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Effect of different diets on growth and development of the two-spotted cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae)

Myung-Ha Song, Moon-Hee Han, Kyu-Won Kwak, Seokhyun Lee, Eun-Sun Kim, Kwan-Ho Park, Won-Tae Kim, and Ji-Young Choi*

Industrial Insect Division, National Institute of Agricultural Sciences, Rural Development Association, 166 Nongsaengmyeong-ro, Wanju, Jeollabuk-do 55365, Republic of Korea

Abstract

Many insects have gained increasing attention as an alternative protein for humans. Among those, the two-spotted cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae), was recently approved as a general food ingredient by the Korean Ministry of Food and Drug Safety. For industrial utilization of *G. bimaculatus*, mass rearing techniques and production system should be standardized first. In this study, we investigated the effects of five different feeds on the growth and development of *G. bimaculatus*. Feed is the one of the key factors that has considerable effects on rearing insects. With five different kinds of feed on 1st, 3rd, and 5th instar nymphs, the change of survival rate, body weight were monitored up to eight wk after hatching. We concluded that 50% of soybean flour, 20% of corn powder, 10% of rice bran, 9% of milk serum, 10% of rice flour, 0.5% of microorganisms, and 0.5% of multivitamins and minerals (diet C) was the best mix for promoting growth and development of 3rd instar nymphs compared to the control diet.

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Introduction

Insects are one of the biggest groups of living organisms. There are approximately one million species of insects, constituting about three-fourths of all organisms (Gullan *et al*., 2005). Depending upon their richness and variety, the resources from insects are enormous and diverse. Nowadays along with the technology development, insect-origin resources are utilized in various fields including agriculture, medicine, pharmaceuticals, and food industry (Berasategui *et al*., 2016; Lokeshwari *et al*., 2010).

In Korea Gryllidae, of the order Orthoptera, is composed of 5 families with 52 species. *Gryllus bimaculatus*, also known as the two-spotted cricket, is an exotic species and has unique two dots on the base of wings discriminating from other *Gryllus* species. *G. bimaculatus* is a subtropical insect and widely distributed from Africa to south Asia (Iba *et al*., 1995). Since they do not enter diapause, it is available all year round (Izumigama *et al*., 1986). Also, they are easily reared in the laboratory and have high productivity and large populations. They have been used as good models for studies such as behavioural ecology, endocrinology, pharmacology, and

***Corresponding author.**
Ji-Young Choi
Industrial Insect Division, National Institute of Agricultural Sciences, Rural Development Association, 166 Nongsaengmyeong-ro, Wanju, Jeollabuk-do 55365, Republic of Korea.
Tel: +82-63-238-2810 / FAX: +82-63-238-3833
E-mail: choijy7@korea.kr

physiology (Alexander, 1991; Gwynne *et al*., 1990; Simmons, 1991; Wagner *et al*., 1999; Yamasaki, 1986). Recently *G. bimaculatus* was approved as a general food ingredient by the Korean Ministry of Food and Drug Safety. However, domestic research on *G. bimaculatus* is still in its beginning stages, and particularly, the investigation for mass rearing remains unexplored.

To standardize the large-scale rearing system, the research on egg taking technique and optimal rearing condition is necessary. Also, it is known that intra- and interspecific competition among the order Orthoptera, especially exhibiting a high cannibalistic behaviour. Because eventually it needs to establish the mass production system and to standardize rearing system of *G. bimaculatus* for industrial utilization, it is important to investigate the mortality, body mass, and oviposition rate on rearing conditions. The aim of the present study is to find the standard feed mix to accelerate the growth of two-spotted crickets from various combination of soybean flour, corn powder, and rice bran in order to enhance the nutritional values and to establish the mass rearing technique.

Materials and Methods

Experimental animals

The *G. bimaculatus* were purchased from a commercial seller, Cricket Farm (Hwaseong-si, Gyeonggi-do, Korea) and a private seller. The purchased crickets were kept together to hybrid for increasing genetic diversity in the laboratory condition at 27±1°C with 60% humidity.

Development of artificial diet

Five types of the artificial diet were formulated in different proportions of ingredients. To test the effects of feed additives, we added soybean flour and/or rice bran based on Table 1. Among the ingredients, soybean flour and milk serum were used as protein sources. The diet containers were sealed and kept at 4°C.

Rearing condition

Adult crickets were reared in the laboratory regulated at 27±1°C and 60% relative humidity under 8h light and 16h dark photoperiod. However, until the adult emergence, the nymphs were reared in white plastic containers under 16:8(L:D) light conditions, because nymphs show the diurnal rhythm rather than the nocturnal rhythm of the adult crickets. The artificial diets were sufficiently fed to the nymphs with fully soaked flower foam, Oasis® for water supply and changed once a week. For each treatment, 100 individuals for the 1st, 3rd, and 5th instar nymphs, respectively, were reared to adult emergence. Each treatment was repeated three times. Averages and standard deviations of each experiment were compared to control feed and tested by ANOVA analysis.

Results and Discussion

The survival rate of *G. bimaculatus* under the five different diets was shown in Table 1. Except for the diet type B, the survival rate from each group of 1st, 3rd, and 5th instar to adult on artificial diets was two times higher than that on control diet (Table 2). The 1st and 3rd instar group showed relatively

Table 1. Composition of five different diets tested for rearing *G. bimaculatus*.

Name of feed	Ingredients
Control	100% Wheat bran
A	60% soybean flour + 40% corn powder
B	60% soybean flour + 20% corn powder + 19% rice bran + 0.5% microorganisms + 0.5% multivitamins and minerals
C	50% soybean flour + 20% corn powder + 10% rice bran + 9% milk serum + 10% rice flour + 0.5% microorganisms + 0.5% multivitamins and minerals
D	40% soybean flour + 25% corn powder + 10% rice bran + 10% milk serum + 10% rice flour + 4% roasted brown rice + 0.5% microorganisms + 0.5% multivitamins and minerals

Table 2. Survival rate (±SD) of the period from each nymphal instar to adult of *G. bimaculatus* reared on five diets.

Diets	Survival rate (%)		
	1st	3rd	5th (n.s.)
Control	8.3±0.58 a	15.3±8.08 a	27.7±2.08
A	19.0±1.73 b	33.3±9.29 c	54.7±14.29
B	8.7±1.53 a	19.0±4.36 ab	29.0±13.23
C	15.3±7.57 ab	30.3±3.06 c	55.0±11.53
D	17.0±6.08 ab	26.0±6.08 bc	48.0±21.93

The mean and standard deviation were analyzed with a confidence interval of 95% by Least Significant Difference (LSD) test.
n.s. No significant difference

low survival rate than 5th instar. And this was probably because the younger nymphs were much more susceptible to stress caused by the rearing condition. Therefore, there needs to be investigated further studied on the effect of rearing conditions, including temperature, humidity, and density on nymphal growth and development.

At the start point of diet treatments, the average body weights of the each instar nymph were similar (ranging from 1.44 to 1.56 mg for 1st instar group, 17.63-18.89 for 3rd instar, 86.28-92.44 for 5th instar, respectively). The final average weight of the diet type A, C, and D was higher than the control (Table 3). Especially, for the 1st instar nymph, those from group A, C, and D grew two wk faster (7 wk) to reach the same level of body weight than control group (9 wk). It seems that certain composition in diet A, C, and D stimulates the physiological mechanisms regulating the body mass of nymphs.

Table 4 showed the total nymphal period and adult weights from each diet group. Only those from group C had average 5 d shorter nymphal period compared to control group. This meaned that they grew faster and somehow their developments were promoted. Also, the average weights of adult crickets emerged from 1st, 3rd, and 5th nymphs fed with diet type C were 21%, 40%, and 14% heavier compared to the control group, respectively (Table 4).

Table 5 showed the oviposition period and number of

Table 3. Average body weight of *G. bimaculatus* at the start of each nymphal instar and at the end of the trial (±SD).

Diets	Weight of nymphal instars (mg)					
	1st (n.s.)		3rd		5th	
	Start of the trial	End of the trial	Start of the trial	End of the trial	Start of the trial	End of the trial
Control	1.5±0.06	396.4±72.68	17.3±7.29	399.2±134.91 a	85.7±12.86	392.2±40.46 a
A	1.5±0.06	460.2±179.46	18.4±4.56	497.3±133.43 b	94.0±7.81	548.1±71.24 b
B	1.5±0.06	229.5±43.27	18.7±4.88	339.4±35.96 a	89.7±3.21	467.4±52.15 ab
C	1.5±0.06	481.4±83.79	18.0±6.03	554.3±24.26 b	89.7±12.70	562.3±30.78 b
D	1.5±0.06	508.7±62.90	18.9±4.80	454.8±107.39 ab	87.7±12.86	494.4±49.90 ab

The mean and standard deviation were analyzed with a confidence interval of 95% by Least Significant Difference (LSD) test.
n.s. No significant difference

Table 4. The nymphal period and adult weight of *G. bimaculatus* on the conditioned diets (±SD).

Diets	No. of individuals	Nymphal period (days)			Weight of newly emerged adult (mg)		
		1st	3rd (n.s.)	5th (n.s.)	1st (n.s.)	3rd	5th
Control	185	64.9±4.12 ab	61.3±11.03	60.3±8.97	525.0±70.71	559.3±17.91 ab	571.0±18.27 ab
A	290	63.1±9.53 ab	59.7±8.30	57.7±7.12	547.1±75.40	648.8±37.46 b	697.9±36.97 c
B	173	68.9±8.56 b	61.6±9.97	59.1±9.85	515.0±28.28	527.9±79.96 a	523.6±95.71 a
C	289	57.4±8.73 a	57.4±7.04	60.9±5.64	602.4±14.15	786.4±34.55 c	694.7±26.74 c
D	274	61.8±8.44 ab	59.1±8.57	58.8±8.79	537.7±36.42	656.3±49.52 b	626.9±17.48 bc

The mean and standard deviation were analyzed with a confidence interval of 95% by Least Significant Difference (LSD) test.
n.s. No significant difference

Table 5. Oviposition periods and number of eggs (±SD) produced by *G. bimaculatus* reared on five diets.

Diets	Duration of oviposition (days)			Number of eggs per female per day		
	1st	3rd	5th	1st (n.s.)	3rd	5th
Control	26.7±3.79 a	41.9±10.06 b	50.2±14.46 b	40.4±2.20	31.2±3.38 ab	25.3±14.60 a
A	41.4±3.78 b	58.0±9.72 c	56.6±5.32 bc	70.6±14.67	48.8±7.16 bc	40.8±5.33 b
B	27.0±7.35 a	31.1±5.67 a	39.3±5.12 a	19.1±10.72	20.8±8.15 a	19.9±6.08 a
C	54.6±7.35 b	59.0±8.26 c	67.8±7.03 d	74.2±6.68	62.4±9.89 cd	64.4±13.46 c
D	47.0±6.22 c	56.3±10.40 c	62.4±7.09 cd	57.5±17.44	67.9±21.01 d	50.6±14.47 b

The mean and standard deviation were analyzed with a confidence interval of 95% by Least Significant Difference (LSD) test.
[n.s.] No significant difference

eggs laid per day. The oviposition periods of adults emerged from diet group A, C, and D were extended dramatically, compared to the control group. Interestingly, those from group B had much shorter oviposition period than control, while their body weights were not much different. Also, the daily egg production was much higher for those reared on diet A, C, and D than control group. Especially, the female emerged from group C laid eggs way over than double compared to the control, daily. These results led us to determine that the fecundity from diet group C was much higher than other diet groups or control. Therefore, the diet type C is the best feed mix not only for the nymphal growth and development but also for the fecundity.

Considering the results above, it was concluded that supplying diet C (50% of soybean flour, 20% of corn powder, 10% of rice bran, 9% of milk serum, 10% of rice flour, 0.5% of microorganisms, and 0.5% of multivitamins and minerals) to the 3rd instar nymph for minimum five to six wk was the most effective diet for the nymphal growth and development.

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