredients than the previous commercial diet for *Diaprepes abbreviates* (L.) (Coleoptera: Curculionidae), and followed that with a geometric design combined with response surface models to identify major nutritive components of the diet (Lapointe *et al*., 2010). Tan *et al.* (2013) investigated an artificial diet for raising *Orius sauteri* (Poppius) (Heteroptera: Anthocoridae) using a microencapsulation technique. They tested 25 combinations of ingredients using an orthogonal experimental design and identified five optimal combinations based on different biological and physiological characters. The results of a follow-up test of locomotory and respiratory capacity indicated that respiratory quotient, metabolic rate, and average creeping speed were all influenced by varying dietary ingredients.

The use of gene expression could be a more direct method to accelerate diet development and identify deficiencies in diet formulations. Nutrigenomics examines how nutrition affects gene expression patterns and offers not only a molecular means to measure an insect's response to changes in the food stream but also provides information on diet limitations (Yocum *et al*., 2006).

Arma chinensis (Fallou) (Hemiptera: Pentatomidae) is a predaceous insect species that can effectively suppress a wide range of agricultural and forest insect pests in the orders Lepidoptera, Coleoptera, Hymenoptera and Hemiptera (Gao *et al*., 1993; Chai *et al*., 2000; Liang *et al*., 2006; Yan *et al*., 2006*a*, *b*; Chen *et al*., 2007; Gao, 2010; Zou *et al*., 2012). An insect-free artificial diet comprised of pig liver and tuna was developed for *A. chinensis* (Zou *et al*., 2013*a*). Several life history parameters were diminished for *A. chinensis* reared on the artificial diet compared to a natural food source like the pupae of Chinese oak silk moth (COSM) *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera: Saturniidae). Fecundity and egg viability was lower, and developmental time from 2nd instar to adult and the preovipositional period were significantly longer for diet-fed *A. chinensis*. Nymphal weight, body length, adult longevity, survival from 2nd instar to adult, and fertility increased, while sex ratio (♂ : ♀) decreased, with the rearing of consecutive generations on the diet. Additionally, the longevity of adults reared on the artificial diet was significantly longer than of those reared on pupae. As a result, the cost to rear *A. chinensis* on the artificial diet approached 2.0 times the cost of rearing *A. chinensis* on pupae of *A. pernyi* (Zou *et al*., 2015).

The molecular mechanisms underlying the nutritive impact of the artificial diet of *A. chinensis* health have been investigated. The biological pathways associated with differentially expressed genes (DEGs) between the pupae-fed and diet-fed treatments were identified by mapping 13,872 DEGs and annotated sequences to the reference canonical pathways in KEGG (Kanehisa *et al*., 2004; Zou *et al*., 2013*b*). In total, 5879 sequences were assigned to 239 KEGG pathways. The pathways most represented by the DEGs were metabolic pathways (891, 15.16%) and pathways in cancer (215, 3.66%).

One group of DEGs that were upregulated in diet-fed vs. prey-fed *A. chinensis* were enriched for seven pathways related to fat metabolism, including adipocytokine signaling pathway, pyruvate metabolism, fatty acid biosynthesis, glycerolipid metabolism, fat digestion and absorption, fatty acid metabolism and fatty acid elongation. These potentially signal excess dietary lipid that may be remediated by reducing the pig liver, tuna and chicken egg in the diet formulation. Another group of DEGs that were upregulated was enriched for four pathways related to starch and sugar metabolism, including carbohydrate digestion and absorption, and fructose and mannose metabolism, sugar-lipase-3, glucose transporter, and insulin and mTOR signaling pathway. These potentially signal excess dietary sugar and carbohydrates that may be remediated.

Most of the up-regulated DEGs associated with fat and sugar metabolism are also related to vitamins, including ascorbate and aldarate metabolism, vitamin digestion and absorption, folate biosynthesis, pantothenate and coenzyme A biosynthesis, nicotinate and nicotinamide metabolism, biotin metabolism, retinol metabolism, thiamine metabolism, vitamin B6 metabolism and riboflavin metabolism, as a singular substance and the results reported here.

The high instance of a DEG relating to cancer is a challenging result due in part to the dearth of information on cancer in insects. However, we speculate that canned tuna fish could have caused the enrichment of DEGs related to cancer pathways because some ingredients are carcinogens, such as acrylamide (Christova-Bagdassarian *et al*., 2012).

Knowing the physiological roles of the DEGs enable us to predict effects of some dietary ingredients and subsequently propose formulation improvements to artificial diets.

Our objective was to compare the performance of *A. chinensis* reared on the previous diet with its performance when reared on a modified version of that diet wherein genome-directed formulation changes had been made to the diet that included reduced sugar, carbohydrate, biotin, nicotinamide, pyridoxine, riboflavin, thiamine, vitamin C, L-glutamine, chicken egg, pig liver, and canned tuna, increased calcium pantothenate, folic acid, and wheat germ oil, and added Ecuadorian shrimp.

Materials and methods

A. chinensis

The *A. chinensis* used to establish a laboratory colony for this study were obtained in July 2015 from Qian'an county (44° 57′ N, 124° 14′ E, 139 m), Songyuan city, Jilin province. Adults were held in 450 ml paper cups, one mated pair per cup, and supplied with distilled water absorbed in a cotton swab. Two soybean seedlings at the two cotyledon stage were provided in the adult rearing chambers in water-filled 6 cm × 1.5 cm-glass tubes. Water was added to the tubes every 2 or 3 days. Female *A. chinensis* laid egg masses on the surface of cage net or soybean leaves. Egg masses of *A. chinensis* were collected from the laboratory colony and transferred onto # 5 qualitative filter paper moistened with distilled water

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in a 9 cm diam. Petri dish under the conditions described above. Distilled water was added to the filter paper once every day. Egg hatch occurred in ca. 6 days. First instars were placed in 310 ml transparent plastic cups containing a piece of moist absorbent cotton. Molt to 2nd instar occurred in ca. 3 days after egg hatch. Immatures were maintained on COSM pupae and held at 27 ± 1 °C, 16 : 8 (L : D) and 75 ± 5% RH for ca. four generations prior to this study.

Food preparation

COSM pupae were purchased from a supermarket in Tianjin and stored at 4 °C until fed to *A. chinensis*. No additional preparation was required. Pupae were not used beyond 15 days of storage.

A comparison of the first artificial diet formulation (FAD) and a reformulation of that diet based on transcriptome information (RFD) are shown in tables 1 and 2. Both diets are comprised of a chemically defined portion (table 1) plus supplemental materials (table 2). The list of ingredients presented in tables 1 and 2 represents the final composition per 220 and 210 ml of FAD and RFD, respectively. The ingredients were blended together, the mixture adjusted to pH 6.8 and stored at 4 °C prior to use. The diet was encapsulated in a composite sheet (15 cm × 10 cm), constructed of Parafilm® and plastic film (Heyuan Evergreen Plastics Mfg. Co., Ltd. Taiwan), sterilized with a 5% solution of sodium hypochlorite, and formed into 40 µl hemispherical domes (Greany & Carpenter, 1998; Coudron *et al.*, 2000). The diet domes were refrigerated at 4 °C for up to 7 days prior to feeding, and new diet was prepared weekly.

In response to the DEGs in diet-fed vs. prey-fed *A. chinensis* (Zou *et al.*, 2013*b*) the following changes were made to the FAD formulation: the quantity of biotin, nicotinamide, vitamin B6, thiamine, riboflavin, and vitamin C were reduced and calcium panthothenate and folic acid were increased in RFD compared with FAD in order to address the upregulated genes related to vitamins; L-glutamine was reduced in order to address the upregulated genes in the pathway of alanine, aspartate and glutamate metabolism; sucrose was reduced in order to address the upregulated genes in the pathways related to starch and sugar metabolism; chicken egg and pig liver were reduced to address the upregulated genes related to fat metabolism; wheat germ oil was increased to address the low performance of males (Yousef *et al.*, 2003); and Ecuadorian shrimp replaced half of the tuna fish in order to decrease carcinogens that may have been in the canned fish.

Table 1. Chemically defined ingredients in 220 ml of the first artificial diet (FAD) and 210 ml of the reformulated diet (RFD).

Ingredient	Amount	
	FAD	RFD
Vitamins		
Biotin	0.04 mg	0.03 mg
Calcium panthothenate	2.00 mg	3.00 mg
Choline chloride	0.10 g	0.10 g
Folic acid	0.50 mg	0.70 mg
Inositol	40.00 mg	40.00 mg
Nicotinamide	2.00 mg	1.60 mg
Pyridoxine	0.50 mg	0.40 mg
Riboflavin	1.00 mg	0.80 mg
Thiamine	0.50 mg	0.40 mg
Para aminobenzoic acid	40.00 mg	40.00 mg
Vitamin B12	4.00 mg	4.00 mg
Vitamin C	0.54 g	0.50 g
Niacin	40.00 mg	40.00 mg
Free amino acids		
L-glutamine	1.00 g	0.80 g
Carbohydrates		
Sucrose	6.00 g	3.00 g
Antibiotics		
Gentamycin sulphate	6.20 mg	6.20 mg
Other		
Casein hydrolyzate	4.00 g	4.00 g

Table 2. Natural product ingredients in 220 ml of the first artificial diet (FAD) and 210 ml of the reformulated diet (RFD).

Supplement	Amount	
	FAD	RFD
Oil		
Wheat germ oil[a]	20 µl	30 µl
Animal protein		
Chicken egg	40 ml	35 ml
Raw pig liver	40 g	30 g
Tuna fish in oil[b]	40 g	20 g
Ecuadorian shrimp	0 g	20 g

[a]K-Lex brand (Alaska, U.S.A.).
[b]Canned Century brand in soybean oil (Thai Union Manufacturing Co., Ltd., Thailand).

Rearing with the reformulated diet

Individual 2nd instars were placed in 300 ml paper cups and maintained through the adult stage as described above. Each day the nymphs and adults were provided with fresh distilled water and encapsulated diet. The 2nd, 3rd, 4th, 5th instars and adult pairs were given 1, 1, 2, 3, and 8 diet domes (40 µl/dome), respectively. Diet domes fed to nymphs and adults were changed every day. *A. chinensis* was reared for six consecutive generations exclusively on the reformulated diet.

Life history evaluation

Daily observations were made to record changes in development for individuals reared on the reformulated artificial diet. Two days after emergence adults were weighed. Weight measurements were made using a Sartorius BP 211D (Sartorius AG, Göttingen, Germany) balance. Eggs were collected and counted from each mated pair daily. Egg hatch (i.e., viability) was determined by counting the number of 1st instars hatched 8 days after oviposition. The number of cannibalized eggs was verified by the empty eggshells. Fecundity was determined by counting the total number of eggs oviposited per female during its entire life and fertility was determined by counting the number of females that laid fertile eggs (Rojas *et al.*, 2000; Wittmeyer *et al.*, 2001). For fertility calculations, females that did not lay eggs because of death due to cannibalism during the 15 days after pairing were removed.

Data analysis

Two sample t-tests for means were used to compare developmental time, body weight, body length, preovipositional

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period, adult longevity, and total fecundity between different treatments in the same generation. The proportion of viable eggs, survival from egg to adult, cannibalism, sex ratio and fertility were compared between different treatments in the same generation by the Chi-squared tests of 2 × 2 tables. SAS (version 8.0) was used to analyze the data.

Results

Development time

A comparison of life history parameters for *A. chinensis* reared on the RFD showed no significant differences in developmental times between males and females of *A. chinensis* in any of the treatments. Therefore, developmental time comparisons were done without sex distinction.

The preoviposition period of females was extended significantly in RFD-fed insects compared with those reared on FAD (F_1, dF = 98; $t = -2.10$ and $P = 0.038$; F_3, dF = 98; $t = -6.29$ and $P < 0.001$; F_4, dF = 98; $t = -13.71$ and $P < 0.001$; F_5, dF = 98; $t = -5.13$ and $P < 0.001$; F_6, dF = 98; $t = -5.16$ and $P < 0.001$). The female reared on RFD required approximately 1.12–5.52 days longer preoviposition time than those fed on FAD. However, the preovipositional period of females was 0.86 day shorter in RFD-fed insects compared with those reared on FAD in F_2, but there was no significant difference between them (dF = 98; $t = 1.26$ and $P = 0.210$) (table 6).

There was no significant difference for time to egg hatch in all generations (F_2, dF = 98; $t = -0.60$ and $P = 0.550$; F_3, dF = 98; $t = -1.94$ and $P = 0.056$; F_4, dF = 98; $t = -1.84$ and $P = 0.069$; F_5, dF = 98; $t = 0.31$ and $P = 0.760$; F_6, dF = 98; $t = 0.15$ and $P = 0.879$). There was no significant difference in developmental time for 1st-instar nymphs in all treatments (F_2, dF = 98; $t = 1.73$ and $P = 0.087$; F_3, dF = 98; $t = 1.34$ and $P = 0.184$; F_4, dF = 98; $t = 0.44$ and $P = 0.659$; F_5, dF = 98; $t = 0.77$ and $P = 0.445$; F_6, dF = 98; $t = 1.00$ and $P = 0.320$). However, developmental time was significantly longer for 2nd-instar nymphs reared on RFD in F_1, F_3, F_4, F_5, and F_6 than those reared on FAD (F_1, dF = 98; $t = -7.81$ and $P < 0.001$; F_3, dF = 98; $t = -3.56$ and $P = 0.001$; F_4, dF = 98; $t = -7.86$ and $P < 0.001$; F_5, dF = 98; $t = -5.79$ and $P < 0.001$; F_6, dF = 98; $t = -4.92$ and $P < 0.001$). But the second generation RFD-fed 2nd instars completed development approximately 0.5 day earlier than those reared on FAD (dF = 98; $t = 2.44$ and $P = 0.017$). Developmental time was significantly longer for 3rd-instar nymphs reared on RFD in F_1, F_2, F_3, F_4, F_5, and F_6 than those reared on FAD (F_1, dF = 98; $t = -7.47$ and $P < 0.001$; F_2, dF = 98; $t = -10.73$ and $P < 0.001$; F_3, dF = 98; $t = -5.19$ and $P < 0.001$; F_4, dF = 98; $t = -10.11$ and $P < 0.001$; F_5, dF = 98; $t = -11.84$ and $P < 0.001$; F_6, dF = 98; $t = -8.19$ and $P < 0.001$). Developmental time was significantly longer for 4th-instar nymphs reared on RFD in each generation than those reared on FAD (F_1, dF = 98; $t = -5.33$ and $P < 0.001$; F_2, dF = 98; $t = -7.37$ and $P < 0.001$; F_3, dF = 98; $t = -2.42$ and $P = 0.017$; F_4, dF = 98; $t = -17.74$ and $P < 0.001$; F_5, dF = 98; $t = -19.55$ and $P < 0.001$; F_6, dF = 98; $t = -11.30$ and $P < 0.001$). For 5th-instar nymphs, the developmental time was significantly longer for those reared on RFD in F_2, F_4, F_5, and F_6 than those reared on FAD (F_2, dF = 98; $t = -7.39$ and $P < 0.001$; F_4, dF = 98; $t = -11.76$ and $P < 0.001$; F_5, dF = 98; $t = -11.87$ and $P < 0.001$; F_6, dF = 98; $t = -9.30$ and $P < 0.001$). But there were no significant differences in developmental times for the first and the third generations RFD-fed 5th instars than those reared on FAD (F_1, dF = 98; $t = -1.97$ and $P = 0.052$; F_3, dF = 98; $t = -0.67$ and $P = 0.506$) (table 3).

Weight

There was no significant difference in egg weight between RFD-fed and FAD-fed *A. chinensis* in F_2, F_3, and F_4 (F_2, dF = 98; $t = 0.46$ and $P = 0.646$; F_3, dF = 98; $t = 1.12$ and $P = 0.266$; F_4, dF = 98; $t = 1.48$ and $P = 0.141$). Egg weights were significantly higher for FAD-fed *A. chinensis* than of RFD-fed *A. chinensis* in F_5 and F_6. These differences, although statistically significant, were small (F_5, dF = 98; $t = 2.05$ and $P = 0.043$; F_6, dF = 98; $t = 3.18$ and $P = 0.002$) (table 4).

The 1st-instar nymphs weighed significantly higher for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_2 (dF = 98; $t = -8.17$ and $P < 0.001$). However, there was no significant difference in weight for 1st-instar nymphs between RFD-fed nymphs and FAD-fed nymphs in other generations (F_3, dF = 98; $t = 0.99$ and $P = 0.326$; F_4, dF = 98; $t = -0.47$ and $P = 0.643$; F_5, dF = 98; $t = 0.84$ and $P = 0.406$; F_6, dF = 98; $t = 0.27$ and $P = 0.785$) (table 4).

The 2nd-instar nymphs weighed significantly higher for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_1 and F_2 (F_1, dF = 98; $t = -5.47$ and $P < 0.001$; F_2, dF = 98; $t = -12.21$ and $P < 0.001$). However, for F_3, F_4, F_5 and F_6, the 2nd-instar nymphs weighed significantly lower for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* (F_3, dF = 98; $t = 7.44$ and $P < 0.001$; F_4, dF = 98; $t = 11.12$ and $P < 0.001$; F_5, dF = 98; $t = 9.89$ and $P < 0.001$; F_6, dF = 98; $t = 9.09$ and $P < 0.001$) (table 4).

The 3rd-instar nymphs weighed significantly higher for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_1 (dF = 98; $t = -5.39$ and $P < 0.001$). However, the 3rd-instar nymphs weighed significantly lower for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_2, F_3, F_4, F_5, and F_6 (F_2, dF = 98; $t = 7.73$ and $P < 0.001$; F_3, dF = 98; $t = 10.66$ and $P < 0.001$; F_4, dF = 98; $t = 8.33$ and $P < 0.001$; F_5, dF = 98; $t = 13.80$ and $P < 0.001$; F_6, dF = 98; $t = 17.82$ and $P < 0.001$) (table 4).

The 4th-instar nymphs weighed significantly higher for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_1 (dF = 98; $t = -3.53$ and $P < 0.001$). For F_3, the 4th-instar nymphs were heavier for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis*, but there was no significant difference between them (dF = 98; $t = -0.86$ and $P = 0.395$). The 4th-instar nymphs weighed significantly lower for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_2, F_4, F_5, and F_6 (F_2, dF = 98; $t = 4.48$ and $P < 0.001$; F_4, dF = 98; $t = 16.48$ and $P < 0.001$; F_5, dF = 98; $t = 20.14$ and $P < 0.001$; F_6, dF = 98; $t = 22.10$ and $P < 0.001$) (table 4).

The 5th-instar nymphs weighed significantly lower for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_1, F_3, F_4, F_5 and F_6 (F_1, dF = 98; $t = 8.58$ and $P < 0.001$; F_3, dF = 98; $t = 5.04$ and $P < 0.001$; F_4, dF = 98; $t = 15.29$ and $P < 0.001$; F_5, dF = 98; $t = 12.58$ and $P < 0.001$; F_6, dF = 98; $t = 14.93$ and $P < 0.001$). However, there was no significant difference in weight between RFD-fed 5th-instar nymphs and FAD-fed 5th-instar nymphs in F_2 (dF = 98; $t = 1.51$ and $P = 0.135$) (table 4).

The female body weights were significantly higher for FAD-fed *A. chinensis* than of RFD-fed *A. chinensis* in each generation (F_1, dF = 98; $t = 11.50$ and $P < 0.001$; F_2, dF = 98; $t = 3.90$ and $P < 0.001$; F_3, dF = 98; $t = 4.64$ and $P < 0.001$; F_4, dF = 98; $t = 9.49$ and $P < 0.001$; F_5, dF = 98; $t = 8.39$ and $P < 0.001$; F_6, dF = 98; $t = 4.69$ and $P < 0.001$). The male body weights were significantly higher for FAD-fed *A. chinensis* than of RFD-fed

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Table 3. Egg and nymphal developmental time (days) of *Arma chinensis* reared on a former artificial diet (FAD) (Zou *et al.*, 2013*a*) and reformulated diet (RFD) for six generations.

Generation	Treatment	*n*	Egg[a]	1st instar[b]	2nd instar	3rd instar	4th instar	5th instar
F_1	FAD	50			4.44 ± 0.13 *	5.18 ± 0.15 *	6.52 ± 0.20 *	9.04 ± 0.22
	RFD	50			5.92 ± 0.14 *	6.96 ± 0.18 *	8.40 ± 0.29 *	9.78 ± 0.31
F_2	FAD	50	6.28 ± 0.08	3.28 ± 0.06	6.12 ± 0.14 *	5.44 ± 0.08 *	6.62 ± 0.38 *	9.54 ± 0.49 *
	RFD	50	6.36 ± 0.11	3.10 ± 0.08	5.62 ± 0.15 *	8.60 ± 0.28 *	10.04 ± 0.27 *	13.64 ± 0.26 *
F_3	FAD	50	6.18 ± 0.08	3.20 ± 0.06	4.96 ± 0.11 *	5.30 ± 0.20 *	7.72 ± 0.57 *	12.84 ± 0.93
	RFD	50	6.42 ± 0.10	3.08 ± 0.07	5.46 ± 0.09 *	6.68 ± 0.18 *	9.24 ± 0.28 *	13.48 ± 0.22
F_4	FAD	50	6.08 ± 0.09	3.16 ± 0.06	5.14 ± 0.11 *	5.00 ± 0.12 *	5.28 ± 0.15 *	8.22 ± 0.34 *
	RFD	50	6.34 ± 0.11	3.12 ± 0.07	6.58 ± 0.14 *	7.86 ± 0.25 *	11.22 ± 0.30 *	13.08 ± 0.24 *
F_5	FAD	50	6.34 ± 0.08	3.22 ± 0.07	5.22 ± 0.11 *	4.30 ± 0.09 *	5.08 ± 0.13 *	7.96 ± 0.33 *
	RFD	50	6.30 ± 0.10	3.14 ± 0.08	6.34 ± 0.16 *	6.86 ± 0.20 *	10.96 ± 0.27 *	12.74 ± 0.23 *
F_6	FAD	50	6.42 ± 0.08	3.20 ± 0.06	5.20 ± 0.11 *	4.28 ± 0.09 *	5.06 ± 0.13 *	7.82 ± 0.26 *
	RFD	50	6.40 ± 0.10	3.10 ± 0.08	6.18 ± 0.17 *	5.64 ± 0.14 *	7.76 ± 0.20 *	11.62 ± 0.31 *

Values are mean ± SE. Asterisks identified the significant difference in development time between FAD and RFD in the same generation ($P < 0.05$).
[a]Egg mass.
[b]1st instar clutch.

Table 4. Egg and nymphal weights (mg) of *Arma chinensis* reared on a former artificial diet (FAD) (Zou, 2013; Zou *et al.*, 2015) and reformulated diet (RFD) for six generations.

Generation	Treatment	*n*	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar
F_1	FAD	50			1.33 ± 0.03 *	3.48 ± 0.10 *	10.41 ± 0.38 *	38.41 ± 1.48 *
	RFD	50			1.58 ± 0.03 *	4.54 ± 0.17 *	12.37 ± 0.40 *	25.05 ± 0.48 *
F_2	FAD	50	0.45 ± 0.00	0.58 ± 0.01 *	1.02 ± 0.03 *	7.28 ± 0.25 *	15.57 ± 0.58 *	33.49 ± 1.18
	RFD	50	0.45 ± 0.00	0.76 ± 0.02 *	1.66 ± 0.04 *	5.12 ± 0.11 *	12.43 ± 0.39 *	31.47 ± 0.64
F_3	FAD	50	0.47 ± 0.01	0.98 ± 0.01	2.79 ± 0.06 *	8.54 ± 0.30 *	14.59 ± 0.56	36.89 ± 1.09 *
	RFD	50	0.46 ± 0.01	0.97 ± 0.01	2.19 ± 0.05 *	5.14 ± 0.11 *	15.11 ± 0.26	30.64 ± 0.60 *
F_4	FAD	50	0.45 ± 0.00	0.95 ± 0.01	3.01 ± 0.07 *	8.21 ± 0.31 *	28.44 ± 0.80 *	57.39 ± 1.08 *
	RFD	50	0.44 ± 0.00	0.96 ± 0.01	2.13 ± 0.04 *	5.49 ± 0.10 *	14.59 ± 0.25 *	35.69 ± 0.92 *
F_5	FAD	50	0.46 ± 0.01 *	0.98 ± 0.01	3.03 ± 0.07 *	9.19 ± 0.18 *	26.52 ± 0.55 *	57.01 ± 1.29 *
	RFD	50	0.45 ± 0.00 *	0.97 ± 0.01	2.30 ± 0.03 *	6.21 ± 0.12 *	13.93 ± 0.30 *	35.88 ± 1.08 *
F_6	FAD	50	0.46 ± 0.01 *	0.96 ± 0.01	3.04 ± 0.07 *	9.54 ± 0.17 *	26.67 ± 0.53 *	57.23 ± 1.06 *
	RFD	50	0.44 ± 0.00 *	0.95 ± 0.01	2.36 ± 0.03 *	5.90 ± 0.11 *	13.54 ± 0.26 *	35.61 ± 0.99 *

Eggs or nymphs per treatment was weighted individually at 2 d after egg deposition for eggs and 2 d after eclosion for nymphs. Values are mean ± SE. Asterisks identified the significant difference in weight between FAD and RFD in the same generation ($P < 0.05$).

A. chinensis in each generation except for F_2 (F_1, dF = 98; t = -8.83 and $P < 0.001$; F_3, dF = 98; $t = 2.85$ and $P = 0.005$; F_4, dF = 98; $t = 6.41$ and $P < 0.001$; F_5, dF = 98; $t = 9.38$ and $P < 0.001$; F_6, dF = 98; $t = 5.68$ and $P < 0.001$). For F_2, there was no significant difference in male body weight between RFD-fed *A. chinensis* and FAD-fed *A. chinensis* (dF = 98; $t = -0.71$ and $P = 0.480$). Adult females and males reared on RFD weighed an average of 8.09 to 19.62 mg and 4.75 to 11.29 mg less than those reared on FAD (table 6).

Length

There was no significant difference in egg length between RFD-fed and FAD-fed *A. chinensis* in F_2 and F_3 (F_2, dF = 98; $t = 1.31$ and $P = 0.192$; F_3, dF = 98; $t = 1.79$ and $P = 0.076$). Egg lengths were significantly shorter for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_4, F_5 and F_6. These differences, although statistically significant, were small (F_4, dF = 98; $t = 5.31$ and $P < 0.001$; F_5, dF = 98; $t = 5.03$ and $P < 0.001$; F_6, dF = 98; $t = 6.30$ and $P < 0.001$) (table 5).

When fed RFD, the body length of F_2 *A. chinensis* 1st-instar nymphs was significantly shorter than those fed FAD (dF = 98; $t = 3.42$ and $P < 0.001$). However, the body length of 1st-instar nymphs was significantly longer for RFD-fed *A. chinensis* than those fed FAD in F_3 (dF = 98; $t = -2.30$ and $P = 0.023$). There was no significant difference in body length for 1st-instar nymphs between RFD-fed nymphs and FAD-fed nymphs in other generations (F_4, dF = 98; $t = 0.71$ and $P = 0.482$; F_5, dF = 98; $t = 0.62$ and $P = 0.537$; F_6, dF = 98; $t = 0.77$ and $P = 0.445$) (table 5).

For 2nd instar nymphs, there was no significant difference in body length between RFD-fed nymphs and FAD-fed nymphs in the first four generations (F_1, dF = 98; $t = -0.96$ and $P = 0.341$; F_2, dF = 98; $t = -0.34$ and $P = 0.736$; F_3, dF = 98; $t = 0.55$ and $P = 0.582$; F_4, dF = 98; $t = 1.39$ and $P = 0.168$). Body lengths of 2nd instar nymphs were significantly shorter for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_5 and F_6. These differences, although statistically significant, were small (F_5, dF = 98; $t = 2.02$ and $P = 0.046$; F_6, dF = 98; $t = 2.63$ and $P = 0.010$) (table 5).

The RFD-fed 3rd-instar nymphs were a little shorter than those fed FAD in F_1, but there was no significant difference between them (dF = 98; $t = 1.41$ and $P = 0.162$). However, the 3rd-instar nymphs were significantly shorter for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in other generations (F_2, dF = 98; $t = 8.79$ and $P < 0.001$; F_3, dF = 98; $t = 8.24$

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Table 5. Egg and nymphal body length (mm) of *Arma chinensis* reared on a former artificial diet (FAD) (Zou, 2013; Zou *et al.*, 2015) and reformulated diet (RFD) for six generations.

Generation	Treatment	*n*	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar
F_1	FAD	50			2.30 ± 0.01	3.53 ± 0.03	5.67 ± 0.05 *	8.46 ± 0.06 *
	RFD	50			2.32 ± 0.02	3.48 ± 0.03	5.35 ± 0.05 *	7.76 ± 0.02 *
F_2	FAD	50	0.88 ± 0.01	1.64 ± 0.01 *	2.51 ± 0.01	3.87 ± 0.03 *	5.35 ± 0.06	8.11 ± 0.06
	RFD	50	0.87 ± 0.00	1.52 ± 0.03 *	2.52 ± 0.03	3.55 ± 0.02 *	5.45 ± 0.04	7.97 ± 0.04
F_3	FAD	50	0.89 ± 0.01	1.68 ± 0.01 *	2.67 ± 0.02	4.03 ± 0.03 *	5.49 ± 0.05 *	8.12 ± 0.05 *
	RFD	50	0.87 ± 0.01	1.71 ± 0.01 *	2.65 ± 0.04	3.61 ± 0.04 *	5.67 ± 0.03 *	7.77 ± 0.03 *
F_4	FAD	50	0.92 ± 0.01 *	1.66 ± 0.01	2.69 ± 0.02	4.04 ± 0.03 *	6.43 ± 0.06 *	8.96 ± 0.02 *
	RFD	50	0.88 ± 0.01 *	1.65 ± 0.01	2.63 ± 0.04	3.89 ± 0.02 *	5.12 ± 0.04 *	7.79 ± 0.03 *
F_5	FAD	50	0.92 ± 0.01 *	1.67 ± 0.01	2.68 ± 0.02 *	4.12 ± 0.02 *	6.42 ± 0.03 *	8.96 ± 0.09 *
	RFD	50	0.88 ± 0.01 *	1.66 ± 0.01	2.62 ± 0.02 *	3.74 ± 0.03 *	5.03 ± 0.03 *	7.76 ± 0.08 *
F_6	FAD	50	0.93 ± 0.01 *	1.66 ± 0.01	2.68 ± 0.02 *	4.12 ± 0.02 *	6.43 ± 0.06 *	8.98 ± 0.08 *
	RFD	50	0.87 ± 0.01 *	1.65 ± 0.01	2.61 ± 0.02 *	3.92 ± 0.02 *	5.02 ± 0.04 *	7.63 ± 0.06 *

Egg and nymphal body length values were measured individually at 2 d after egg deposition for eggs and 2 d after eclosion for nymphs. Values are mean ± SE. Asterisks identified the significant difference in body length between FAD and RFD in the same generation ($P < 0.05$).

and $P < 0.001$; F_4, dF = 98; $t = 4.17$ and $P < 0.001$; F_5, dF = 98; $t = 11.14$ and $P < 0.001$; F_6, dF = 98; $t = 7.68$ and $P < 0.001$) (table 5).

For F_2, the 4th-instar nymphs were longer for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis*, but there was no significant difference between them (dF = 98; $t = -1.54$ and $P = 0.127$). The RFD-fed 4th-instar nymphs were significantly longer than those fed FAD in F_3 (dF = 98; $t = -2.93$ and $P = 0.004$). However, the 4th-instar nymphs were significantly shorter for RFD-fed *A. chinensis* than of FAD-fed *A. chinensis* in F_1, F_4, F_5 and F_6, (F_1, dF = 98; $t = 4.55$ and $P < 0.001$; F_4, dF = 98; $t = 18.35$ and $P < 0.001$; F_5, dF = 98; $t = 32.35$ and $P < 0.001$; F_6, dF = 98; $t = 19.78$ and $P < 0.001$) (table 5).

The RFD-fed 5th-instar nymphs were a little shorter than those fed FAD, but there was no significant difference between them in F_2 (dF = 98; $t = 1.85$ and $P = 0.067$). For other generations, the RFD-fed 5th-instar nymphs were significantly shorter than those fed FAD (F_1, dF = 98; $t = 11.00$ and $P < 0.001$; F_3, dF = 98; $t = 5.51$ and $P < 0.001$; F_4, dF = 98; $t = 31.46$ and $P < 0.001$; F_5, dF = 98; $t = 10.52$ and $P < 0.001$; F_6, dF = 98; $t = 13.24$ and $P < 0.001$) (table 5).

The body length of RFD-fed female was significantly shorter than those fed FAD for each generation in a manner similar to the female body weight (F_1, dF = 98; $t = 6.50$ and $P < 0.001$; F_2, dF = 98; $t = 8.84$ and $P < 0.001$; F_3, dF = 98; $t = 12.43$ and $P < 0.001$; F_4, dF = 98; $t = 16.17$ and $P < 0.001$; F_5, dF = 98; $t = 18.62$ and $P < 0.001$; F_6, dF = 98; $t = 17.79$ and $P < 0.001$). The body length of RFD-fed male was significantly shorter than those fed FAD for each generation (F_1, dF = 98; $t = 18.46$ and $P < 0.001$; F_2, dF = 98; $t = 11.80$ and $P < 0.001$; F_3, dF = 98; $t = 11.31$ and $P < 0.001$; F_4, dF = 98; $t = 16.06$ and $P < 0.001$; F_5, dF = 98; $t = 23.19$ and $P < 0.001$; F_6, dF = 98; $t = 10.88$ and $P < 0.001$). Adult females and males reared on RFD were 0.52 to 1.22 mm and 0.82 to 1.41 mm shorter on average than those reared on FAD (table 6).

Survival

The survival from 1st to 2nd-instar nymphs of F_2, F_3, and F_6 for RFD-fed *A. chinensis* was significantly lower than those of nymphs reared on FAD (F_2, dF = 1; $\chi^2 = 13.67$ and $P < 0.001$; F_3, dF = 1; $\chi^2 = 5.02$ and $P = 0.025$; F_6, dF = 1; $\chi^2 = 4.84$ and $P = 0.028$). These differences, although statistically significant, were small. There was no significant difference in survival from 1st to 2nd-instar nymphs of F_4 and F_5 between RFD and FAD treatments (F_4, dF = 1; $\chi^2 = 1.39$ and $P = 0.238$; F_5, dF = 1; $\chi^2 = 2.43$ and $P = 0.119$) (table 7).

There was no significant difference in the survival from 2nd instar to adult in F_1 and F_3 for RFD-fed and FAD-fed treatments (F_1, dF = 1; $\chi^2 = 0.48$ and $P = 0.488$; F_3, dF = 1; $\chi^2 = 0.01$ and $P = 0.906$). The survival from 2nd instar to adult of F_2, F_4, F_5 and F_6 for RFD-fed *A. chinensis* was significantly lower than for those reared on FAD (F_2, dF = 1; $\chi^2 = 7.89$ and $P = 0.005$; F_4, dF = 1; $\chi^2 = 18.71$ and $P < 0.001$; F_5, dF = 1; $\chi^2 = 8.47$ and $P = 0.004$; F_6, dF = 1; $\chi^2 = 13.45$ and $P < 0.001$) (table 7).

Cannibalism did not occur in nymphs because of isolation. However, adults reared on both FAD and RFD in all generations were observed cannibalizing eggs. There were significantly more eggs from RFD-fed *A. chinensis* that were cannibalized by adults than those from FAD-fed in F_2 (dF = 1; $\chi^2 = 596.37$ and $P < 0.001$). The egg cannibalism decreased from F_3 to F_6 in RFD-fed treatments and was significant lower for RFD-fed *A. chinensis* than those fed FAD in F_4, F_5 and F_6 (F_4, dF = 1; $\chi^2 = 7.77$ and $P = 0.005$; F_5, dF = 1; $\chi^2 = 19.92$ and $P < 0.001$; F_6, dF = 1; $\chi^2 = 20.20$ and $P < 0.001$). However, there was no significant difference in egg cannibalism between RFD-fed *A. chinensis* and FAD-fed *A. chinensis* in F_3 (dF = 1; $\chi^2 = 1.21$ and $P = 0.272$) (table 8).

There was no significant difference in the proportion of cannibalized females between RFD-fed adults and FAD-fed adults in F_1, F_3, F_4, F_5, and F_6 (F_1, dF = 1; $\chi^2 = 1.50$ and $P = 0.221$; F_3, dF = 1; $\chi^2 = 0.03$ and $P = 0.869$; F_4, dF = 1; $\chi^2 = 1.39$ and $P = 0.239$; F_5, dF = 1; $\chi^2 = 1.95$ and $P = 0.163$; F_6, dF = 1; $\chi^2 = 0.13$ and $P = 0.720$). However, there were less cannibalized females in RFD-fed than in FAD-fed insects in F_2 (dF = 1; $\chi^2 = 21.99$ and $P < 0.001$). A similar situation occurred in males of all groups. There was no significant difference in the proportion of cannibalized males between RFD-fed adults and FAD-fed adults in F_1, F_3, F_4, and F_6 (F_1, dF = 1; $\chi^2 = 0.68$ and $P = 0.411$; F_3, dF = 1; $\chi^2 = 0.19$ and $P = 0.663$; F_4, dF = 1; $\chi^2 = 1.00$ and $P = 0.318$; F_6, dF = 1; $\chi^2 = 1.01$ and $P = 0.316$). However, there were less cannibalized males in RFD-fed than in FAD-fed insects in F_2 and F_5 (F_2, dF = 1; $\chi^2 = 24.84$ and $P < 0.001$; F_5, dF = 1; $\chi^2 = 4.83$ and $P = 0.028$) (table 8).

The male adults lived longer than female adults in all of the treatments. The longevity of female adults reared on RFD was significantly shorter than for those reared on FAD in F_2, F_3, F_4,

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Table 6. Adult weight (mg), body length (mm), preoviposition (days), total fecundity and longevity (days) of *Arma chinensis* reared on a former artificial diet (FAD) (Zou, 2013; Zou *et al.*, 2013*a*, 2015) and reformulated diet (RFD) for six generations.

Generation	Treatment	*n*	Female body weight[a]	Female body length[a]	Male body weight[a]	Male body length[a]	Preoviposition	Female longevity[b]	Male longevity[b]	Total fecundity[c]
F_1	FAD	50	67.52 ± 1.33 *	11.96 ± 0.04 *	47.67 ± 1.00 *	10.87 ± 0.03 *	11.60 ± 0.33 *	34.94 ± 1.32	51.74 ± 2.41	181.82 ± 10.82 *
	RFD	50	50.45 ± 0.65 *	11.44 ± 0.07 *	37.88 ± 0.49 *	9.85 ± 0.05 *	12.72 ± 0.41 *	32.72 ± 1.03	50.12 ± 1.50	112.16 ± 4.60 *
F_2	FAD	50	59.42 ± 1.88 *	12.06 ± 0.07 *	42.72 ± 1.15	10.92 ± 0.04 *	13.96 ± 0.61	38.38 ± 1.31 *	45.92 ± 2.94 *	140.86 ± 10.85
	RFD	50	51.33 ± 0.86 *	11.28 ± 0.06 *	43.70 ± 0.76	10.10 ± 0.05 *	13.10 ± 0.30	28.44 ± 0.62 *	38.72 ± 1.57 *	134.94 ± 5.31
F_3	FAD	50	57.08 ± 1.61 *	12.56 ± 0.08 *	45.63 ± 1.37 *	11.23 ± 0.06 *	10.94 ± 0.39 *	37.34 ± 1.18 *	63.16 ± 3.86 *	187.44 ± 10.94 *
	RFD	50	48.59 ± 0.86 *	11.50 ± 0.04 *	40.90 ± 0.93 *	10.19 ± 0.07 *	13.88 ± 0.26 *	29.92 ± 0.82 *	45.74 ± 1.97 *	138.96 ± 5.93 *
F_4	FAD	50	69.46 ± 1.62 *	12.56 ± 0.05 *	53.34 ± 1.34 *	11.19 ± 0.06 *	9.50 ± 0.22 *	45.40 ± 1.78 *	54.92 ± 1.63 *	269.48 ± 14.74 *
	RFD	50	52.05 ± 0.86 *	11.48 ± 0.04 *	43.05 ± 0.88 *	9.98 ± 0.05 *	15.02 ± 0.34 *	31.04 ± 0.65 *	38.98 ± 1.43 *	142.20 ± 5.73 *
F_5	FAD	50	69.00 ± 2.03 *	12.52 ± 0.04 *	51.01 ± 0.91 *	11.21 ± 0.04 *	12.98 ± 0.42 *	44.36 ± 1.64 *	53.36 ± 1.46	261.78 ± 13.93 *
	RFD	50	49.38 ± 1.16 *	11.42 ± 0.04 *	39.72 ± 0.79 *	9.80 ± 0.04 *	16.74 ± 0.60 *	30.42 ± 0.78 *	51.70 ± 2.10	122.64 ± 5.01 *
F_6	FAD	50	69.27 ± 1.60 *	12.59 ± 0.05 *	52.94 ± 1.26 *	11.22 ± 0.04 *	13.14 ± 0.39 *	45.24 ± 1.57 *	54.02 ± 1.66 *	257.16 ± 13.45 *
	RFD	50	59.76 ± 1.25 *	11.37 ± 0.04 *	44.53 ± 0.77 *	10.35 ± 0.07 *	15.98 ± 0.39 *	35.90 ± 0.73 *	46.26 ± 1.58 *	121.78 ± 5.19 *

Values are mean ± SE. Asterisks identified the significant difference between FAD and RFD in the same generation ($P < 0.05$).
[a]Measured 2 days after emergence of adults.
[b]Adults died naturally.
[c]Average eggs per fertile female.

F_5 and F_6 (F_2, dF = 98; $t = 6.85$ and $P < 0.001$; F_3, dF = 98; $t = 5.14$ and $P < 0.001$; F_4, dF = 98; $t = 7.59$ and $P < 0.001$; F_5, dF = 98; $t = 7.66$ and $P < 0.001$; F_6, dF = 98; $t = 5.40$ and $P < 0.001$). RFD-fed females lived approximate 7.42–14.36 days shorter, respectively, than those reared on FAD. However, there was no significant difference in female longevity between RFD-fed adults and FAD-fed adults in F_1 (dF = 98; $t = 1.32$ and $P = 0.190$). The longevity of male adults reared on RFD was significantly shorter than for those reared on FAD in F_2, F_3, F_4, and F_6 (F_2, dF = 98; $t = 2.16$ and $P = 0.033$; F_3, dF = 98; $t = 4.02$ and $P < 0.001$; F_4, dF = 98; $t = 7.36$ and $P < 0.001$; F_6, dF = 98; $t = 3.39$ and $P = 0.001$). RFD-fed males lived approximate 7.20–15.94 days shorter, than those reared on FAD. However, there were no significant differences in male longevity between RFD-fed adults and FAD-fed adults in F_1 and F_5 (F_1, dF = 98; $t = 0.57$ and $P = 0.570$; F_5, dF = 98; $t = 0.65$ and $P = 0.518$) (table 6).

Reproductive capacity

The difference in total fecundity between RFD-fed and FAD-fed treatment in F_1, F_3, F_4, F_5, and F_6 was highly significant (F_1, dF = 98; $t = 5.93$ and $P < 0.001$; F_3, dF = 98; $t = 3.90$ and $P < 0.001$; F_4, dF = 98; $t = 8.05$ and $P < 0.001$; F_5, dF = 98; $t = 9.40$ and $P < 0.001$; F_6, dF = 98; $t = 9.39$ and $P = 0.001$). RFD-fed females laid significantly less eggs than those reared on FAD. For F_2, there was no significant difference in total fecundity between RFD-fed and FAD-fed treatment (dF = 98; $t = 0.49$ and $P = 0.625$) (table 6).

The viability of F_2, F_5, and F_6 eggs from RFD-fed females was significantly higher than those of females reared on FAD (F_2, dF = 1; $\chi^2 = 18.86$ and $P < 0.001$; F_5, dF = 1; $\chi^2 = 18.31$ and $P < 0.001$; F_6, dF = 1; $\chi^2 = 4.96$ and $P = 0.026$). However, the egg viability of F_3 from RFD-fed females was significantly lower than those of females reared on FAD (dF = 1; $\chi^2 = 59.93$ and $P < 0.001$) and there was no significant difference in egg viability of F_4 between RFD-fed *A. chinensis* and FAD-fed *A. chinensis* (dF = 1; $\chi^2 = 0.05$ and $P = 0.826$). Further, the viability decreased for eggs from RFD-fed females over F_3 to F_6 generations (table 7).

There was no significant difference in the proportion of fertile females (i.e., females that oviposited eggs) between RFD-fed females and FAD-fed females in F_1, F_2, and F_3 (F_1, dF = 1; $\chi^2 = 0.16$ and $P = 0.691$; F_2, dF = 1; $\chi^2 = 1.49$ and $P = 0.222$; F_3, dF = 1; $\chi^2 = 1.74$ and $P = 0.187$). The proportions of fertile females decreased from F_4 to F_6, and there was significant difference in the proportion of fertile females between RFD-fed females and FAD-fed females (F_4, dF = 1; $\chi^2 = 9.66$ and $P = 0.002$; F_5, dF = 1; $\chi^2 = 8.01$ and $P = 0.005$; F_6, dF = 1; $\chi^2 = 8.75$ and $P = 0.003$) (table 8).

The food source impacted the sex ratio (♂ : ♀). There were significantly more females than males in the RFD-fed F_1 than those reared on FAD (dF = 1; $\chi^2 = 8.03$ and $P = 0.005$). However, there was no significant difference in sex ratio between RFD-fed and FAD-fed treatments from F_2 to F_6 (F_2, dF = 1; $\chi^2 = 0.09$ and $P = 0.768$; F_3, dF = 1; $\chi^2 = 0.75$ and $P = 0.386$; F_4, dF = 1; $\chi^2 = 0.03$ and $P = 0.870$; F_5, dF = 1; $\chi^2 = 0.03$ and $P = 0.870$; F_6, dF = 1; $\chi^2 = 1.04$ and $P = 0.309$) (table 7).

Discussion

From a perspective of nutrigenomics, nutrients act as dietary signals that are detected by cellular sensors and

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Table 7. Egg viability, survival and sex ratio of *Arma chinensis* reared on a former artificial diet (FAD) (Zou *et al.*, 2013*a*, 2015) and reformulated diet (RFD) for six generations.

Generation	Treatment	*N*	Egg Viability[a]	*n*	Survival[b] to 2nds	*n*	Survival[c] to adults	*n*	Sex ratio (♂ : ♀)
F_1	FAD					150	0.493	353	1 : 0.657 *
	RFD					150	0.533	308	1 : 1.026 *
F_2	FAD	11,607	0.811 *	1106	0.974 *	150	0.787 *	368	1 : 1.044
	RFD	8651	0.835 *	1400	0.944 *	150	0.640 *	372	1 : 1.000
F_3	FAD	3312	0.833 *	1140	0.968 *	150	0.620	227	1 : 0.876
	RFD	4632	0.761 *	1237	0.950 *	150	0.613	305	1 : 1.020
F_4	FAD	7493	0.708	1033	0.978	150	0.867 *	332	1 : 1.259
	RFD	5327	0.710	977	0.970	150	0.653 *	334	1 : 1.227
F_5	FAD	11,081	0.691 *	1017	0.973	150	0.847 *	281	1 : 1.195
	RFD	7742	0.720 *	1225	0.962	150	0.707 *	292	1 : 1.163
F_6	FAD	6356	0.661 *	933	0.979 *	150	0.853 *	348	1 : 1.217
	RFD	4559	0.681 *	756	0.960 *	150	0.673 *	318	1 : 1.038

Asterisks identified the significant difference between FAD and RFD in the same generation ($P < 0.05$).
[a]Proportion of eggs not cannibalized that successfully hatched.
[b]Proportion of 1st instars developing to 2nd instars.
[c]Proportion of 2nd instars developing to adults.

Table 8. Cannibalism of eggs and adults of *Arma chinensis* reared on a former artificial diet (FAD) (Zou *et al.*, 2013*a*, 2015) and reformulated diet (RFD) for six generations.

Generation	Treatment	*N*	Life stages cannibalized				Fertility[a]
			Egg cannibalism[b]	*n*[c]	Adult female cannibalism[d]	Adult male cannibalism[e]	
F_1	FAD			150	0.300	0.387	0.900
	RFD			150	0.367	0.433	0.913
F_2	FAD	12,589	0.078 *	150	0.547 *	0.600 *	0.733
	RFD	7867	0.193 *	150	0.280 *	0.313 *	0.793
F_3	FAD	3981	0.168	120	0.183	0.283	0.875
	RFD	4032	0.159	120	0.192	0.258	0.927
F_4	FAD	8316	0.099 *	120	0.150	0.258	0.975*
	RFD	7426	0.086 *	120	0.208	0.317	0.867*
F_5	FAD	11,979	0.075 *	140	0.164	0.264 *	0.964*
	RFD	5407	0.056 *	140	0.107	0.157 *	0.871*
F_6	FAD	6834	0.070 *	130	0.146	0.277	0.969*
	RFD	4530	0.049 *	130	0.131	0.223	0.869*

Asterisks identified the significant difference between FAD and RFD in the same generation ($P < 0.05$).
[a]Proportion of females that oviposited. Dead males were replaced with virgin males of the same age.
[b]Proportion of eggs cannibalized.
[c]Total number of adult pairs.
[d]Proportion of females cannibalized in the total number of adult pairs (n^c), verified by the empty corpse of the females.
[e]Proportion of males cannibalized in the total number of adult pairs (n^c), verified by the empty corpse of the males.

influence gene and protein expression and, subsequently, metabolite production (Müller & Kersten, 2003). The mechanism of action of nutrients is strongly related to their capacity to modulate gene expression. However, in terms of gene expression-based biomarker development, progress to date has been limited. Although a number of potential gene expression-based nutrient sensitive biomarkers have been identified, these suffer from potential confounding effects that undermine their value. This reflects a fundamental problem with the specificity of single genes as biomarkers since expression of most individual genes are regulated by more than one environmental factor. Expression profile 'signatures,' defined as the characteristic patterns of differential gene expression, can help to overcome this problem and be used to measure responses to different levels of nutrients (Elliott, 2008).

Transcriptomes in responses to different foods had been analyzed in *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) (Zhang *et al.*, 2013), *Locusta migratoria* (Linnaeus) (Orthoptera: Acrididae) (Spit *et al.*, 2014), *Coleomegilla maculata* (DeGeer) (Coccinellidae: Coleoptera) (Allen, 2015) and *Spodoptera* spp (Lepidoptera: Noctuidae) (Roy *et al.*, 2016). However, an effort to reformulate diets according to transcriptome data have not been reported.

We report prominent performance improvements over six generations that resulted from the formulation changes based on transcriptome data. Those included increased adult body weight, shortened preoviposition period, and higher egg viability. Other improvements, such as shortened developmental time and increased weight during nymphal stages, were intermittent and interspersed with no change or a comparable decrease in performance within a generation and across generations. The most prominent decline in performance over six generations was lowered fecundity and survival from 2nd instar to adults.

The improvements we observed are encouraging. However, overall the formulation changes did not result in the level of

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improvement we want to achieve. If we accept the premise that transcriptome information accurately identifies nutritional shortfalls then it remains possible that changes we made may not have been extensive enough (e.g., requires more reduction of sugar and lipid, etc.) or that a more complex design may be needed to maximize the ingredients (Lapointe *et al.*, 2008) identified by the transcriptome information. It is also possible that Ecuadorian shrimp was not a good substitute for tuna fish.

Another possibility is that transcriptome results provide partial information about nutritional deficiencies but lack important post-transcriptional information that would be revealed via proteome analysis (Greenbaum *et al.*, 2003; de Godoy *et al.*, 2008; Maier *et al.*, 2009). Thus, expression profile 'signatures', differences in protein expression and metabolic profiling in diet-fed and prey-fed *A. chinensis* may provide valuable dietary insight that could complement, as well as extend, the transcriptome information and thereby aid in directing formulation improvements. These will be explored in future studies.

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