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Article

How Does Locally Produced Feed Affect the Chemical Composition of Reared House Crickets (*Acheta domesticus*)?

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ABSTRACT: The interest in eating insects has grown in Western countries. Insects are on the market in some European countries due to the reinterpretation of EU legislation. In this study, house crickets (*Acheta domesticus*) were reared with two homemade and one commercial feed with different chemical compositions. Commercial chicken feed, which contained the highest levels of protein and minerals, produced the biggest and the most protein- and mineral-containing crickets. The protein content varied between 50.2 and 64.2 g/100 g (dry weight, DW). The examination of the amino acid profiles showed that the feed had a smaller effect on them than the amount of protein. Crickets, which received the most carbohydrate-rich feed, were highest in fat and lowest in protein. The fat content of all crickets was high (25.0−33.7 g/100 g, DW), and an average fatty acid profile was 40% saturated fatty acids (SFAs), 31% monounsaturated fatty acids (MUFAs), and 27% polyunsaturated fatty acids (PUFAs). A cricket's diet has a significant effect on its composition.

KEYWORDS: *farmed crickets, edible insects, production test, protein, amino acids, fat, fatty acids, moisture, ash, trace elements, heavy metals, crude fiber, dietary fiber, carbohydrates, nitrogen-to-protein conversion factor*

INTRODUCTION

Meat is an important source of protein for humans in the Western world.[1,2] The trend of growing meat consumption puts pressure on the production of feed. The production of 1 kg of high-quality animal protein (cattle, poultry, and pigs) typically needs 6−8 kg of plant protein in OECD countries.[3] Furthermore, livestock husbandry causes approximately 14.5% of the global human-induced greenhouse gas emissions.[4] When compared to traditional livestock, insects can produce protein with a lower use of natural resources due to their short life cycle, low space requirement, and capacity to utilize many kinds of agricultural side streams.[2,5] When crickets are farmed on side streams, environmental contamination can be reduced, while side streams replace more expensive feed, such as chicken feed, which has been used to rear crickets successfully.[2] Crickets can reach a feed conversion ratio (FCR) of about 1.7 kg feed per one kilogram of live weight gain, which is low compared to traditionally farmed animals such as the FCR of 2.5 for chicken and 10 for beef.[2]

The interest in entomophagy, the practice of eating insects, is increasing in the Western world.[6] There has been a change in posture toward insects since some EU member states made a reinterpretation of the European regulation (EC) No 258/97[7] concerning novel food products and ingredients thereof. Following the entry into force of the new novel food regulation ((EU) 2015/2283[8]) in 2018, there are currently eight species on the EU list of approved insects for food, one of which is a house cricket (*Acheta domesticus*; order: *Orthoptera*; family: *Gryllidae*).[9] The judicial reinterpretation has resulted in an elevated societal and commercial interest in insects as food and feed.[10] Globally, the edible insect market was valued at 33 million USD in 2015 and 55 million in 2017 with a future estimated growth of 43.5% by 2024, which makes research in this area even more interesting.[11] In the U.S. market, grasshoppers, locusts, and crickets are expected to be the most profitable insects, and especially, house crickets are commonly reared.[2]

Insects have been proposed as an alternative protein source for humans due to their high nutritional value, particularly in proteins, fats, minerals, vitamins, and energy.[5] Crickets (and other *Orthopterans*) have been proved to be the favorite edible insects of Europeans from the acceptance point of view.[12] *Orthoptera* insects are reported to have protein contents between 6.25% and 77.13% on dry matter.[5] Because of the high amount of chitin in the cricket exoskeleton, cricket consumption may also have a positive effect on the human gut microbiota.[6] On the other hand, chitin is known to cause an overestimation of protein content.[28,40] According to Paul et al., house crickets contain a high amount of polyunsaturated fatty acids (PUFAs) and a low amount of saturated fatty acids (SFAs), a combination that has been found to lower the risk for coronary artery diseases.[13] Feed can have a strong affect on the nutritional composition of house crickets. For example, an improved fatty acid composition would be achieved by feeding the crickets with omega 3-enriched feed.[14−16]

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Little is known about variations in composition of the house cricket when fed different diets. Knowledge of the composition of the house cricket is important to be able to design their appropriate use in human diets. Therefore, the aim and contribution of this study to the current literature was to compare the chemical composition of house crickets fed with three different diets and to identify possible differences, depending on what feed the crickets were reared on. The aim was to design and pilot test feeds, which were composed of locally available materials and prepared without artificial ingredients, such as synthetic amino acids or other feed additives. The locally available feed materials were cereals, peas, and rapeseeds as well as potato peels obtained as byproducts from local biobased industrial processes.

MATERIALS AND METHODS

Rearing Practices. Crickets were reared indoors in a privately operated 12 m^2 insect rearing facility that had been renovated one and half years before the trial for insect rearing purposes and that was used for commercial insect rearing. The rearing facility was based in Kurikka, Finland. House crickets were reared in transparent plastic storage boxes (72 × 40 × 39 cm) of the Finnish industry standard. A similar material has been used in previous studies.[17] Lids of the boxes were modified by replacing the plastic top by an insect net in order to facilitate air flow and prevent condensation. A net, made of plastic and available from the local hardware store, was attached to the lids of the boxes with glue. To enlarge a crawlable surface area, egg cartons (29.6 cm × 29.6 cm) were added in the boxes, as has been done in several experiments.[14,18] Lighting in the rearing room was automated and adjusted to a 12 h/12 h day/night ratio.[19] The source of light was two 1500 mm fluorescent lamps (58 W and 5000 lm each) installed in the ceiling. Temperature and relative humidity were measured every 5 min by using programmable sensors. During the trials, the average room temperature was 31.5 °C, and the minimum and maximum temperatures were 26.3 and 33.9 °C, respectively. The room was insulated, and the heating was arranged by an electric radiator that was located on the floor and equipped with an electric thermostat. An electric fan was used to ensure air circulation and stable temperature in different parts of the facility. Rearing boxes for each feed treatment were distributed evenly across three levels so that the temperature was stable across the feeding groups. Inside the boxes, the average temperature was 30.7 °C, varying from 27.7 to 32.5 °C. The temperatures of the experiment were in accordance with the literature, where cricket rearing is recommended to take place above 25 °C and below 35 °C.[18,20] The relative humidity in the rearing room was 49.8% (min 37.8% to max 58.2%) and in the rearing boxes, 61.3% (min 48.2% to max 69.7%) during the trials, which is around the typical humidity range recommended for crickets.[21]

The study included a cricket rearing study with three different feeds and prestudy with seven different feeds as specified below. In both cases, a commercial chicken feed was used as a control. A pretrial was carried out in the following order. All tested feeds were placed in two separate rearing boxes throughout the pretrial period. In the pretrial, eight different feeds were tested: test feeds TF1−TF7 and a control. House crickets reared in the pretrial originated from a single hatching box from where hatched crickets were transferred into each rearing box. In accordance with Van Broekhoven et al., crickets were fed *ad libitum*.[22] Feed and water were checked and distributed twice a day. Water was dispensed by using moistened hand towels in order to prevent crickets from drowning. Crickets were weighed at the beginning of the trial, after 3 and 5 weeks, and after harvesting (7 weeks). Weighing was done by randomly taking a sample of 10 crickets from each rearing box instead of weighing crickets individually due to the small size of crickets and the measuring range of the scales used.

The pretrial feeds manufactured from locally sourced materials (Table S1) were designed to resemble the nutritional values of broiler chicken starter feed as per Finnish feeding recommendations. All feed materials were ground before the feed was manufactured. Cereals, peas, and dried potato peels were ground by one of authors, whereas rapeseed was obtained readily as meal. The particle size of the feed was 2 mm. The desired feed contained 12.4 MJ of metabolizable energy, 220 g of crude protein, 12 g of lysine, 4.8 g of methionine, 7.5 g of threonine, and 9.0 g of methionine + cysteine per kg dry matter (DM).[23] This was selected as a starting point because the literature suggested that crickets require a feed with a high level of protein.[14] A standard recommended feed would also have contained micronutrients such as 10 g of calcium, 4.1 g of phosphorus, and 1.6 g of natrium per kg DM. A commercial chicken feed (Milka Chicken Feed, Biofarm Oy, Finland) was used as a control because it had been used on Finnish cricket farms. According to its product label, Milka Chicken Feed contains 12.4 MJ of metabolizable energy, 173 g of crude protein, 7.9 g of lysine, 3.9 g of methionine, 6.9 g of threonine, 6.7 g of methionine + cysteine, 41 g of calcium, 6.0 g of phosphorus, and 1.7 g of natrium per kg DM. The composition of feeds tested in the experiment was determined by using a linear programming (LP) model developed in Microsoft Excel. The objective of the LP model was to minimize the price of feed in relation to the target energy and protein content, which should correspond to the concentrations of broiler chicken starter feed provided by the Finnish feeding recommendations.[23] Hence, micronutrient composition was not optimized in the LP model, and there was no mineral supplementation in the manufactured feeds, which may have resulted in undesirable levels of micronutrients in feed.

The best-performing pretrial feed was selected (TF5 → F3) for the main study by comparing house cricket weight gain and mortality rates during pre-experiments. The commercial chicken feed (F2) was used as a control. On the basis of insights gained in the pretrial, the third feed (F1) was designed by using nearly the same raw materials as F3 but in different amounts. In F1, peas and part of the fava beans in F3 were replaced by oats and a larger amount of barley (Table S1). The three final feeds used in the main study are specified below (ingredients are listed in order of predominance):

- Feed F1: Oat, wheat, barley, turnip rapeseed meal, potato, and fava beans. Manufactured by the researchers.
- Feed F2: Wheat, barley, oat, calcium carbonate, textured soy protein granules, vegetable oil, turnip-rapeseed pellets, calcium sodium phosphate, vitamin and trace element supplementation, sodium chloride (salt), and amino acids. Vitamin and trace element supplementation included vitamins A, D3, and E, iron, iodine, copper, manganese, zinc, and selenium. Commercial chicken feed (control).
- Feed F3: Wheat, turnip-rapeseed meal, potato, fava beans, peas, and barley. Manufactured by the researchers.

Nutritional values of feed materials used in the feed design were obtained from the Finnish feed tables, except for the contents of potato peel and potato protein, which were taken from Feedbase.[23,24] Cereal prices for the LP model were obtained from the Official statistics and legume prices from The Finnish Cereal Committee.[25,26] Prices of other feed materials were collected in April 2017 from companies selling similar products.

The main rearing study was carried out according to the farmer's practices. While the pretrial included only 100 newly hatched house crickets per rearing box, the number of hatched crickets per rearing box was substantially higher in the main study where feeds F1, F2, and F3 were tested. First, crickets were laid into the rearing box of the main study by inserting a full hatching box diagonally at the back of the box. The boxes were started during a period of 16 days so that an even number of boxes per treatment was started on the same day. While there is some inevitable natural within-box variation (mean ±1 day) in the age of the hatched crickets because of the egg laying, this variation was similar in each rearing box and treatment, and the average age of the crickets in each rearing box that was started on the same day was identical.

Water was dispensed as in the pretrial and after 14 days by placing grated cucumber in the rearing boxes. Cucumbers (that were graded unfit to retail due to physical appearance) were collected from a

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nearby greenhouse. During the primary trial, crickets (of the classifications F1−F3) were reared on all three feeds in six rearing boxes (six replicates). The insects were fed according to their appetite by adding a small amount of feed upon the daily care routine of the rearing facility. The standard feeding practice of the facility was that both added feed and feed that had not yet been eaten remained in the feed tray. The leftover feed was removed from the feed dispensers and weighed at the end of the trial. The insects appeared not to be selective regarding the feed they ate.

The crickets were harvested at the age of 7 weeks (49 days). After harvesting, the crickets were weighed and frozen to −20 °C. These samples were stored in the freezer from August 2017 to September 2017, when they were transported to Evira (known since January 1, 2019 as the Finnish Food Authority) for chemical analysis.

Sample Pretreatment. The cricket samples were freeze-dried for 5 days on average (<−90 °C, $<10^{-4}$ hPa (mbar); Scanvac CoolSafe, LaboGene ApS, Lynge, Denmark). The samples were weighed prior to and after freeze-drying to determine the weight loss during drying. All the samples were milled to achieve a particle size under 0.5 mm. All chemical analyses were performed on freeze-dried and milled crickets, except for moisture analysis, which was performed on a wet sample.

Chemical Analyses for Feed and Crickets. The moisture of feeds was analyzed by drying them in the oven at 103 °C for 4 h, and the moisture of crickets was analyzed by drying them in an oven at 102 °C for at least 3 h in order to make their weight constant, according to ISO 1442:1997.[27] The crude ash of the feeds was analyzed by heating it in the oven at 550 °C for 3 h and weighed in accordance to Commission Regulation (EC) No 152/2009, annex III.[28]

The nitrogen content of the feeds and crickets was analyzed using the Kjeldahl method as described in Commission Regulation (EC) No 152/2009, annex III.[28] The sample was digested by concentrated sulfuric acid using copper as a catalyst. The acid solution was made alkaline with sodium hydroxide. The liberated ammonia was distilled into a boric acid solution and determined by titration with sulfuric acid. The traditional nitrogen-to-protein conversion factor of 6.25 was used to calculate the level of protein.[29,30] However, this conversion factor has been stated to overestimate the result, and therefore, a 5.09 conversion factor was also used, which has been determined specifically for house crickets.[31]

The fat content of feeds and crickets was analyzed according to Commission Regulation (EC) No 152/2009, annex III, by acid digestion with 3 M hydrochloric acid and solvent extraction with light petroleum and a boiling range from 40 to 60 °C.[28]

Fatty acids were analyzed in duplicate and only from crickets. For fatty acid analysis, the lipids of crickets were first extracted using the Schmid-Bondzynski-Ratzlaff method (ISO 2004).[32] The sample was first digested with hydrochloric acid (ρ = 1.125 g/mL). After the addition of ethanol, lipids were extracted with diethyl ether and light petroleum. The extracted lipid fraction was saponified, and fatty acids were esterified using methanol in the presence of boron trifluoride as described in AOCS Ce 2-66:1997.[33] Methyl esterified fatty acids (FAMEs) were analyzed by gas chromatography (GC; Agilent 6890, Santa Clara, CA) employing a DB-23 capillary column (60 m, 250 μm, 0.15 μm; Agilent J&W, Santa Clara, CA) and mass selective (MSD) detection. GC parameters were as follows: carrier gas He with flow of 1.7 mL/min, split (55:1) injection volume of 1 μL, injector temperature of 250 °C, oven initial temperature of 45 °C hold 1 min, then 25 °C/min to 175 and 1.5 °C/min to 230 °C, hold 1 min, and transfer line temperature of 150 °C. MSD parameters were as follows: scan mode of 40−400, threshold of 100, MSD quadrupole temperature of 150 °C, and MSD source temperature of 230 °C. Individual FAMEs were identified on the basis of retention times compared to FAME standard mixtures (GLC 85: Nu-Chek-Prep, Inc., Elysian, MN; Qualmix Fish S: Larodan, Solna, Sweden) and on their specific mass fraction profile. FAs are reported on the basis of their FAME peak area as a proportion of the total area of all the integrated chromatographic peaks.

The amino acid content of feeds and crickets was analyzed according to Commission Regulation (EC) No 152/2009, annex III.[28] A single analysis was performed on feeds while crickets were analyzed as six replicates (one analysis from each rearing box). The analysis included alanine (Ala), arginine (Arg), asparagine and aspartic acid (Asn+Asp), glutamine and glutamic acid (Glu+Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val). The sample was hydrolyzed with 6 mol/L hydrochloric acid at 110 °C for 24 h. The hydrolyzed sample was adjusted to pH 7 and derivatized according to the Waters AqqcTag (Milford, MA) derivatization kit. Amino acids were analyzed with Waters Acquity UHPLC (Milford, MA) with a photodiode array detector using a Waters AqqcTag Ultra C18 column (1.7 μm, 2.1 × 100 mm) and detected at 260 nm.

Cysteine (Cys) and methionine (Met) were oxidized to cysteic acid and methionine sulfone with performic acid at 0 °C for 16 h before hydrolysis. After oxidation, the sample was analyzed as described above. The tryptophan (Trp) levels of the feeds were analyzed according to Commission Regulation (EC) No 152/2009, annex III[28] with the following modifications: The sample was hydrolyzed with 5 mol/L sodium hydroxide at 110 °C for 16 h. After hydrolysis, Trp was analyzed with Waters Acquity UHPLC using a Waters AqqcTag Ultra C18 column (1.7 μm, 2.1 × 100 mm) and detected with fluorescence (ex 285 nm, em 340 nm).

The amino acid score was calculated from the amino acid profile with the following equation.

$$\text{amino acid score} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in requirement pattern}}$$

Reference values for children aged 3 to 10 years were used for the calculation of the amino acid scores for all the population groups above 3 years, as recommended by FAO/WHO.[34]

The crude fiber of feeds was analyzed using FibreBags (Gerhardt, Königswinter, Germany). The sample was boiled for 30 min in sulfuric acid (H_2SO_4; 0.13 M), after which it was rinsed with water and boiled for 30 min in potassium hydroxide (KOH; 0.23 M). The sample was rinsed again with water until the pH was neutral. The sample was dried in 105 °C overnight, after which the residue was weighed. The result was corrected by ash value.[28]

Prior to dietary fiber (DF) analysis, the cricket samples were defatted by treating the samples with petroleum ether, since the dried crickets contained >10% fat. The DF was analyzed according to the method AOAC 2011.25.[35] The sample (an exact amount of 1.000 ± 0.005 g) was weighed in an incubation bottle. An enzyme mixture of pancreatic α-amylase/amyloglucosidase (AMG) was added, and the sample was incubated for 16 h at 37 °C in a shaking water bath to remove starch. Next, protease was added, and the sample was incubated for 30 min at 60 °C to remove proteins. The enzymatically hydrolyzed sample was filtered twice, first to separate water-insoluble dietary fiber (IDF) from soluble DF (SDF) and second to isolate water-soluble polysaccharides (SDFPs; DF soluble in water and precipitated by 78% aqueous ethanol) from oligosaccharides (SDFSs; DF soluble in water and not precipitated by 78% aqueous ethanol). IDF and SDFP residues were dried, weighed, and corrected for protein and ash values. SDFSs were further hydrolyzed by AMG and analyzed by high performance liquid chromatography (HPLC) after deionization, as described in Rainakari et al.[36] Sorbitol was used as an internal standard for SDFS analysis. The total DF amount is the sum of IDF, SDFP, and SDFS.

Elements in feeds and crickets were analyzed with inductively coupled plasma optical emission spectrometry (ICP-OES) using an iCAP 6500 DUO (Thermo Scientific, Waltham, MA) and inductively coupled plasma mass spectrometry (ICP-MS) using a NexION 300X (PerkinElmer, Waltham, MA). The sample was weighed (0.5 g) and placed into a PTFE microwave digestion vessel, and 5 mL of nitric acid, 2 mL of hydrogen peroxide, and 3 mL of deionized water were added. For sample digestion, the microwave digestion system Mars 5 (CEM Corporation, Matthews, NC) was used. The program was as

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Table 1. Overall Consumption of Feeds 1−3 (F1−F3, Correspondingly) and Weight Gain of the Crickets Based on Means of Each Feed[a]

	F1	F2	F3
amount of feed distributed (g/box)	571.6 ± 22.4$^{¤¤¤}$	1350.5 ± 143.6***§§§	614.6 ± 97.6$^{¤¤¤}$
amount of residual feed after harvest (g/box)	376.8 ± 13.4$^{¤¤¤}$	834.8 ± 125.5***§§§	447.3 ± 66.3$^{¤¤¤}$
consumption of feed (g/box)	194.8 ± 33.3$^{¤¤¤}$	515.7 ± 100.7***§§§	167.2 ± 25.1$^{¤¤¤}$
weight of harvested crickets (g/box)	109.5 ± 7.7$^{¤¤¤}$	422.6 ± 93.6***§§§	145.4 ± 37.0$^{¤¤¤}$
feed consumption per cricket weight gain (kg feed consumed per kg harvested crickets)	1.8 ± 0.3$^{¤¤§§}$	1.2 ± 0.1**	1.2 ± 0.3**
feed distributed per cricket weight gain (kg feed distributed per kg harvested crickets)	5.2 ± 0.3$^{¤¤¤}$	3.4 ± 0.8***	4.4 ± 0.7

[a]Mean ± SD. The amount of consumed feed equals the amount of distributed feed minus residual feed. The residual feed may also contain other elements than feed. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs F1; $^{¤}p < 0.05$, $^{¤¤}p < 0.01$, $^{¤¤¤}p < 0.001$ vs F2; $^{§}p < 0.05$, $^{§§}p < 0.01$, $^{§§§}p < 0.001$ vs F3 with Tukey's post hoc test.

follows: 5 min to 100 °C, 5 min to 130 °C, 5 min to 160 °C, 7 min to 200 °C, 10 min at 200 °C, and cooling down to 80 °C. The digested sample was transferred into a 50 mL volumetric flask, which was filled to the top with deionized water. For ICP-MS analysis, the sample was further diluted; 3.4 mL of sample was transferred to a 10 mL volumetric flask, and internal standards bismuth (Bi), germanium (Ge), and rhodium (Rh) (Romil, Cambridge, United Kingdom) were added before filling the flask to the top with deionized water. Single-element standard solutions of arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorus (P), potassium (K), selenium (Se), and zinc (Zn) (Merck, Darmstadt, Germany) and the multielement standard solution of As, Cd, Cr, Cu, Mn, Ni, Pb, Se, and Zn (Romil, Cambridge, United Kingdom) were used for calibration.

Accredited methods and normal quality assurance procedures were used in the laboratory analyses. Therefore, the number of replicates was very small ($n = 1−2$), especially in feed analyzes, and no standard deviation or range is shown in the results.

The percent of population reference intake (PRI) and adequate intake (AI) was calculated according to the latest EFSA recommendations for adults.[37]

Statistical Analyses. The chemical composition of three cricket samples fed with the different feeds (F1−F3 crickets), each in six replicates, was compared using a one-way analysis of variance (ANOVA) test conducted in IBM SPSS Statistics 25. The level of statistical significance was set to $p < 0.05$. The significance grows with decreasing p-value (shown as three p-value areas: $p < 0.05$, $p < 0.01$, and $p < 0.001$). A Tukey's post hoc test was used when the ANOVA test yielded an overall significant variation.

RESULTS AND DISCUSSION

The results of the main feeding trial indicated that feed F2 yielded a larger cricket mass than the two other feeds tested and a lower amount of feed distributed per kg of weight gain than F1 (Table 1). Crickets reared with feed F1 were measured to have a higher feed consumption per kg of cricket mass gained than the other feeds and a lower amount of residual feed and lower total weight of harvested crickets compared to feed F2. Feed intake was significantly higher with F2 than F1 and F3 (Table 1). Crickets developed well with feed F1, but an increased mortality rate was observed during the last 2 weeks of rearing. In contrast, feed F3 resulted in poor development, but the cricket's survival rate did not rise at the end of the rearing period.

Table 2. Analyzed Macronutrient (Moisture, Fat, Protein, Crude Fiber, and Ash) Levels (%) in Feeds 1−3 (F1−F3, correspondingly) as Fresh Weights[a]

feed	moisture (%)	fat (%)	protein (%)	crude fiber (%)	ash (%)	carbohydrates (%)
F1	10.7	1.9	10.2	3.9	3.0	70.3
F2	10.7	4.1	16.1	3.7	10.4	55.0
F3	11.0	1.7	15.1	4.4	3.4	64.4

[a]$n = 2$. The amount of carbohydrates has been calculated as 100 − other macronutrients.

The feed conversion rates (FCRs; kg feed per kg weight gain) of feeds F2 and F3 weight gain were unexpectedly low (Table 1). Bawa et al. reported FCR values between 1.5% and 1.8% for house crickets in rearing experiments with feed protein levels of 16−22%.[38] Lower FCRs in this study may be due to the overestimation of the weighed residual feed, as it included feces, possibly some dead crickets, and pieces of hard exoskeleton, which young crickets (nymphs) must shed several times during growth. Thus, when feed consumption was estimated by subtracting the amount of residual feed from distributed feed, the consumption may be underestimated. In addition, during the experiment, it was ensured that the crickets did not experience hunger, which is why the feed was given more than necessary, resulting in very high feed distribution per cricket weight gain (Table 1). The amount of cucumber used as a water source was not considered in the calculation of feed consumption.

Nutritional Values of Feed. The analyzed results for crude fat, crude protein, crude ash, crude fiber, and moisture levels of feeds are presented in Table 2. The commercial chicken feed F2 clearly had the highest protein, fat, and ash levels compared to the other two feeds studied, while F1 had the lowest protein and ash levels. F3 had a higher crude fiber content than feeds F1 and F2, and it had a protein content only one percentage point lower than in F2. Moisture content was similar between feeds. The available carbohydrates in feeds were estimated by the method of difference based on the analyzed nutrients. The highest carbohydrate content was found in F1 (Table 2). The compositions of macronutrients in the test feeds used only in the preliminary test are given in Table S2.

According to the product's nutritional information, the protein content of commercial feed F2 is 173 g/kg dry weight (17.3%), which was well in line with the analytical result (18%, DW). The protein content of F3 was very close to that of commercial feed F2 (17%, DW).

Amino Acids in Feed. The sums of amino acids in F1−F3 were 10.1, 16.6, and 15.2 g/100 g, respectively. These results were in line with the protein levels of the feed samples (Table 2). The concentrations of individual amino acids are presented in Figure 1. Although there were some differences in amino acid concentrations between feed samples, the amino acid profiles (relative amounts) were rather similar. Thus, the

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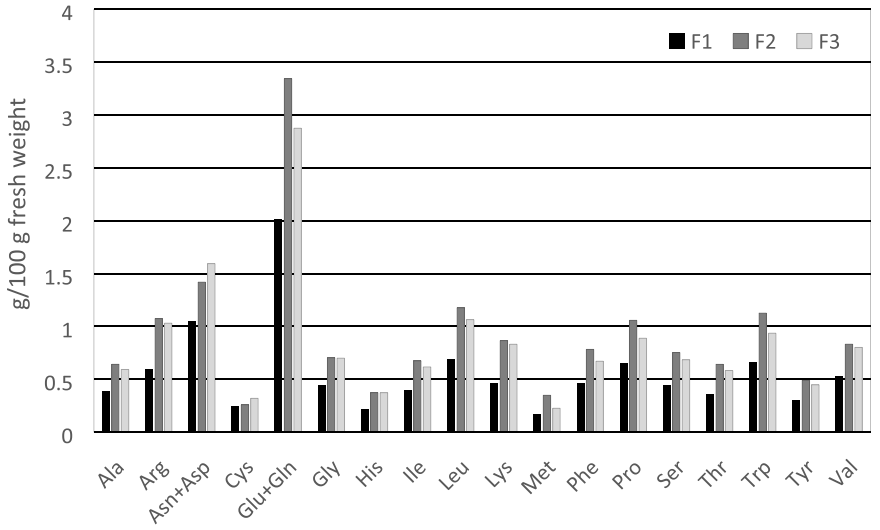

Figure 1. Concentrations of amino acids in feeds F1−F3 used in the main rearing experiments (n = 1). Ala = alanine, Arg = arginine, Asn+Asp = asparagine and aspartic acid, Cys = cysteine, Glu+Gln = glutamine and glutamic acid, Gly = glycine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Pro = proline, Ser = serine, Thr = threonine, Trp = tryptophan, Tyr = tyrosine, and Val = valine.

Table 3. Heavy Metal Content in Feed Samples 1−3 (F1−F3, Correspondingly)[a]

feed	As[b]	Cd[b]	Cr[b]	Ni	Pb[b]
F1	nd	nd	nd	0.097	nd
F2	nd	nd	0.13	0.12	nd
F3	nd	nd	nd	0.068	nd

[a]mg/100 g of fresh weight; n = 1. nd = not detected. [b]Limit of quantification (LOQ): As, 0.050 mg/100 g; Cd, 0.010 mg/100 g; Cr, 0.050 mg/100 g; Pb, 0.050 mg/100 g.

differences were mostly related to the amount of protein in feed samples not differences in the amino acid level of protein. On the basis of these results, it is not possible to comment on the effect of individual amino acid levels on cricket growth. The compositions of amino acids in the test feeds used only in the preliminary test are presented in Table S3.

Elements in Feed. The summarized results of the elements in feeds F1−F3 are presented in Tables 3 and 4. According to Directive 2002/32/EC, the level of undesirable substances in animal feed cannot exceed the maximum permissible levels.[39] The concentrations of arsenic (As), cadmium (Cd), and lead (Pb) are under the limit of quantification in all feed samples and thus do not exceed the maximum level of undesirable substances (Table 3). The level of chromium (Cr) and nickel (Ni) varied slightly between the feed samples with commercial broiler feed F2 containing the highest concentration. Currently, there is no directive for the maximum permissible levels of chromium and nickel.

All mineral concentrations in feed F2, which contained added minerals, were noticeably higher than in the other two feeds used in the final rearing experiments, except for potassium (K). As shown in Table 4, feed F3 had a slightly higher mineral level than F1, except for cobalt (Co). Without further research on the amounts of minerals that support cricket growth, no direct conclusions can be drawn. However, the results suggest that low concentrations of some minerals in feeds F1 and F3 may have had a retarding effect on cricket growth. Bawa et al. reported that the crude ash level was 6−8% (DW) in their house cricket rearing experiments.[38] The crude ash content reflects the total elemental content in the feed, and in this study, the concentrations ranged from 3% to 12% (DW; Table 2). Of the individual elements, P, K, Na, and Ca are the most abundant minerals in crickets in most studies, suggesting their importance. However, more research is needed on the significance of the elements present in lower concentrations in the feeds. The elemental composition of test feed samples TF1−TF7 used in the preliminary study is presented in Tables S4 and S5.

Nutritional Values of Crickets. The levels of macronutrients (fat, protein, dietary fiber) and moisture in crickets are presented in Table 5. All the studied crickets contained a remarkable amount of fat (25.0−33.7% in dry weight/5.4−9.5% in fresh weight; data not shown). This may have an influence on a person's daily energy intake, if consumed in large quantities, and emphasizes the relevance of fatty acid composition. Crickets fed with F3 had a lower fat and higher moisture level than the other crickets. The cricket's diet is known to affect its composition.[5] In this experiment, however, the difference between feeds F3 and F1 in terms of fat and moisture content was marginal. It is therefore unclear why the difference in the composition of the crickets fed with feeds F1

Table 4. Mineral Content in Feed Samples 1−3 (F1−F3, Correspondingly)[a]

feed	Ca	K	Mg	Na[b]	P	Co	Cu[b]	Fe	Mn	Mo[b]	Zn
F1	61	1100	130	nd	290	0.021	nd	3.9	1.5	nd	2.0
F2	2700	730	200	190	670	0.027	2.1	30	15	0.083	13
F3	120	1300	170	nd	390	0.016	nd	5.9	1.6	0.064	2.6

[a]mg/100 g of fresh weight, n = 1. nd = not detected. [b]Limit of quantification (LOQ): Na, 10 mg/100 g; Cu, 0.50 mg/100 g; Mo, 0.050 mg/100 g.

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Table 5. Macronutrient Levels of House Crickets as g/100 g in Dry Weights[a]

cricket	fat	protein factor 6.25[b]	protein factor 5.09[c]	nitrogen	moisture	dietary fiber
CF1	32.8 ± 0.8	53.2 ± 1.9	43.3 ± 1.5	8.5 ± 0.3	71.9 ± 0.5	
CF2	30.6 ± 2.6	58.4 ± 1.5	47.6 ± 1.2	9.3 ± 0.2	72.2 ± 0.4	5.2 ± 0.1
CF3	25.2 ± 0.3	59.9 ± 2.6	48.8 ± 2.1	9.6 ± 0.4	78.2 ± 0.5	

[a]Moisture content as fresh weight; mean ± sd; n = 2−6. CF1−CF3 = crickets fed with corresponding feeds. [b]Commonly used nitrogen-to-protein conversion factor.[30] [c]Specific nitrogen-to-protein conversion factor for house crickets.

Table 6. Fatty Acid Profiles (% of Total Sum of Fatty Acids) of Crickets[a]

fatty acid class	F1 crickets	F2 crickets	F3 crickets
MUFAs (%)	32.1 ± 0.2$^{¤¤¤}$	28.7 ± 0.4§§§***	32.6 ± 0.3$^{¤¤¤}$
PUFAs (%)	25.0 ± 0.6$^{¤¤¤}$	29.7 ± 0.8§§§***	25.1 ± 0.6$^{¤¤¤}$
n-3 fatty acids (%)	*0.6 ± 0.1*$^{¤¤¤§}$	*1.1 ± 0.1*§§§***	*0.7 ± 0.0*$^{¤¤¤}$*
n-6 fatty acids (%)	*24.0 ± 0.7*$^{¤¤¤}$	*28.5 ± 0.8*§§§***	*24.1 ± 0.5*$^{¤¤¤}$
SFAs (%)	40.7 ± 0.9	39.0 ± 1.2	40.2 ± 0.4
TFAs (%)	0.1 ± 0.1	0.1 ± 0.0	<0.1 ± 0.0

[a]Presented as mean ± sd, n = 3. MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids, SFAs = saturated fatty acids, TFAs = *trans* fatty acids. *p < 0.05, **p < 0.01, ***p < 0.001 vs Feed 1; $^{¤}p$ < 0.05, $^{¤¤}p$ < 0.01, $^{¤¤¤}p$ < 0.001 vs Feed 2; $^{§}p$ < 0.05, $^{§§}p$ < 0.01, $^{§§§}p$ < 0.001 vs Feed 3 with Tukey's post hoc test.

Table 8. Amino Acid Scores in Crickets[a]

amino acid	FAO requirement, mg/g[34]	amino acid score		
		F1 crickets	F2 crickets	F3 crickets
histidine	16	1.4	1.41	1.39
isoleucine	30	1.32	1.34	1.30
leucine	61	1.13	1.15	1.11
lysine	48	1.15	1.15	1.16
methionine + cysteine	23	1.22	1.13	1.08
phenylalanine + tyrosine	41	2.26	2.16	2.22
threonine	25	1.44	1.44	1.42
tryptophan	6.6	1.41	1.42	1.35
valine	40	1.54	1.55	1.50

[a]FAO requirement for 3 to 10 year-old children was used as the reference value.

and F3 was so obvious. The fat content in this study was found to be slightly higher than in a review by Payne et al.,[40] where the mean fat content (±sd) of the crickets in six studies was 4.56 (±2.15) g/100 g. It is possible that the crickets analyzed in different studies were not all in the same stage of life. Larval forms commonly contain more fat than those in the adult stage due to the high energy requirement during development.[40] Bawa et al. reported that crickets fed with a high-protein diet (22%) had a high-protein and low-fat content.[38] Furthermore, crickets had high-fat and low-protein levels when fed low-protein and high-carbohydrate diets, because excess carbohydrates that are not used by the crickets for energy are stored as fat. The relatively high-fat levels analyzed in this study are in line with the literature. Crickets fed with the most carbohydrate-rich feed F1 had the highest fat content. However, in our study, the diet with 18% protein (F2) produced a clearly higher protein and lower fat content in

Table 7. Amount of Amino Acids (g/100 g in Dry Weight and mg/g Protein) in Crickets[a]

amino acid	g/100 g (in dry weight)			mg/g protein		
	F1 crickets	F2 crickets	F3 crickets	F1 crickets	F2 crickets	F3 crickets
alanine	4.23 ± 0.20	4.72 ± 0.13	4.69 ± 0.24	79.5 ± 2.4	80.9 ± 3.0	78.3 ± 2.0
arginine	3.34 ± 0.16	3.69 ± 0.14	3.77 ± 0.19	62.8 ± 1.3	63.2 ± 1.4	62.9 ± 0.6
asparagine + aspartic acid	4.16 ± 0.22	4.79 ± 0.25	4.66 ± 0.25	78.2 ± 2.4	82.1 ± 2.8*§	77.7 ± 1.5
cysteine[b]	0.46 ± 0.04	0.48 ± 0.06	0.53 ± 0.03	8.6 ± 0.6	8.3 ± 1.0	8.9 ± 0.5
glutamine + glutamic acid	5.56 ± 0.24	6.14 ± 0.20	6.14 ± 0.30	105 ± 2.2	105 ± 2.1	103 ± 1.1
glycine	2.79 ± 0.11	2.98 ± 0.06	3.03 ± 0.14	52.6 ± 1.2	51.1 ± 1.2	50.6 ± 0.5*
histidine[b]	1.19 ± 0.05	1.32 ± 0.04	1.33 ± 0.06	22.4 ± 0.4	22.5 ± 0.4	22.3 ± 0.4
isoleucine[b]	2.11 ± 0.10	2.35 ± 0.08	2.33 ± 0.13	39.7 ± 1.0	40.2 ± 1.3	38.9 ± 1.0
leucine[b]	3.68 ± 0.18	4.11 ± 0.15	4.06 ± 0.23	69.2 ± 1.8	70.4 ± 2.2	67.8 ± 1.8
lysine[b]	2.95 ± 0.14	3.22 ± 0.12	3.33 ± 0.20	55.4 ± 1.3	55.2 ± 1.9	55.6 ± 1.1
methionine[b]	1.01 ± 0.06	1.03 ± 0.14	0.96 ± 0.05	19.1 ± 0.7	17.7 ± 2.4	16.0 ± 0.4**
phenylalanine[b]	1.77 ± 0.09	1.93 ± 0.12	1.96 ± 0.12	33.3 ± 1.2	33.0 ± 2.0	32.7 ± 1.3
proline	2.90 ± 0.11	3.25 ± 0.07	3.17 ± 0.13	54.6 ± 0.8	55.6 ± 1.1	52.9 ± 1.3*$^{¤¤¤}$
serine	2.08 ± 0.11	2.48 ± 0.17	2.28 ± 0.10	39.1 ± 1.1	42.4 ± 02.3**§§§	38.1 ± 0.7
threonine[b]	1.92 ± 0.09	2.11 ± 0.08	2.13 ± 0.12	36.1 ± 0.9	36.1 ± 1.2	35.6 ± 0.9
tryptophan[b]	0.50 ± 0.04	0.54 ± 0.02	0.53 ± 0.04	9.3 ± 0.8	9.4 ± 0.4	8.9 ± 0.9
tyrosine[b]	3.14 ± 0.14	3.24 ± 0.21	3.48 ± 0.21	59.2 ± 2.2	55.6 ± 4.2	58.2 ± 2.7
valine[b]	3.27 ± 0.14	3.61 ± 0.08	3.6 ± 0.19	61.4 ± 1.3	61.9 ± 1.6	60.1 ± 1.7
total EAA[b]	22.0 ± 1.0	23.9 ± 0.9	24.2 ± 1.2	414 ± 10	410 ± 15	405 ± 11
total	47.8 ± 1.2	51.5 ± 0.6	51.9 ± 0.8	887 ± 25	889 ± 13	874 ± 16

[a]Presented as mean ± sd. n = 6 (tryptophan, n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 vs Feed 1; $^{¤}p$ < 0.05, $^{¤¤}p$ < 0.01, $^{¤¤¤}p$ < 0.001 vs Feed 2; $^{§}p$ < 0.05, $^{§§}p$ < 0.01, $^{§§§}p$ < 0.001 vs Feed 3 with Tukey's post hoc test [b]EAA = essential amino acid.

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Table 9. Heavy Metal Levels in *Acheta domesticus*[a]

heavy metals	F1 crickets	F2 crickets	F3 crickets
As	<0.0010	0.0011 ± 0.0002	<0.001
Cd	0.0020 ± 0.0005	0.0022 ± 0.0004	0.0024 ± 0.0002
Cr	0.099 ± 0.18	0.048 ± 0.023	0.040 ± 0.022
Ni	0.041 ± 0.021	0.025 ± 0.011	0.029 ± 0.012
Pb	0.0078 ± 0.001	0.0090 ± 0.0032	0.0093 ± 0.0022

[a]mg/100 g dry weight; $n = 6$ (presented as mean ± sd). The groups were not statistically different ($p > 0.05$).

house crickets than the corresponding protein diet in the study of Bawa et al.[38] This indicates that other nutrients also play a significant role in cricket development.

Compared to other protein-rich foods (e.g., pork sirloin contains 6.9% fat, chicken breast strips without skin, 2.0%, beef sirloin, 4.0%, and fresh broad bean, 0.6%, all as fresh weights), the fat content of house crickets was high.[41] However, the studied crickets as well as most of the crickets sold on the modern Western market are dried, not fresh. Therefore, the nutritional values of meat presented in fresh weight are not directly comparable with our own results, as the values represent the foods in the form of consumption (fresh vs dried). The information on moisture content enables the recalculation of results in fresh weight, which is needed for standardized information on the nutritional composition of food.[42]

Crickets that were fed feeds F2 and F3 had an equal, higher nitrogen value than crickets that were fed feed F1 (Table 5). When one considers crickets as a protein source, there is an ongoing debate over which nitrogen-to-protein conversion factor should be used in the protein calculation of insects, as there is not yet enough research on the actual protein content of crickets.[43] The average nitrogen level of proteins has been found to be 16%, leading to a nitrogen-to-protein conversion factor of 6.25.[44] However, in crickets and other insects protein is not the only nitrogen source. Chitin, which forms the exoskeleton of insects, is an *N*-acetylglucosamine polymer in its chemical structure and contains nitrogen in each of its structural units. Therefore, the factor 6.25, which is the standard nitrogen-to-protein conversion factor in, e.g., several food composition databases, cannot be considered suitable for insects, as it will overestimate the protein content due to chitin.[43] In the literature, other factors for insects have also been presented, e.g., 5.09 for *A. domesticus*, 5.00 for *Gryllus bimaculatus*, and 4.76 for three edible larvae (*Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*).[31,45] On the basis of the literature, the suitable nitrogen-to-protein conversion factor for several insect species is closer to five than six. In addition to 6.25, the factor 5.09 has been used in Table 5 to calculate protein content, because it has been specifically determined for house crickets. The protein results calculated with these two factors differ by almost 20%, representing a huge overestimation of the protein content of house crickets when the factor 6.25 is used.

The protein content (factor 6.25) of cricket samples in this study varied from 12.5% to 16.9% in fresh weight (data not shown). These were in line with Payne et al., who found 15.6 g/100 g (FW) crude protein in house crickets as a mean value for three studies.[40] Also, similar and slightly higher protein values (55.00−70.75%, DW) were reported by Rumpold and Schlüter for *A. domesticus*.[5] A considerable variation in protein as well as fat levels of crickets was noticed in different studies. There are a lot of parameters, e.g., feed and the developmental stage of the insects, that affect the final nutrient composition. Therefore, to compare nutritional results from different growth experiments, there is a need to study, control, and document those parameters.

Despite the factor used, the crude protein contents of the studied crickets, as dry weight (as consumed), were higher than the crude protein levels of traditional protein-rich items, e.g., protein content of 22.9 g/100 g for beef sirloin, 21.5 g/100 g for pork fillet, 22.4 g/100 g for chicken breast fillet without skin, and 8.8 g/100 g for fresh broad bean, which are consumed fresh.[41] In fresh weight, for the comparison, the mean protein content of the crickets in this study was 14.7 g/100 g. The importance of crickets as a source of protein cannot be assessed solely on the basis of protein content but must also be considered in relation to the amounts and forms (fresh vs dried) of consumption.

Fatty Acid Profiles in House Crickets. Fatty acid profiles of house crickets are presented in Table 6. Altogether, 19 different fatty acids were detected. The most abundant fatty

Table 10. Trace Element Levels in *Acheta domesticus*[a]

		F1 crickets		F2 crickets		F3 crickets	
minerals and trace elements	PRI/AI, mg/day	mg/100 g	PRI/AI, %	mg/100 g	PRI/AI, %	mg/100 g	PRI/AI, %
Ca	950	81 ± 8.0	9	200 ± 32****	21	84 ± 6.5	9
K	3500	740 ± 42	21	680 ± 140	19	750 ± 140	21
Mg	M 350/F 300	65 ± 3.6	M 19/F 22	78 ± 9.5**	M 22/F 26	66 ± 4.8	M 19/F 22
Na		190 ± 21		260 ± 52***		150 ± 30	
P	550	660 ± 40	120	730 ± 52*	133	690 ± 38	125
Co		0.021 ± 0.023		<0.010		<0.010	
Cu	M 1.6/F 1.3	1.9 ± 0.11	M 119/F 146	2.7 ± 0.27****	M 169/F 208	2.0 ± 0.22	M 125/F 154
Fe	M 11/F 16	5.8 ± 1.0	M 53/F 36	7.1 ± 0.37**	M 65/F 44	5.6 ± 0.66	M 51/F 35
Mn	3.0	1.8 ± 0.09	60	3.6 ± 0.58****	120	1.7 ± 0.19	57
Mo	0.065	0.051 ± 0.0081	78	0.050 ± 0.010	77	0.057 ± 0.0065	88
Se	0.070	0.027 ± 0.0088	39	0.038 ± 0.0037**	54	0.024 ± 0.0032	34
Zn	M 13/F 10[b]	16 ± 0.98	M 123/F 160	22 ± 0.78****	M 169/F 220	16 ± 1.8	M 123/F 160

[a]mg/100 g dry weight; $n = 6$ (presented as mean ± sd). M = male, F = female. All statistical differences were between CF2 and other crickets: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. [b]Averaged value.

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acids in cricket fat were oleic acid C18:1n-9 (26.8−30.4%), linoleic acid C18:2n-6 (23.6−29.4%), and stearic acid C18:0 (6.7−9.5%) (data not shown). The proportions of saturated fatty acid residues (SFAs) were high in all cricket samples, varying from 37.7% to 41.3% in single samples. Together with high levels of fat, this finding is in contrast with Payne et al., who presented low levels of saturated fat in crickets.[40] However, the ratio of SFAs to unsaturated fatty acids (UFAs) varies from 0.63 to 0.73 in all samples and, thus, is in the range suggested by Rumpold and Schlüter.[5] The fat in crickets that were fed feed F2 had higher proportions of polyunsaturated fatty acids (PUFAs; 29.7% versus 24.9% in F1 crickets and 25.1% in F3 crickets) and lower proportions of monounsaturated fatty acids (MUFAs; 28.7% versus 32.1% in F1 crickets and 32.6% in F3 crickets) than the other crickets. The EFSA recommends that the intake of saturated fat be as low as possible.[37] In addition, long-chain omega-3 fatty acids are recommended, as they are well-known for their positive effect to cardiovascular health. Unfortunately, these studied crickets were not a remarkable source of omega-3 fatty acids, as the proportion of α-linolenic acid C18:3n-3, the only omega-3 fatty acid detected in crickets, varied from 0.6% to 1.2%. The proportions of trans fatty acids were low in all cricket samples, as they varied from not detectable (<0.08%) to 0.2%.

Studies have shown that the fatty acid composition of crickets could be affected by changing their diet.[5,46] When comparing the analyzed house cricket fatty acid profiles to beans, beef, pork fillet, and chicken breast fillet, cricket fat most closely resembles chicken and pork fat, which contain proportionally 21% and 14% PUFAs (respectively), 47% MUFAs (for both), and 32% and 37% SFAs (respectively).[41] Crickets had more PUFAs than beef (5%) and pork (14%) but less MUFAs (40% and 47% in beef and pork, respectively) and also less SFAs than beef but approximately an equal proportion of SFAs as pork. Trans fatty acids were at a level typical of meat.

The dietary fiber content was analyzed only from the crickets fed with commercial chicken feed (F2). Crickets can be considered a source of dietary fiber, since the content was over 5 g/100 g (as dry weight; Table 5). The majority of dietary fiber in crickets is water-insoluble chitin. Due to the high level of nitrogen in chitin, the dietary fiber level analyzed in this study was most likely an underestimation. In the analysis, the residual protein content is measured, and the detected amount is subtracted from the dietary fiber content. Thus, the same challenge with the widely used nitrogen-to-protein correction factor 6.25, discussed earlier in this paper, also affects the dietary fiber analysis.

Very limited information on the dietary fiber levels of crickets can be found in the literature. Adult field crickets (*Gryllus testaceus* Walker) were reported to be composed of 8.7% chitin,[47] which is more but in the same order of magnitude as the dietary fiber content of *A. domesticus* obtained in this study. The differences can be explained by different analytical methods and the fact that the size of the exoskeleton can vary between species and stage of development. The levels of chitin in cultivated house crickets ranged from 2.0 to 12.0 g/100 g in a study by Kipkoech et al.[48] The amount of chitin increased steadily as the house crickets aged, being about 5−9 g/100 g during weeks 4−7, which is consistent with the dietary fiber content analyzed in this study.

Amino Acids in House Crickets. The concentrations of individual amino acids are presented in Table 7. The results are expressed in two formats: as g/100 g in dry weight and as mg/g protein. The results without protein conversion (g/100 g, DW) give information on the total amount of amino acids and thus the amount of protein, while results expressed with protein conversion (mg/g protein) provide information about the amino acid profile and thus the quality of the protein.

The sums of amino acids (in dry weight) in crickets fed with feeds F1, F2, and F3 were 47.8 ± 1.2 g/100 g, 51.5 ± 0.6 g/100 g, and 51.9 ± 0.8 g/100 g, respectively, which are in agreement with the protein results (Table 5). The amount of amino acids in F1 crickets was significantly lower ($p < 0.01$) than in F2 crickets (control) or F3 crickets, which was also in line with the lower protein content of F1 crickets (Table 5). Furthermore, the amino acid levels of F1 crickets were significantly lower for almost all amino acids (g/100 g, DW; Table 7).

The differences in amino acid profiles (mg/g protein) between crickets were minor; the profiles of F2 crickets and F3 crickets were especially similar. Only the amount of serine in F2 crickets was significantly higher than in the others (Table 7). These findings indicate that the feed used mainly affected the total amount of protein, not its quality so much. The measured amino acid profiles were comparable to previously published amino acid compositions of *Acheta domesticus*.[5,49]

Amino acids are classified as essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and nonessential (alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine) according to whether or not they can be synthesized by the human body.[50] Methionine is a substrate for cysteine, and under insufficient supply of methionine, cysteine can become essential; thus, it is considered semiessential. The same applies for phenylalanine and tyrosine, tyrosine being a metabolic product of phenylalanine. Essential amino acids must be obtained from food. All of the studied house crickets contained all of the essential amino acids (EAA), and the sum of EAA exceeded 277 mg/g protein, meeting the nutritional criteria of WHO/FAO/UNU (2007) for adults.[50] In all feeding studies, the amount of EAAs was 46% of the total amino acids, just meeting the criteria of WHO. All EAAs met the nutritional requirement of protein, as all EAAs have an amino acid score greater than 1 (Table 8). Tryptophan has been reported as the limiting amino acid in *Acheta domestica* by Köhler et al.[49] However, on the basis of this study, methionine + cysteine was the limiting EAA (Table 8).

Elements in Crickets. The summarized results of trace elements in house crickets are presented in Tables 9 and 10. A high variability in elemental concentrations was observed within the groups. The use of different feeds (F1−F3) did not affect the heavy metal concentration of crickets. No maximum levels for heavy metals have been set in the Commission Regulation (EC) No 1881/2006 for insects.[51] If the corresponding limits for meat (excluding offal) of bovine animals, sheep, pigs, and poultry were applied to the crickets, the concentrations would not exceed the limits.

Profiles for other trace elements in crickets were also in line with the elemental composition of the feeds, suggesting that feeding affects elemental levels. The use of feeds F1 and F3 showed very similar results in the composition of trace elements in crickets. F2 crickets contained higher concentrations of almost all trace elements than F1 and F3 crickets. Potassium (K) and molybdenum (Mo) were the exceptions as

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these concentrations were lower in F2 crickets. The level of K was also the lowest in feed F2, which can explain the result. The differences between Mo levels in the feeds were minor.

The concentrations of iron (Fe), manganese (Mn), and zinc (Zn) were about 5−10 times higher in F2, compared to other feeds. Interestingly, in crickets, the differences in concentrations were only 1.2−2.1-fold in favor of F2 crickets, and a similar change in level was also seen for Ca. Hunt et al. have shown that feeding insects with a calcium-rich diet increases their overall levels of calcium (Ca).[52] Our study supports this finding as crickets fed with Ca-rich F2 had a much higher Ca concentration ($p < 0.0001$). However, the Ca content in F2 was 23−44 times higher than in other feeds but only 2.5 times higher in F2 crickets. On the basis of these results, it appears that crickets cannot utilize large excesses of minerals, and it is important to optimize the feed according to their needs. More research is needed on the minerals that promote and limit cricket growth.

When the results of trace elements are compared to EFSA recommendations (mg/day) for adults,[37] it can be observed that house crickets are a source of potassium and magnesium and rich in phosphorus, copper, iron, manganese, molybdenum, selenium, and zinc.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.0c00083.

Table S1, a detailed list of the feed materials together with the feed prices, calculated protein, and energy contents of each feed used in the preliminary and main rearing experiments; Tables S2−S5, analyzed results for macronutrients, amino acids, heavy metals, and minerals of feeds used in the preliminary tests (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

FCR, feed conversion ratio (used feed per live weight gain); TF1−TF7, test feeds 1−7 used in the preliminary experiments; F1−F3, feeds 1−3 used in the actual rearing experiments; the LP model, the linear programming model in Excel; F1, F2, and F3 crickets, house crickets (*Acheta domesticus*) fed in a rearing experiment with feeds F1−F3; HPLC, high-pressure liquid chromatography; GC, gas chromatography; MSD, mass selective detection; RI, refractive index; ICP-OES, inductively coupled plasma optical emission spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; AMG, amyloglucosidase; IDF, water-insoluble dietary fiber; SDFP, dietary fiber soluble in water and precipitated by 78% aqueous ethanol; SDFS, dietary fiber soluble in water and not precipitated by 78% aqueous ethanol; EAA, essential amino acid; EC, European Commission; EU, European Union; EFSA, European Food Safety Authority; FAs, fatty acids; FAMEs, methyl esterified fatty acids; DF, dietary fiber; DM, dry matter; DW, dry weight; FW, fresh weight; SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, trans fatty acids; Cys, cysteine; His, histidine; Ser, serine; Arg, arginine; Gly, glycine; Asp+Asn, aspartic acid and asparagine; Met, methionine; Glu+Gln, glutamine and glutamic acid; Thr, threonine; Ala, alanine; Pro, proline; Lys, lysine; Tyr, tyrosine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Trp, tryptophan

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