Journal of Insect Science, (2025) 25(3): 21; ieaf059
https://doi.org/10.1093/jisesa/ieaf059
Research

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OXFORD

Responses of house crickets (Orthoptera: Gryllidae) to various dietary gross energy levels: effects on growth performance and nutrient deposition

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Subject Editor: Christos Athanassiou

Received on 29 January 2025; revised on 24 April 2025; accepted on 29 April 2025

Crickets present a sustainable protein alternative with a lower ecological footprint compared to traditional animal proteins. This research aimed to investigate the effect of dietary energy density on feed intake, growth, and body composition in house crickets (*Acheta domesticus* L., Orthoptera: Gryllidae) up to 45 d after hatching. The study consisted of 2 phases (7 to 20 and 21 to 45 d of age), with house crickets randomly assigned to 5 dietary treatments, each with six replicates. Dietary energy levels ranged from 3,819 to 4,265 kcal gross energy (GE)/kg in phase 1 and from 3,978 to 4,405 kcal GE/kg in phase 2. As dietary energy density increased, feed intake linearly decreased, while body mass linearly increased. In phase 1, protein retention increased from 72.1% to 85.5% as GE increased from 3,819 to 4,265 kcal /kg. Similarly, in phase 2, protein retention increased from 53.3% to 59.3% as GE increased from 3,978 to 4,379 kcal/kg. Correspondingly, the feed conversion ratio (FCR) improved with increasing dietary GE values. Broken-line analysis revealed the lowest FCR at 4,158 and 4,382 kcal GE/kg feed for house crickets from 7 to 20 and 21 to 45 d after hatching, respectively. These findings confirm the relevance of energy density in achieving optimal growth performance and provide valuable insights for formulating nutritious cricket diets. However, caution is warranted when extrapolating these results, as diets were formulated using GE instead of metabolizable energy (ME). Future studies should determine cricket-specific ME values to fine-tune dietary energy density.

Keywords: gross energy requirement, body composition, efficiency, feed conversion ratio

Introduction

To date, the ecological footprint of animal protein production is of great public concern. From this perspective, crickets are considered an important alternative protein source because the environmental impact of raising crickets is lower compared to traditional livestock (Fernandez-Cassi et al. 2019, Udomsil et al. 2019). Furthermore, house crickets are considered nutritious for humans (Oonincx et al. 2015, 2020), and protein extracted from the house cricket (*Acheta domesticus* L., Orthoptera: Gryllidae) is widely used as an ingredient in food processing for products, such as pasta, chocolate, bakery items, and whey protein preparations (Melgar-Lalanne et al. 2019). In view of the sustainable and efficient production of cricket protein, nutrition plays a key role, but the nutrition of crickets is still in its infancy. To illustrate the latter, Bawa et al. (2020), Ssepuuya et al. (2021), and Vaga et al. (2021), for instance, reported only the ingredient composition and macro-nutrient profiles of diets. The dietary ingredients included commercially available cricket and poultry diets and pumpkin pulp (Bawa et al. 2020), locally available leaves of, amongst others, cassava and pumpkin (Ssepuuya et al. 2021), and various flowering plants locally available in Sweden (Vaga et al. 2021). Clearly, cricket-specific dietary recommendations on ingredient composition, profiles of digestible nutrients, and energy density are not yet established. These factors are considered of great

importance in relation to feed intake and thus the economic viability of cricket farming.

In poultry and swine, for instance, it is well known that the energy density of feed plays a crucial role in regulating feed intake. This can be illustrated by data from broilers reported by Maliwan et al. (2018), who demonstrated an inverse relationship between the energy density of feed and feed intake in indigenous Thai crossbred broilers (*Gallus gallus* domesticus). Consequently, a high energy density of feed may result in a lower feed conversion ratio (FCR, calculated as kg feed/kg growth) which in turn has a major impact on the economic return of meat production of animal protein. In contrast to poultry and swine, cricket-specific recommendations on the optimum energy density of cricket feed are scarce owing to a dearth of studies addressing this issue. The objective of the current research was therefore to investigate the effect of dietary energy density on feed intake and growth performance in house crickets up to 45 d after hatching. In practice, commercial cricket diets can be formulated using the nutrient requirements of broilers as a model (Nakagaki and Defoliart 1991, Halloran et al. 2017). Therefore, we used the same approach to formulate the experimental diets used in the current study. However, unlike poultry diet formulation, which uses metabolizable energy (ME) to express dietary energy density, we expressed the energy content of the experimental diets in terms of gross energy (GE) due to the current lack of data on cricket-specific ME or digestible energy (DE) values for the feed ingredients. In addition, we considered it opportune to analyze the body composition of the house crickets as well, so as to gain insight into the net requirements of protein and energy of growing crickets. It was anticipated that such information would be instrumental in designing future studies on the energy and nutrient requirements of crickets.

Materials and Methods

Cricket Management and Housing

House cricket eggs were obtained from the F&J Insect Farm, Sukhothai, Thailand and were kept with spawning material (coconut dust) in a plastic tray (40 × 25 × 9 cm, L × W × H). The temperature and relative humidity in the trays were maintained between 34–36 °C and 85–90%, respectively. Water was sprayed daily onto the trays to maintain the aforementioned high relative humidity. After approximately 7 d, the house cricket eggs hatched and 3 d afterward the unhatched eggs were removed. The nymphs were reared either until 7 or 21 d after hatching thereby allowing us to investigate the growth performance in response to the experimental diets from 7 to 20 d (phase 1) and from 21 to 45 d (phase 2) after hatching. During the rearing period, the house crickets were fed a commercial cricket feed containing 21% crude protein (Pure Pride feed 7001, TFMs Company Limited, Saraburi, Thailand), and fresh water was provided ad libitum through a drinking pipe with cotton wool balls soaked in water.

During the experimental periods (eg phases 1 and 2), the house crickets were raised in floor-stand smart-board containers (60 × 120 × 60 cm, L × W × H, stand 20 cm) equipped with 28 cardboard egg trays to provide shelter. Each container consisted of 3 tray feeders (30 × 23 × 2.5 cm, L × W × H) and a drinking pipe with cotton wool balls soaked in water. The house crickets were housed in a naturally ventilated system and surrounded by nets to prevent infestation by other insects. The lighting schedule was based on natural lighting (approximately 12 h/day).

Experimental Design and Diets

At the start of each phase, a pre-determined number of house crickets (detailed in the subsequent section on data and sample collection) were placed in each container. Then, each container was randomly allocated to one of the 5 dietary treatments/phase, with 6 containers/dietary treatment. During phase 1, the experimental diets contained 3,800, 3,900, 4,000, 4,100, or 4,200 kcal gross energy (GE)/kg feed, while during phase 2 the diets contained 4,000, 4,100, 4,200, 4,300, or 4,400 kcal GE/kg feed. The ingredient and analyzed composition of the experimental diets is shown in Tables 1 and 2. Feed ingredients (except for corn gluten meal) were ground to pass a 1 mm sieve using a hammer mill (SM-100 Retsch GmbH, Haan, Germany) and were subsequently mixed (RM-100, Mill Power Tech, Tainan, Taiwan). All diets were offered ad libitum and supplied to the house crickets in a mash form.

Data and Sample Collection

In this type of the current research, it was not possible to count the dead house crickets daily during each experimental phase. It was therefore impossible to express daily feed intake and body weight gain in terms of a mass unit/house cricket. Moreover, it is known crickets engage in cannibalism (Takacs et al. 2023) thereby hindering the interpretation of growth performance data when expressed per individual cricket. Therefore, the total house cricket mass/container, at the start and the end of each phase, along with the total feed intake/container were used to calculate the growth performance.

At the start of each phase, an aliquot of house crickets was euthanized using chloroform (99.8%, RCI LABSCAN, Bangkok, Thailand). Thereafter, house crickets were counted manually so as to obtain exactly 4,500 individuals. These 4,500 individuals were then weighed. This procedure was performed in triplicate. The mean, total body weights of the 4,500 individuals were 9.05 g (SD = 0.02, n = 3) and 43.95 g (SD = 0.01, n = 3) on days 7 and 21, respectively. Thus, we placed 9.1 or 44.0 g of house crickets (phases 1 and 2, respectively) in the experimental containers, assuming these weights corresponded with 4,500 individual house crickets. The latter value and the number of dead house crickets at the end of each phase were used to calculate the survival rate.

Each day, a known amount of feed was supplied to each container and leftover feed was recorded at the end of each phase so as to calculate total feed intake. The leftover feed was separated from feces using a mesh, and feces were removed with forceps to avoid contamination. In line with common practice in poultry research, feed moisture content was not taken into account to calculate feed intake because all of the experimental diets used in this study contained less than 10% moisture (Tables 1 and 2) (6.61–7.33% and 7.55–8.18% in phase 1 and 2, respectively).

Next to the preparation of the containers allocated to the experimental diets, both on days 7 and 21 after hatching, 3 extra containers with ~ 4,500 house crickets were prepared, and the crickets were killed directly thereafter with chloroform (99.8%, RCI LABSCAN, Bangkok, Thailand). Then, the carcasses were stored at – 20 °C pending further processing. On days 20 and 45, all house crickets from each container were harvested after fasting for 24 h and killed as described above. The house cricket carcasses were then stored at – 20 °C until further processing. During fasting, house crickets had free access to water. Prior to the chemical analysis of the carcasses, all frozen carcasses obtained at the start and the end of each phase were freeze-dried (Alpha 2 – 4 LSCplus, Christ, Germany), and subsequently ground to approximately 1 mm with

Table 1. Ingredient and analyzed macro nutrient composition of the experimental diets fed to house crickets from 7 to 20 d of age

	Dietary gross energy density (kcal/kg)				
Items	3,800	3,900	4,000	4,100	4,200
Ingredients, % as fed					
Corn	7.84	13.52	19.27	25.07	30.82
Cassava chip	15.00	15.00	15.00	15.00	15.00
Cassava pulp	15.00	11.25	7.50	3.75	0.00
Defatted rice bran	18.00	13.50	9.00	4.50	0.00
Soybean meal (44% CP)	36.00	36.00	36.00	36.00	36.00
Corn gluten meal (60% CP)	4.11	4.60	5.20	5.70	6.30
Palm oil	0.00	1.94	3.75	5.58	7.38
Sodium chloride (NaCl)	0.42	0.42	0.42	0.42	0.42
Calcium carbonate ($CaCO_3$)	1.20	1.30	1.38	1.47	1.56
Monocalcium phosphate ($Ca(H_2PO_4)_2$)	1.37	1.42	1.46	1.50	1.53
Premix[1]	0.50	0.50	0.50	0.50	0.50
DL-methionine (99% purity)	0.32	0.31	0.29	0.28	0.27
L-lysine (78% purity)	0.11	0.11	0.11	0.12	0.12
L-threonine (98.5% purity)	0.13	0.13	0.12	0.11	0.10
Analyzed composition (g/kg as fed)					
Gross energy (kcal/kg)[2]	3,819	3,965	4,098	4,167	4,265
Dry matter	927	928	934	932	927
Crude protein	234	234	236	225	239
Ether extract	10	30	54	76	92
Ash	85	79	84	77	68

[1]The premix provided per kg of diet: vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

[2]For sake of reference, the corresponding calculated ME values (kcal/kg of feed) in poultry diets are 2,400, 2,600, 2,800, 3,000, and 3,200, respectively.

Table 2. Ingredient and analyzed macro nutrient composition of the experimental diets fed to house crickets from 21 to 45 d of age

	Dietary gross energy density (kcal/kg)				
Items	4,000	4,100	4,200	4,300	4,400
Ingredient, % as fed					
Corn	26.42	29.02	31.44	33.91	36.45
Cassava chip	15.00	15.00	15.00	15.00	15.00
Cassava pulp	10.00	7.50	5.00	2.50	0.00
Defatted rice bran	12.00	9.00	6.00	3.00	0.00
Soybean meal (44% CP)	30.00	30.00	30.00	30.00	30.00
Corn gluten meal (60% CP)	2.96	3.50	4.04	4.56	5.08
Palm oil	0.00	2.30	4.74	7.16	9.56
Sodium chloride (NaCl)	0.31	0.31	0.30	0.30	0.30
Calcium carbonate ($CaCO_3$)	1.30	1.36	1.42	1.48	1.53
Monocalcium phosphate ($Ca(H_2PO_4)_2$)	0.98	1.00	1.06	1.10	1.10
Premix[1]	0.50	0.50	0.50	0.50	0.50
DL-methionine (99% purity)	0.20	0.19	0.18	0.17	0.16
L-lysine (78% purity)	0.17	0.17	0.17	0.18	0.18
L-threonine (98.5% purity)	0.16	0.15	0.15	0.14	0.14
Analyzed composition (g/kg as fed)					
Gross energy (kcal/kg)[2]	3,978	4,162	4,261	4,379	4,405
Dry matter	925	919	918	918	921
Crude protein	206	206	209	207	209
Ether extract	17	41	67	92	113
Ash	71	68	63	65	62

[1]The premix provided per kg of diet: vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

[2]For sake of reference, the corresponding calculated ME values (kcal/kg of feed) in poultry diets are 2,600, 2,800, 3,000, 3,200 and 3,400, respectively.

Table 3. Growth performance and survival rate of house crickets fed the experimental diets from 7 to 20 d of age. Unless stated otherwise, units are expressed as g /container

Items	Dietary gross energy density (kcal/kg)					Pooled SEM	P
	3,800	3,900	4,000	4,100	4,200		
Total cricket weight							
7 d	9.05	9.05	9.05	9.05	9.05	0.01	1.00
20 d	69.0ab	69.7a	67.6ab	54.8b	67.0ab	1.76	0.05
Total weight gain	59.9ab	60.6a	58.6ab	45.8b	58.0ab	1.76	0.05
Mean daily weight gain	4.61ab	4.66a	4.50ab	3.52b	4.46ab	0.14	0.05
Cumulative feed intake	58.8a	55.9a	48.4ab	36.4b	46.9ab	2.00	0.001
Feed conversion ratio[1]	0.98a	0.92a	0.83b	0.79b	0.81b	0.02	<0.001
Survival rate, %[2]	72.8	76.7	77.2	70.4	72.1	1.17	0.30

[a-b]values within each row with different superscript differ significantly ($P < 0.05$).

[1]Calculated as g of feed per container / g of body weight gain.

[2]Calculated as the number of living crickets on day 20 / number of living crickets on day 7 × 100.

the use of a laboratory mill (MRC Sample mill SM-450, Holon, Israel). Then, the samples were stored again at – 20 °C until a chemical analysis was conducted.

Chemical Analysis

The dry matter contents (method number 930.15), ether extracts (method number 920.39), and the ash contents (method number 942.05) of the experimental diets and the freeze-dried carcasses were determined according to the standard methods of the AOAC (1990). The nitrogen content was determined using a Dumas combustion technique (AOAC 2006, ID 990.03) with the use of an MAX N exceed, N/protein Analyzer (Elementar Analysen Systeme GmbH, Hanua, Germany). The combustion values of the experimental diets and that of the house cricket carcasses were measured using an adiabatic colorimeter bomb (C6000, IKA, Staufen, Germany).

Calculations and Statistical Analysis

The chemical composition of the gain in-house cricket mass/container was calculated as the difference between the absolute amounts of carcass water, -protein, -fat, and -ash at the end and start of each experimental phase. The energy retention was calculated likewise. The chemically unidentified fraction of the carcass was calculated as: 100% – %water – %protein – %fat – %ash.

Each individual container was considered as an experimental unit. All data were subjected to one-way analysis of variance using SAS software (SAS Institute 1996), after checking for normality of the distributions (SPSS version 18.0, SPSS Inc. 2010). Tukey's *t*-test was used to identify diets with different effects on the variable involved. Linear regression analysis was used to determine the relationships between the energy density of the diet (ie analyzed values, Tables 1 and 2), feed intake, weight gain, and weight gain of body water, protein, ash, and fat. Broken-line regression analysis (NLIN procedure of SAS software) was used to estimate the required GE content of the feed to provide constant values of FCR using the following model (Robbins et al. 2006):

$y = L + U \times (R—x)$

where y = FCR (dependent variable), x = dietary GE density (kcal/kg) (independent variable), R = required dietary GE density (kcal/kg), L is the response at x = R, and U is the slope of the curve. In this model, y = L when x > R. Throughout, differences were considered statistically significant when $P \leq 0.05$.

Results

Growth Performance and Nutrient Deposition from 7 to 20 d of Age

Initial house cricket mass (Table 3) was similar across the experimental diets ($P = 1.00$). However, after 13 d, house crickets fed a 3,900 kcal/kg diet exhibited a 27.2% greater weight gain ($P = 0.05$) compared to those fed a 4,100 kcal/kg diet. Total weight gain and mean daily weight gain were likewise affected ($P = 0.05$). Except for the diet with 4,100 kcal/kg ($P = 0.001$), feed intakes were similar across the experimental diets. The FCR was influenced by the energy density of the diet ($P < 0.001$) and the lowest values were observed when the energy density was at least 4,000 kcal/kg. The survival rate was not affected by the energy density of the diet ($P = 0.30$).

The proportions of protein, ash, and the chemically unidentified fraction were similar across treatments ($P \geq 0.23$). In contrast, the water and fat contents of the carcasses were significantly influenced by the experimental diets ($P < 0.05$). As dietary GE values increased, water content decreased while fat content increased (Table 4).

The absolute retentions of water, protein, fat, and ash, but not the unidentified fraction ($P = 0.15$), were affected by the energy density of the experimental diets ($P \leq 0.043$). House crickets fed the 4,100 kcal/kg diet exhibited the lowest absolute retention values for water, protein, and ash ($P \leq 0.043$), while fat retention was lowest in crickets-fed diets with the lowest energy density (3,800 kcal/kg) ($P = 0.011$). In contrast to the absolute retention of protein, the relative retention of protein (expressed as a % of protein intake) increased with increasing dietary energy values ($P < 0.001$). Absolute energy retention of energy was similar across experimental diets ($P = 0.13$), but when calculated as a % of intake, the lowest values were observed in diets containing 3,800 or 3,900 kcal/kg ($P < 0.001$).

Growth Performance and Nutrient Deposition from 21 to 45 d of Age

Initial house cricket mass (Table 5) was almost identical across experimental diets ($P = 0.98$). In contrast, after 24 d, house cricket mass varied inversely with dietary density, with those fed a 4,400 kcal/kg diet exhibiting a 23% lower mass compared to those fed a 4,000 kcal/kg diet ($P < 0.001$). Total weight gain, mean daily weight gain, and feed intake were likewise affected ($P < 0.001$). The feed conversion ratio was at least 9.4% greater ($P < 0.001$) in diets containing 4,000 or 4,100 kcal/kg compared to those containing 4,200, 4,300,

Table 4. Chemical composition of whole carcass on day 20 and macro nutrient retention[1] of house crickets fed the experimental diets from 7 to 20 d of age

	Dietary gross energy density (kcal/kg)						
Items	3,800	3,900	4,000	4,100	4,200	Pooled SEM	P
Carcass, % fresh weight							
Water	76.8a	76.7ab	75.7ab	76.3ab	75.4b	0.17	0.020
Protein	16.6	16.5	16.3	16.0	16.3	0.08	0.23
Fat	2.96b	3.55b	4.57a	4.83a	4.94a	0.19	<0.001
Ash	1.35	1.36	1.36	1.35	1.37	0.01	0.73
Unidentified	2.27	1.97	2.23	1.86	1.81	0.09	0.32
Absolute retention, g/container							
Water	46.4a	46.7a	44.7ab	35.4b	43.9ab	1.31	0.043
Protein	10.2a	9.2a	9.9ab	7.0b	9.2ab	0.33	0.027
Fat	1.82b	2.12ab	3.11a	2.26ab	2.93a	0.15	0.011
Ash	0.80a	0.72ab	0.78a	0.56b	0.75ab	0.03	0.039
Unidentified	1.22	0.91	1.26	0.58	0.75	0.10	0.15
Energy, kcal/container	71.9	75.1	77.7	60.3	80.5	5.71	0.13
Relative retention, % of intake							
Protein	72.1c	77.5bc	82.8ab	84.2a	85.5a	1.29	<0.001
Energy	31.9b	33.8b	39.0a	39.9a	40.3a	0.81	<0.001

[a-c]values within each row with different superscript differ significantly ($P < 0.05$).

[1]The chemical composition of the whole carcass of the house crickets measured on day 7 was used to calculate the initial, absolute amounts of whole carcass macro nutrients. The values (% of fresh weight) were as follows: water, 73.66; protein, 16.70; fat, 3.24; ash, 1.74, unidentified, 4.66.

Table 5. Growth performance and survival rate of house crickets fed the experimental diets from 21 to 45 d of age. Unless stated otherwise, units are expressed as g /container

	Dietary gross energy density (kcal/kg)						
Items	4,000	4,100	4,200	4,300	4,400	Pooled SEM	P
Body weight							
21 d	43.9	43.9	43.9	43.9	43.9	0.01	0.98
45 d	666.3a	640.2ab	631.4ab	572.4bc	513.2c	12.65	<0.001
Total body weight gain	622.4a	596.3ab	587.5ab	528.5bc	469.2c	12.65	<0.001
Mean daily gain	25.9a	24.8ab	24.5ab	22.0bc	19.6c	0.53	<0.001
Cumulative feed intake	898.8a	832.8ab	742.4bc	675.8cd	591.3d	22.93	<0.001
Feed conversion ratio[1]	1.44a	1.40a	1.27b	1.28b	1.26b	0.02	<0.001
Survival rate, %[2]	82.6a	80.8ab	77.9abc	73.2bc	70.2c	1.21	0.002

[a-c]values within each row with different superscript differ significantly ($P < 0.05$).

[1]Calculated as mg of feed / mg of body weight gain.

[2]Calculated as the number of living crickets on day 45 / number of living crickets on day 21 × 100.

and 4,400 kcal/kg. The survival rate was inversely related to the dietary GE content ($P = 0.002$).

The water content of the carcasses (Table 6) was affected by the experimental diets ($P = 0.001$), with the greatest water content observed in house crickets-fed diets containing either 4,000 or 4,100 kcal/kg. The proportion of protein in the carcasses decreased with increasing GE values of the diet ($P = 0.002$), while the fat content increased as the energy density of the diet increased ($P < 0.001$). The ash content was similar across dietary treatments ($P = 0.77$), but the chemically unidentified fraction of the carcass was affected by the dietary GE content: i.e., the value was 23.3% lower in diets with 4,200 compared to those with 4,000 kcal/kg.

The absolute retention of water, protein, ash, and the chemically unidentified fraction responded similarly to the experimental diets; all values decreased with increasing dietary GE values ($P \leq 0.008$). When protein retention was expressed as a % of protein intake, the highest value was observed in house crickets fed a diet with 4,300 kcal/kg, which was 6.0 percentage units greater compared to the diet with the lowest GE value. In contrast to the absolute retention of water, protein, and ash, the absolute retention of fat responded differentially to the experimental diets, with the greatest values ($P = 0.019$) observed in house crickets fed a diet containing 4,200 kcal/kg. The latter value was 36.6% greater compared to the value observed after feeding with a diet of 4,000 kcal/kg. The absolute retention of energy was not affected by the dietary GE content ($P = 0.09$), but the relative retention of energy (as a % of GE intake) increased with increasing GE values of the diet ($P = 0.001$).

Broken-line Analysis

The estimated dietary GE content to achieve minimum FCR values for house crickets from 7 to 20 and 21 to 45 d after hatching, was found to be 4,158 and 4,382 kcal GE /kg, respectively (Table 7, Fig. 1).

Table 6. Chemical composition of whole carcass on day 45 and macro nutrient retention[1] of house crickets fed the experimental diets from 21 to 45 d of age

Items	Dietary gross energy density (kcal/kg)					Pooled SEM	P
	4,000	4,100	4,200	4,300	4,400		
Carcass, % fresh weight							
Water	75.7a	75.3a	74.9ab	74.1b	74.2b	0.16	0.001
Protein	16.0a	15.8a	15.7ab	15.7a	15.3b	0.07	0.002
Fat	4.27c	4.88c	6.11b	6.72ab	7.20a	0.23	<0.001
Ash	1.29	1.26	1.26	1.27	1.26	0.01	0.77
Unidentified	2.62a	2.23ab	2.01b	2.54ab	2.51ab	0.07	0.022
Absolute retention, g/container							
Water	473.9a	470.4a	440.7ab	389.7bc	333.2c	13.09	<0.001
Protein	100.4a	94.9ab	91.4ab	84.4b	68.7c	2.50	<0.001
Fat	27.3b	30.3ab	37.3a	37.2a	34.6ab	1.21	0.019
Ash	7.90a	7.24ab	7.10ab	6.43bc	5.46c	0.19	<0.001
Unidentified	15.8a	12.6ab	11.0c	12.6ab	10.7c	0.97	0.008
Energy, kcal/container	855	890	883	871	713	55.70	0.09
Relative retention, % of intake							
Protein	53.3c	54.6bc	59.0ab	59.3a	57.2abc	0.66	0.002
Energy	23.6c	24.2bc	27.9ab	28.8a	29.2a	0.61	0.001

[a-c]values within each row with different superscript differ significantly ($P < 0.05$).

[1]The chemical composition of the whole carcass of the house crickets measured on day 21 was used to calculate the initial, absolute amounts of whole carcass macro nutrients. The values (% of fresh weight) were as follows: water, 73.68; protein, 17.06; fat, 3.25; ash, 1.88, unidentified, 4.13.

Table 7. Estimated gross energy requirement of house crickets based on broken-line model analyses from 7 to 20 d and from 21 to 45 d of age

Items	Regression equations[1]	SE[2]	P	R^3
7–20 d	$y = 0.8017 + 0.000553 \times (4{,}158.4 - x)$	34.42	<0.0001	0.80
21–45 d	$y = 1.26 + 0.00046 \times (4{,}381.5 - x)$	64.52	<0.0001	0.63

[1]The linear broken-line model is $y = L + U \times (R - x)$, where y = feed conversion ratio (FCR); x = dietary gross energy (GE) content (kcal/kg); R = required dietary GE content (kcal/kg) to become constant values of the FCR; L = the response at x = R; and U = the slope of the curve. In this model, y = L when $x > R$.

[2]SE = standard error.

[3]R = The percentage of variance in FCR that is explained by the broken-line model.

Discussion

Dietary Energy Density, Feed Intake, Growth Performance, and Survival

During phase 1, the linear relationship between the energy density of the diet and feed intake was not found to be significant (Fig. 2A). However, upon visual inspection of the data, the feed intake associated with a dietary energy density of 4,167 kcal GE/kg appears to be out of the range, which is consistent with the results shown in Table 3. This notion is also in line with the finding that omission of this data improves the relationship ($n = 4$, $R^2_{adj} = 87.7\%$, $P = 0.042$) between the energy density of the diet and feed intake during phase 1. The observed low feed intake in house crickets supplied with 4,167 kcal GE/kg feed cannot be explained; therefore, we have to consider the low feed intake as an aberrant value. Although less pronounced during phase 1, it thus appears that the energy density of the diet inversely affects the feed intake in house crickets (Fig. 2A and C). The current result on feed intake is consistent with results from poultry (Leeson and Summers 2005, Maliwan et al. 2018, 2022) and swine (Quiniou and Noblet 2012), which also showed an inverse relationship between the energy density of the diet and the feed intake.

For obvious reasons, total weight gain of the house crickets responded positively to an increase in feed intake (Fig. 2B and D), but the relationship between feed intake and weight gain during phase 2 was more evident compared to phase 1. In fact, when the observation on total weight gain related to the house crickets supplied with 4,167 kcal GE/kg feed is omitted, the remaining total weight gain values were found to be similar across the dietary treatments (Table 1). Nevertheless, the current results on feed intake and weight gain indicate an inverse relationship between dietary energy density and FCR. Indeed, during phase 1, the variation in dietary energy density explained 87.4% of the variation in FCR (data not shown, $n = 5$, FCR = 2.67 – 0.00044 × GE/kg, $P = 0.013$), while during phase 2, this variation accounted for 80.2% of the variation in FCR (data not shown, $n = 5$, FCR = 3.25 – 0.00045 × GE/kg, $P = 0.025$). The latter results also are in line with results obtained from poultry (Maliwan et al. 2018, 2022, Hu et al. 2019) indicating that a greater energy content of the diet results in a lower FCR.

During phase 2, but not phase 1, the survival rate was lowest when the diet containing the greatest energy content was fed. This observation, however, is difficult to explain. Perhaps, the high dietary fat content played a role. In the current study, palm oil was used to adjust the energy density of the diet, and the diet with the lowest observed survival rate (70.2%) contained 4,400 kcal GE/kg and 9.56% palm oil. Previously, Adamo et al. (2010) reported that feeding high-fat diets (34% as fed) was associated with reduced resistance to bacterial infections. In addition, Adamo et al. (2008) reported that high levels of hemolymph lipids in cricket diets led to a reduction in monomeric apolipophorin III concentrations, resulting in reduced resistance to bacteria. However, to the best of our knowledge, we are not aware of any increased risk of a bacterial infection during phase 2, thereby hindering any substantiation of the aforementioned notion.

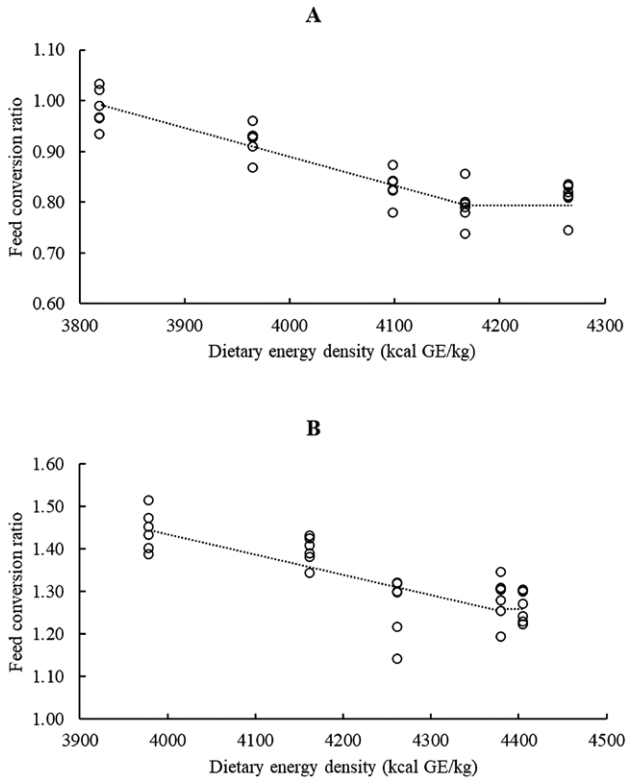

Fig. 1. Relationship between the dietary gross energy (GE) density and the feed conversion ratio (FCR) in house crickets from 7 to 20 (panel A) and 21 to 45 (panel B) d of age. The solid line represents the FCR estimated with the use of the linear broken-line models shown in Table 7. The data points (o) represent the least square means of each dietary treatment ($n = 6$).

Body Composition and Weight Gain

Across the 2 phases of the experiment, the variation in absolute retention of water, protein, and ash collectively explained the 99.7% variation in total weight gain (Fig. 3A–C, respectively). The high value on the explained variance is due, at least partly, to the wide range of data points, with no data falling within the range of 100 to 450 g weight gain/container. On the other hand, the data points obtained from phase 2 closely fit the regression line, and the intercepts of all 3 regression lines were near zero, thereby implying that the regression lines were plausible from a physiological perspective. The current data align with the findings from broilers, cattle, and pigs, showing that the water retention is quantitatively the most important factor in explaining the cricket weight gain, followed by protein retention (Owens et al. 1995, Schiavon and Emmans 2000, Caldas et al. 2019). In contrast to water, protein, and ash, the relationship between fat retention and body weight gain is less straightforward (Fig. 3D). The high value of the explained variance (90.5%) is mainly due to the wide range of data points. This notion is fueled by the observation that, with the exception of observation in each phase, the absolute fat retention was basically similar across the dietary treatments during both experiment phases (Table 1).

It is well established that the retention of both water and protein, rather than fat, decreases the FCR. In the current study, however, within each phase, the relationships between either absolute water or protein retention and FCR, were not found to be significant (ie $n = 5$, $R^2_{adj} \leq 45.4\%$, $P \geq 0.13$). Likewise, absolute fat retention was not significantly related to FCR during phase 1 (ie $n = 5$, $R^2_{adj} = 39.1\%$, $P = 0.16$). However, during phase 2, the variation in absolute fat retention explained 87.8% of the variation in FCR (data not shown, $n = 5$, FCR = 1.95 – 0.019 × GE/kg, $P = 0.012$). Clearly, the current results regarding the relationships among water, protein, and fat deposition in the body of crickets are not straightforward. This is most likely due to the fact that within each phase, the FCR clustered primarily with the 2 diets that had the lowest energy density and the 3 diets that had the highest energy density. It is speculated that using a wider range of GE content in the experimental diets within each phase would have yielded less ambiguous results.

In view of the nature of the chemical analysis of protein, fat, and ash, it seems fair to assume that the chemically unidentified fraction likely represents the carbohydrate fraction of the body, which in vertebrates primarily consists of glycogen and, to a lesser extent, glucose (Toldrá et al. 2014). To the best of the author's knowledge, the chemical nature of the nitrogen-free extract fraction in crickets is not yet completely characterized, but it is likely similar to that found in vertebrates (Hansen et al. 2020, Zafar et al. 2023).

Relative Protein and Energy Retention (% of intake)

Within each phase, the greatest values on the relative retention of energy and protein were found when house crickets were fed diets with the highest GE content; ie ≥ 4,000 Kcal/kg in phase 1 and ≥ 4,200 Kcal/kg in phase 2. The increased efficiency of energy retention with high-energy diets can be explained by the ratio of energy required for maintenance (including movement) compared to that needed for growth. Indeed, it is well known that, at least in poultry and swine, in case of a relative low energy intake, a greater amount of dietary energy is used proportionally for maintenance (Korver and Angle 2019, Liu et al. 2019, Zuidhof 2019), which cannot then be used to support the synthesis of body protein and fat. From the perspective of protein synthesis, this also explains why the efficiency of protein deposition is greater when high-energy diets are fed (Lundy and Parrella 2015, Kaewtapee et al. 2024).

It must be kept in mind that cricket-specific data on metabolizable energy and digestibility of nutrients are currently not available. Thus, caution is warranted with respect to the interpretation of the values on the relative protein and energy retention currently reported.

Chemical Composition of Body Gain

The current data on the chemical composition of the weight gain are provided in Table 8, and it is anticipated that the reported values can serve as a benchmark for future studies on nutrient and energy requirements of crickets. Across the 2 experimental phases, the chemical composition of the body weight gain was found to be fairly constant. When the chemical composition of weight gain is expressed as % of the fat-free gain, the values are similar, with the exception of the percentage of water. This observation indicates that, at least within the current life stages of the house crickets, fat deposition is relatively low compared to protein deposition. The mean water-to-protein ratio of the gain was found to be 4.8, which is almost 20% greater compared to the value calculated for the data from broilers reported by Tran et al. (2021), i.e., 4.0. The latter value is similar to the values reported in shrimp by van Ruth et al. (2014). The similar water-to-protein ratio in crickets and shrimp is likely due to adaptations related to their exoskeletons, which contain chitin and require a high moisture content for flexibility and successful molting processes (Roer et al. 2015, Campli et al. 2024).

Broken Line

The current estimates on the energy requirements were found to be 4,158 and 4,382 kcal GE/kg feed for house crickets from 7 to

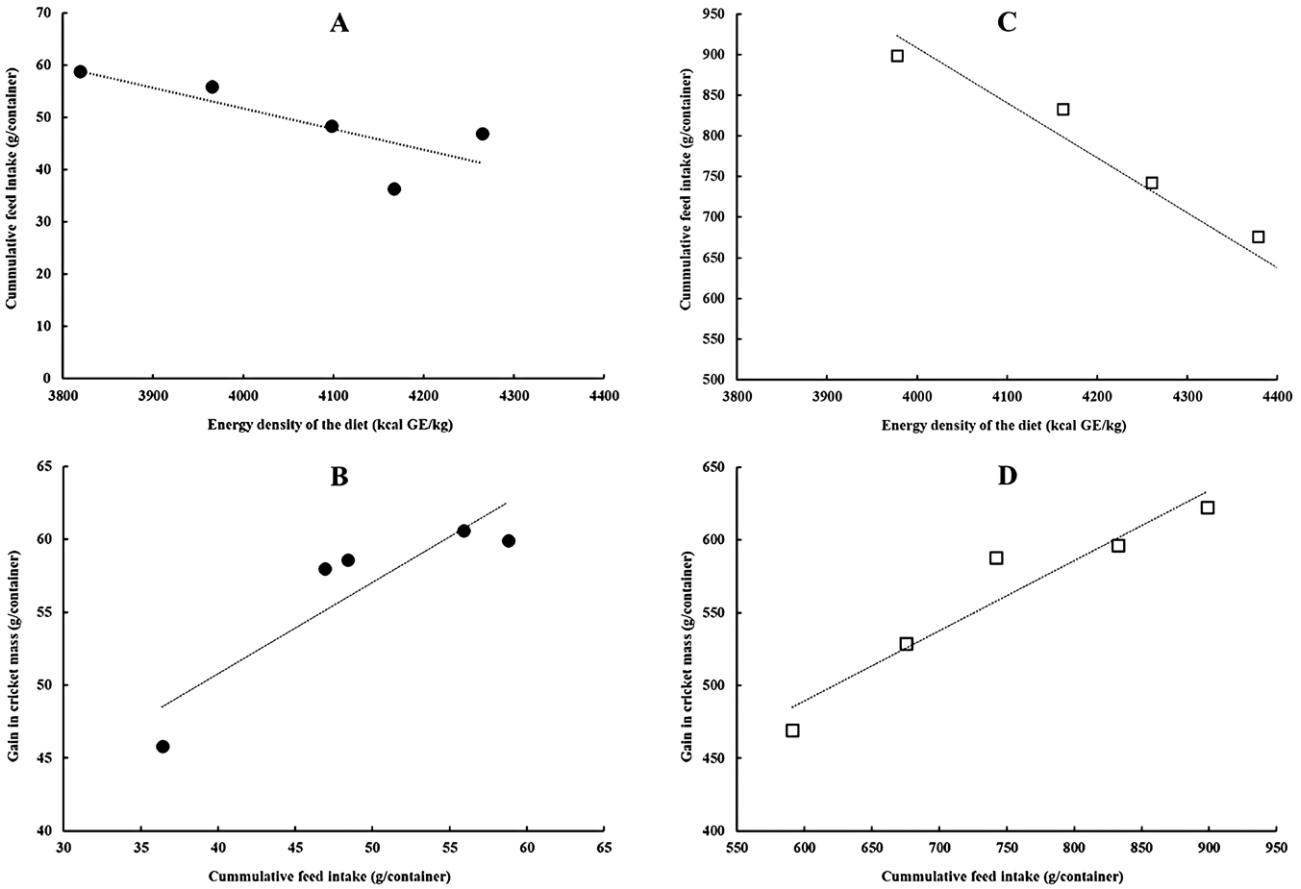

Fig. 2. Relationships between the analyzed gross energy density of the diet (GE, kcal/kg) and the cumulative feed intake (g/container) during phase 1 (panel A) and phase 2 (panel C). Panels B and D show the relationships between the cumulative feed intake (g/container) and weight gain of the house crickets (g/container) during phase 1 and 2, respectively. The linear regression formulas are: Panel A, $y = 210 - 0.04 \times (n = 5, R^2_{adj} = 49.5\%, P = 0.11)$; Panel B, $y = 25.8 + 0.63 \times (n = 5, R^2_{adj} = 73.7\%, P = 0.040)$; Panel C, $y = 3608 - 0.68 \times (n = 5, R^2_{adj} = 90.4\%, P = 0.008)$; Panel D, $y = 199.6 + 0.48 \times (n = 5, R^2_{adj} = 88.8\%, P = 0.011)$. The dotted lines represent the regression line. Symbols: ● phase 1; □: phase 2.

20 and 21 to 45 d after hatching, respectively. In contrast to the house crickets raised from 7 to 20 d of age, the result of the broken-line analysis is less straightforward for those raised from 21 to 45 d after hatching (Fig. 1). This notion can be illustrated by the fact that a simple linear regression between FCR and dietary GE content yielded a similar slope (ie – 0.00045) and R^2 (ie 62.7%, $P < 0.001$). It thus appears that the upper dietary GE values were not sufficiently high to establish a reliable recommendation for the optimum dietary GE density to achieve a minimum FCR. Thus, the current result on optimum dietary GE density should be interpreted with caution. Moreover, caution is also warranted when extrapolating these estimates for practical application, as it is clear that the ingredient composition of the diet plays an important role in determining not only the GE content but also the ME content. In the current study, palm oil was the main ingredient of the GE content of the feed. This approach was considered opportune because the GE content of tri-acylglycerols (TAG, the mayor lipid in plant oils) is generally ~ 2.5 times greater compared to that of starch. Typically, TAG and starch are well digested, and, at least in for instance pigs and poultry, their metabolites are not excreted via the urinary tract. Thus, the GE-, DE-, and ME values of starch and TAG are quite similar. In contrast, the replacement of starch by fiber does not alter the dietary energy content when expressed as kcal GE/kg, but it will most likely have a negative effect on the DE and therefore the ME value of the feed. Furthermore, the oxidation of absorbed amino acids (AA) is associated with nitrogen loss via the urinary tract. Since crickets do not possess a urea cycle (Silva Martin et al. 2025), nitrogen originating from oxidized AA is excreted in the form of uric acid thereby causing a loss of energy. Thus, in protein-rich feedstuffs, the ME value is substantially lower than not only the GE but also the DE value. These points reinforce the need for future studies to obtain cricket-specific ME values for feed ingredients. Because crickets excrete uric acid together with feces, estimating ME values of feed is actually easier compared to estimating DE values. Woodring et al. (1979) described cricket feces as emerging "as a moist but firm pellet that quickly dried and became hard." Furthermore, the feces pellets neither broke up nor dispersed, making them easy (Woodring et al. 1979).

In view of the wide array of potential feedstuffs that can be used in the nutrition of crickets, an energy evaluation system based on ME allows both farmers and industry to select feed ingredients that accurately reflect the energy can utilize, thus being instrumental in achieving a low FCR. Moreover, an ME evaluation system provides a rationale for feed ingredients pricing and is therefore economically relevance for the cricket industry. Just for the sake of reference, the estimated energy requirements, expressed in poultry-based ME values, were found to be 2,875 and 3,270 kcal/kg feed for house crickets during the periods of 7 to 20 and 21 to 45 d post-hatching, respectively. From a practical viewpoint, we consider it opportune to inform the reader of these values, as they can serve as a benchmark

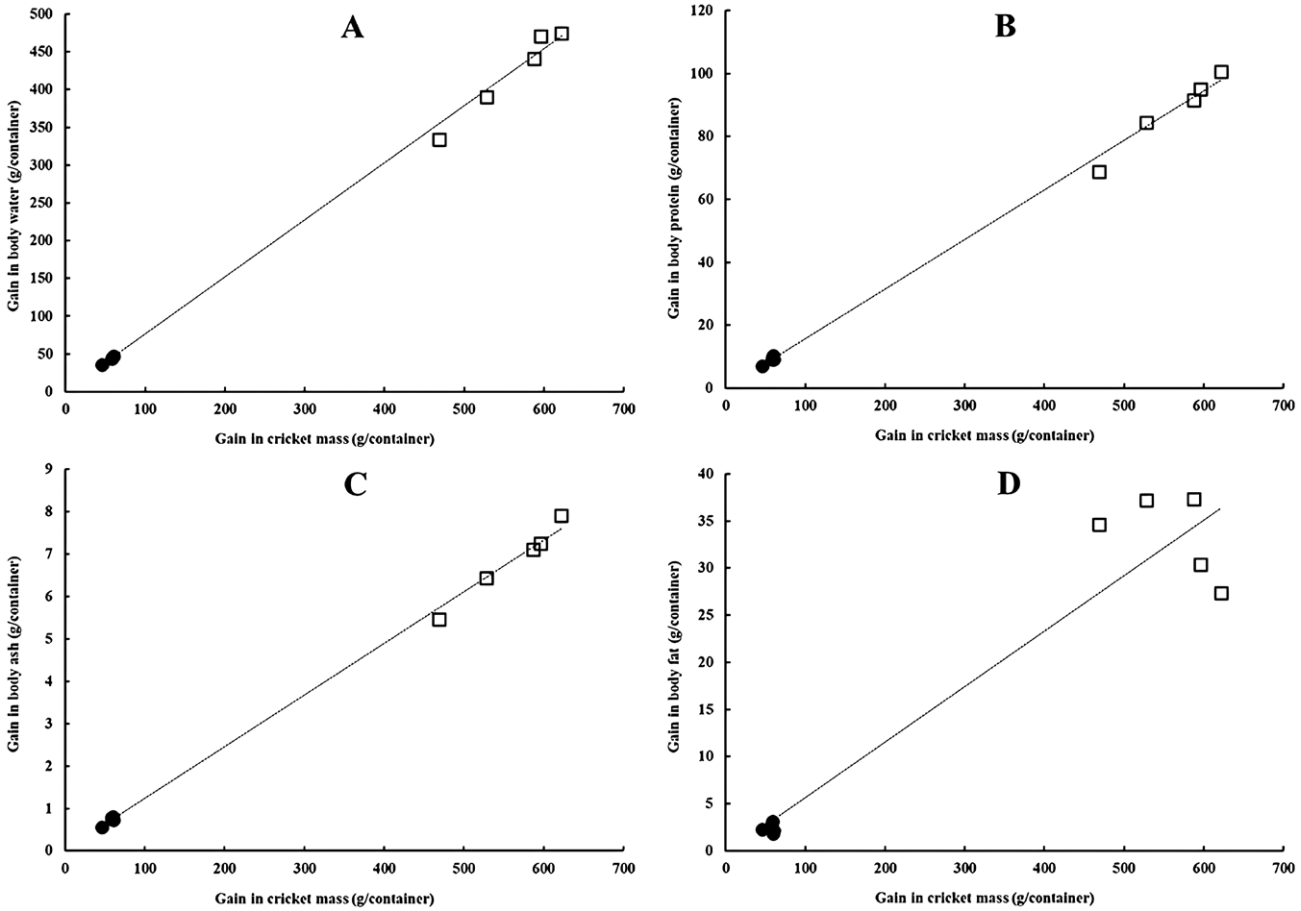

Fig. 3. Relationships between the weight gain of the house crickets (g/container) and body water gain (g/container, panel A), body protein gain (g/container, panel B), body ash gain (g/container, panel C), and body fat gain (g/container, panel D). The linear regression formulas are: Panel A, $y = -0.54 + 0.75 \times (n = 10, R^2_{adj} = 99.7\%, P < 0.001)$; Panel B, $y = -0.03 + 0.16 \times (n = 10, R^2_{adj} = 99.7\%, P < 0.001)$; Panel C, $y = 0.02 + 0.012 \times (n = 10, R^2_{adj} = 99.7\%, P < 0.001)$; Panel D, $y = -0.28 + 0.06 \times (n = 10, R^2_{adj} = 90.5\%, P = < 0.001)$. The dotted lines represent the regression line. Symbols: ● phase 1; □: phase 2.

Table 8. Chemical composition of body weight gain of house crickets fed the experimental diets from 7 to 20 d (phase 1) and from 21 to 45 d of age (phase 2)

Phase	Dietary GE[1]	Water	Protein	Fat	Ash	Rest[2]	Water	Protein	Ash	Rest	Water:Protein
	Kcal/kg	% of fresh carcass					% of fat-free carcass				ratio
1	3819	76.7	16.9	3.0	1.3	2.0	79.1	17.5	1.4	2.1	4.5
1	3965	78.2	15.5	3.5	1.2	1.5	81.1	16.0	1.3	1.6	5.1
1	4098	74.8	16.5	5.2	1.3	2.1	79.0	17.5	1.4	2.2	4.5
1	4167	77.3	15.2	4.9	1.2	1.3	81.4	16.0	1.3	1.3	5.1
1	4265	76.3	16.0	5.1	1.3	1.3	80.4	16.9	1.4	1.4	4.8
2	3978	75.8	16.1	4.4	1.3	2.5	79.2	16.8	1.3	2.6	4.7
2	4162	76.4	15.4	4.9	1.2	2.1	80.4	16.2	1.2	2.2	5.0
2	4261	75.0	15.6	6.4	1.2	1.9	80.1	16.6	1.3	2.0	4.8
2	4379	73.5	15.9	7.0	1.2	2.4	79.0	17.1	1.3	2.6	4.6
2	4405	73.6	15.2	7.6	1.2	2.4	79.7	16.4	1.3	2.6	4.8
	Mean	75.8	15.8	5.2	1.2	1.9	79.9	16.7	1.3	2.1	4.8
	SD[3]	1.55	0.57	1.45	0.05	0.45	0.87	0.53	0.05	0.48	0.20

[1]GE = Gross energy.
[2]Rest = Chemically unidentified fraction.
[3]SD = Standard deviation.

for feed formulation, at least until cricket-specific ME values for feed ingredients become available.

In conclusion, this study is the first to provide insight into the dietary GE content required for house crickets from 7 to 20 and 21 to 45 d of age to achieve optimum FCR, i.e., 4,158 and 4,382 kcal GE/kg, respectively. We believe that these findings provide opportunities to formulate nutritious diets for crickets and benefits for the cricket industry.

Acknowledgments

This work was supported by the Suranaree University of Technology (SUT), Thailand Science Research and Innovation (TSRI) National Science, Research and Innovation Fund (NSRF), and the National Research Council of Thailand (NRCT) (No: NRCT.MHESI. A309/2021).

Author contributions

Sutisa Khempaka (Conceptualization [Lead], Data curation [Equal], Formal analysis [Equal], Funding acquisition [Lead], Investigation [Lead], Project administration [Lead], Resources [Lead], Supervision [Lead], Validation [Lead], Visualization [Lead], Writing - review & editing [Lead]), Supattra Okrathok (Data curation [Equal], Formal analysis [Equal], Methodology [Equal], Writing - original draft [Equal]), J. T. Schonewille (Supervision [Equal], Validation [Equal], Writing - review & editing [Equal]), Chayanant Pukkung (Data curation [Equal], Formal analysis [Equal], Writing - original draft [Equal]), M. Sirisopapong (Data curation [Equal], Formal analysis [Equal], Visualization [Equal]), O. Jantasaeng (Data curation [Equal], Investigation [Equal]), and P. Pasri (Formal analysis [Equal], Methodology [Equal])

Ethics approval

The current study was approved by the Animal Care and Use Committee of Suranaree University of Technology (approval number: SUT-IACUC-014/2021).

Conflicts of interest. None declared.

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