

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Lowering Chitin Content of Cricket (*Gryllus assimilis*) Through Exoskeleton Removal and Chemical Extraction and its Utilization as a Ruminant Feed *in vitro*

Anuraga Jayanegara, Mohammad M. Sholikin, Della A.N. Sabila, Sri Suharti, Dewi Apri Astuti

Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, 16680 Bogor, Indonesia

Abstract

Background and Objective: Cricket contains high crude protein level but it also contains considerable amount of chitin that may impede nutrient digestion and decrease production performance of animal. This experiment aimed to decrease chitin content of cricket (C) through exoskeleton removal (CER) or by chemical extraction (CCE). **Materials and Methods:** Nutritional evaluation of cricket was performed in two experiments. In experiment 1, three forms of cricket were prepared, i.e., C, CER and CCE. These were subjected to chemical composition determination and *in vitro* rumen fermentation incubation as individual substrates. In experiment 2, C and CER were included in concentrate rations at different proportions to substitute soybean meal (SBM), i.e., R1 (concentrate containing 30% SBM), R2 (50% SBM was substituted by C), R3 (100% SBM was replaced by C) and R4 (100% SBM was replaced by CER). The concentrates were then evaluated *in vitro* for their rumen fermentation and digestibility characteristics. Data were analyzed with analysis of variance and Duncan's test. **Results:** Cricket was high in crude protein (CP), ether extract (EE) and chitin contents. Removal of exoskeleton decreased CP and chitin contents of cricket. Chemical extraction of cricket increased its CP and completely removed its chitin. Main fatty acids observed in cricket were linoleic acid, palmitic acid, oleic acid and stearic acid and the composition was unaltered due to exoskeleton removal or chemical extraction. Cricket was relatively highly digestible and exoskeleton removal and chemical extraction did not further improve *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of cricket. The R1 and R2 revealed similar IVDMD and IVOMD, but R3 and R4 resulted in lower values for both parameters than those of R1 and R2 ($p < 0.05$). **Conclusion:** Exoskeleton removal or chemical extraction effectively reduced chitin content of cricket and the insect may be used to substitute SBM up to 50% in concentrate for ruminant.

Key words: Cricket, chitin, exoskeleton, extraction, *in vitro* rumen

Citation: Anuraga Jayanegara, Mohammad M. Sholikin, Della A.N. Sabila, Sri Suharti, Dewi Apri Astuti, 2017. Lowering chitin content of cricket (*Gryllus assimilis*) through exoskeleton removal and chemical extraction and its utilization as a ruminant feed *in vitro*. Pak. J. Biol. Sci., 20: 523-529.

Corresponding Author: Dewi Apri Astuti, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, 16680 Bogor, Indonesia Tel/Fax: +62 251 8626213

Copyright: © 2017 Anuraga Jayanegara *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feeding management in traditional livestock farming system faces a number of problems especially for finding high quality and affordable protein sources with continuous supply. Cereals as conventional ingredients for concentrates are typically more expensive as protein sources as compared to legumes. It is, therefore, necessary to explore and find alternative protein sources with relatively low price, high quality and save to consume. Use of insects as feed ingredients has been introduced in recent years both for monogastric and ruminant livestock¹. Insects possess some comparative advantages over protein from plant and animal origins such as low conversion ratio from organic substrate to insect biomass with very short period of time, less demand for water, low greenhouse gas emissions² and contain high protein with relatively balance amino acid composition³. Insect species that have been tested as feed resources for domesticated animals are mealworm, black soldier fly larvae, house fly maggot, silkworm, grasshopper, locust and cricket^{1,4}.

Cricket is a promising insect species to be used as an alternative feed resource for animal. Normally cricket is reared until 5-6 times of producing eggs and then would be discarded due to its low productivity, such discarded cricket may then be used as animal feed¹. With regard to nutritional value of cricket, it contains high crude protein content, i.e. above 55% from dry matter (DM)⁵ and it is higher in comparison to some other insect species such as mealworm and black soldier fly larvae⁴. However, cricket also contains considerable amount of chitin, i.e., 8.7% DM⁵ which is a nitrogenous substance (long-chain polymer of N-acetylglucosamine) and usually found in insect exoskeleton⁶. Chitin may impede nutrient digestion, absorption and decrease production performance of particularly monogastric animals^{7,8}. Therefore, any treatment to remove or at least to decrease chitin content of cricket would potentially increase nutritive value of cricket.

This experiment was aimed to decrease chitin content of cricket through exoskeleton removal or by chemical extraction. Further, whole cricket (C), cricket after exoskeleton removal (CER) and cricket after chemical extraction (CCE) were evaluated for their proximate composition, amino acid and fatty acid profiles and *in vitro* rumen fermentation and digestibility characteristics, either when incubated as a single substrate or as an ingredient in formulated concentrates.

MATERIALS AND METHODS

Experimental setup: Evaluation of cricket was performed in two experiments. In experiment 1, three forms of cricket were prepared, i.e., C, CER and CCE. These were subjected to chemical composition determination (proximate composition, amino acid composition and fatty acid profile) and *in vitro* rumen fermentation incubation as individual substrates. In experiment 2, C and CER were included in concentrate rations at different proportions to substitute soybean meal, either partially or totally. The concentrates were then evaluated *in vitro* for their rumen fermentation and digestibility characteristics. These experiments were conducted from March-June, 2017 at Laboratory of Dairy Nutrition, Faculty of Animal Science, Bogor Agricultural University, Indonesia.

Cricket preparation and treatment: To obtain whole cricket (C) meal, discarded cricket from a commercial cricket farm in Bogor, Indonesia, was oven-dried at 50°C for 48 h and ground by a hammer mill to pass a 1 mm screen. The CER was obtained by manually removing head, legs and wings of the oven-dried cricket. The CCE was obtained through delipidation, extraction and precipitation processes of cricket using chemical solvents according to procedures from Agboola *et al.*⁹ and De Souza *et al.*¹⁰. In brief, delipidation was performed by solubilizing cricket meal into n-hexane (1:4 w/v) and homogenized for 30 min. Sample was centrifuged thereafter at 5,000 rpm, maintenance temperature was 4°C for 10 min and supernatant was discarded. Pellet was solubilized by using NaOH 2 N for 60 min to shift pH from neutral to alkali (pH around 12). Precipitation was conducted at isoelectric pH by adding HCl 2 N and kept for 30 min. Precipitate was centrifuged twice at 5,000 rpm, maintenance temperature was 4°C for 10 min and dried at 50°C for 8 h to obtain CCE.

Chemical composition determination: Samples were determined for their proximate composition, i.e. organic matter (OM), ash, crude protein (CP), ether extract (EE) and crude fiber (CF) according to AOAC¹¹. Total digestible nutrient (TDN) content was estimated from proximate composition by using an equation from Wardeh¹². Procedure for chitin determination was based on No *et al.*¹³. Analyses of amino acid and fatty acid profiles in samples were conducted by employing high performance liquid chromatography and gas chromatography (Shimadzu Corp., Kyoto, Japan), respectively, according to AOAC¹¹. Chemical composition determination for each parameter was performed in duplicate.

In vitro rumen fermentation: Samples were incubated *in vitro* with buffered rumen fluid by following the procedure of Tilley and Terry¹⁴. An amount of 0.5 g sample was put into a glass tube and mixed with 10 mL rumen fluid and 40 mL McDougall buffer. The buffer was composed of (per liter solution) 9.8 g NaHCO₃, 2.44 g Na₂HPO₄, 0.57 g KCl, 0.47 NaCl, 0.12 g MgSO₄·7H₂O and 0.16 g CaCl₂·2H₂O. Rumen fluid was taken from three Ettawa crossbred goats approximately 4 h after morning feeding by employing a stomach-tube method. The tube was continuously flushed with CO₂ to ensure anaerobic environment, closed with a ventilated rubber and put into a shaking water bath (Memmert GmbH+Co. KG, Schwabach, Germany) at 39°C to start the incubation. After 48 h incubation period, the ventilated rubber was opened and added with 3 drops of HgCl₂ to terminate the first incubation step. The tube was centrifuged at 4,000 rpm for 10 min to separate residue and supernatant. Supernatant was discarded and the residue was further incubated for 48 h with 50 mL pepsin-HCl 0.2%. Residue obtained from the second *in vitro* incubation step was filtered with Whatman paper No. 41 (Sigma-Aldrich, St. Louis, MO, USA) under vacuum and subjected to DM and OM determination. Delta between initial amounts of DM and OM and their corresponding residues were used for, corrected with blank, calculating *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD), respectively. Concentrations of total volatile fatty acid (VFA) and ammonia (NH₃) were measured after 4 h incubation period by using steam distillation and Conway micro-diffusion methods, respectively, as described in Jayanegara *et al.*¹⁵.

Another *in vitro* incubation procedure was performed to measure gas production during fermentation according to Menke and Steingass¹⁶. Samples (200 mg DM) were incubated with 10 mL rumen fluid and 20 mL buffer solution in glass syringes at 39°C for 24 h. Volume of fermentation gas was manually read from the calibrated scale printed on the glass syringes. Supernatant obtained after 24 h incubation was further determined for proteolytic bacteria and protozoa population and protease activity. Population of proteolytic bacteria was determined through roller tube method¹⁷ whereas, protozoa population was counted by using Fuchs-Rosenthal counting chamber (Brand GmbH+Co. KG, Wertheim, Germany). Protease activity was determined by following the procedure of Brock *et al.*¹⁸. *In vitro* incubations were performed in three runs and each run was represented by two incubation tubes or syringes.

Statistical analysis: Data obtained were subjected to two-way analysis of variance (ANOVA) based on a randomized complete block design with the following statistical model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where, Y_{ij} is observed value, μ is overall mean, α_i is treatment effect, β_j is block effect (replicate) and ε_{ij} is random residual error. Different *in vitro* runs were considered as block effect in the statistical model. Duncan's multiple range test was employed for comparison among different treatment means when ANOVA result for a certain parameter significant at $p < 0.05$. Statistical analysis was performed by using IBM SPSS Statistics software version 20 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Experiment 1: Cricket was high in CP, EE and chitin contents (Table 1). Removal of exoskeleton decreased CP and chitin contents of cricket. Chemical extraction of cricket increased its CP and TDN but decreased EE and CF contents and such procedure completely removed its chitin. With regard to amino acid composition, cricket was particularly high in glutamic acid and histidine proportions but low in methionine (Table 2). Exoskeleton removal and chemical extraction generally did not lead to major changes in amino acid profiles

Table 1: Proximate composition (in percent dry matter) of whole cricket (C), cricket after exoskeleton removal (CER) and cricket after chemical extraction (CCE)

Components	C (%)	CER (%)	CCE (%)
Organic matter	95.3	95.2	93.4
Ash	4.7	4.8	6.6
Crude protein	54.1	50.3	62.0
Ether extract	26.9	29.5	11.1
Crude fiber	6.9	6.6	1.6
Chitin	7.7	3.5	nd
TDN	78.9	80.8	89.5

TDN: Total digestible nutrient, nd: Not detected

Table 2: Amino acid profile (in percent total amino acid) of whole cricket (C), cricket after exoskeleton removal (CER) and cricket after chemical extraction (CCE)

Amino acids	C (%)	CER (%)	CCE (%)
Essential			
Methionine	1.88	1.73	1.84
Valine	6.28	6.76	7.21
Tyrosine	6.10	7.24	10.91
Histidine	11.09	2.39	2.26
Lysine	6.59	6.61	5.83
Threonine	4.61	4.22	4.19
Phenylalanine	4.09	4.47	4.73
Isoleucine	4.53	4.75	5.07
Leucine	7.49	8.41	8.79
Non-essential			
Aspartic acid	8.80	9.58	9.19
Glutamic acid	13.00	14.56	12.36
Glycine	6.36	6.05	5.39
Arginine	6.90	7.77	6.53
Alanine	8.13	10.32	10.95
Serine	4.14	5.16	4.76

Table 3: Fatty acid profile (in percent total fatty acid) of whole cricket (C), cricket after exoskeleton removal (CER) and cricket after chemical extraction (CCE)

Fatty acids	C (%)	CER (%)	CCE (%)
Lauric acid (C12:0)	0.04	0.04	0.03
Myristic acid (C14:0)	0.76	0.71	0.51
Myristoleic acid (C14:1)	0.04	0.01	<0.01
Pentadecanoic acid (C15:0)	0.07	0.11	0.07
Palmitic acid (C16:0)	30.15	28.60	30.60
Palmitoleic acid (C16:1)	0.73	0.87	0.76
Heptadecanoic acid (C17:0)	0.16	0.28	0.18
Cis-10-Heptadecanoic acid (C17:1)	0.07	0.03	0.06
Stearic acid (C18:0)	7.33	8.28	7.83
Elaidic acid (trans-C18:1)	0.05	0.28	0.13
Oleic acid (C18:1)	28.19	27.12	26.80
Linoleic acid (C18:2)	31.05	32.63	32.26
γ-Linolenic acid (C18:3)	0.09	0.08	0.06
Arachidonic acid (C20:4)	0.42	0.89	0.63

Table 4: *In vitro* rumen fermentation and digestibility of whole cricket (C), cricket after exoskeleton removal (CER) and cricket after chemical extraction (CCE)

Parameters	C	CER	CCE
NH ₃ (mmol L ⁻¹)	17.5±4.23	18.3±4.35	17.3±5.59
Total VFA (mmol L ⁻¹)	156.0±4.0 ^a	165.0±1.4 ^b	156.0±0.5 ^a
IVDMD (%)	72.9±2.91	74.8±2.32	75.5±0.31
IVOMD (%)	72.0±3.23	74.0±2.26	73.7±0.48

Different superscripts within the same row are significantly different at p<0.05. NH₃: Ammonia, VFA: Volatile fatty acid, IVDMD: *In vitro* dry matter digestibility, IVOMD, *in vitro* organic matter digestibility

Table 5: Concentrate formula containing cricket meal or cricket after exoskeleton removal (CER) and their chemical composition

Component	R1	R2	R3	R4
Ingredient (% DM)				
Cassava by-product	32.80	32.80	32.80	32.80
Copra meal	34.40	34.40	34.40	34.40
Soybean meal	30.00	15.00	0.00	0.00
Cricket meal	0.00	15.00	30.00	0.00
CER	0.00	0.00	0.00	30.00
CaCO ₃	1.40	1.40	1.40	1.40
NaCl	0.70	0.70	0.70	0.70
Premix	0.70	0.70	0.70	0.70
Chemical composition (% DM)				
Crude protein	22.30	23.10	24.30	25.80
Ether extract	2.79	3.12	3.46	2.44
Crude fiber	7.97	8.64	8.87	8.75
TDN	72.00	70.20	68.00	68.00
Calcium	0.98	0.91	0.86	0.86
Phosphorus	0.58	0.39	0.33	0.33

DM: Dry matter, NFE: Nitrogen free extract, TDN: Total digestible nutrient

of cricket except for histidine that markedly reduced due to both treatments. Main fatty acids observed in cricket were (ordered from the highest proportion) linoleic acid, palmitic acid, oleic acid and stearic acid and the composition was unaltered due to exoskeleton removal or chemical extraction (Table 3). Cricket was relatively highly digestible, i.e., IVDMD and IVOMD values were higher than 70% (Table 4).

Exoskeleton removal and chemical extraction did not further improve IVDMD and IVOMD of cricket. This was also the case with ruminal ammonia concentration. Exoskeleton removal increased total VFA concentration of cricket in the rumen *in vitro* than that of control (p<0.05).

Experiment 2: In this experiment, R1 was a concentrate formula containing 30% soybean meal and it was substituted by cricket or CER in R2-R4 (Table 5). In R2 and R3, soybean meal was replaced with cricket by 50 and 100%, respectively, whereas, in R4 soybean meal was completely substituted with CER. Crude protein contents of rations increased from R1-R4 but their TDN contents decreased. *In vitro* ruminal ammonia concentrations of cricket or CER containing rations were higher than that of control (p<0.05, Table 6). Total VFA concentration and protease activity were similar among treatments. Treatment R2 produced similar gas production than that of R1, but gas production in R3 or R4 incubation was lower in comparison to R1 (p<0.05). Populations of proteolytic bacteria and protozoa revealed similar patterns, R2 increased both populations (p<0.05) whereas, R3 and R4 decreased them (p<0.05). With regard to *in vitro* digestibility, R1 and R2 revealed similar IVDMD and IVOMD, but R3 and R4 resulted in lower values for both parameters than those of R1 and R2 (p<0.05).

Chemical composition of cricket: Cricket used in the present study contained considerable amounts of CP, EE and chitin. Such high CP and EE contents of cricket were in agreement with other studies^{19,20}, whereas, the high chitin was in agreement with that of Wang *et al.*⁵. Exoskeleton removal decreased chitin content of cricket by approximately half due to the fact that chitin in insect is mainly deposited in its exoskeleton⁶. This simple approach may, therefore, be used to decrease chitin content of cricket that limits its utilization as an animal feed. A decrease of CP content of cricket after exoskeleton removal is apparently related to chitin decrease since chitin is a nitrogenous molecule with N-acetylglucosamine monomer²¹. Lower EE content after chemical extraction was expected since extraction procedure involved delipidation of cricket by using an organic solvent n-hexane and thus partially removed the fat. Further chemical extraction step after delipidation was solubilization and precipitation by using NaOH and HCl, respectively, mainly aimed to separate cricket protein from other organic molecules particularly fiber; such procedure was successful in decreasing CF. Since chitin is recovered as fiber in the proximate analysis²², concentration of the compound seems

Table 6: *In vitro* rumen fermentation and digestibility of concentrate containing cricket meal or cricket after exoskeleton removal (CER)

Parameters	R1	R2	R3	R4
NH ₃ (mM)	4.87±0.26 ^a	6.16±0.54 ^b	7.07±0.95 ^c	6.98±0.51 ^c
Total VFA (mM)	153.00±0.93	146.00±14.5	147.00±15.0	153.00±2.40
Gas production (mL/200 mg DM)	43.70±1.87 ^{ab}	44.90±2.57 ^b	40.60±1.29 ^a	40.80±1.07 ^a
Proteolytic bacteria (log CFU mL ⁻¹)	7.91±1.21 ^b	8.15±0.45 ^c	7.48±0.55 ^{ab}	7.01±0.90 ^a
Protease activity (U mL ⁻¹)	0.69±2.18	1.56±1.90	1.28±0.32	1.10±0.92
Protozoa (log cell mL ⁻¹)	4.79±0.11 ^b	5.00±0.09 ^c	4.23±0.1 ^a	4.36±0.23 ^a
IVDMD (%)	77.90±1.61 ^b	75.80±3.16 ^b	69.40±1.45 ^a	71.30±3.60 ^a
IVOMD (%)	83.80±2.37 ^b	81.70±3.75 ^b	74.50±1.44 ^a	77.20±4.11 ^a

Different superscripts within the same row are significantly different at $p < 0.05$, R1: Control ration containing 30% soybean meal, R2: Ration containing 15% cricket, R3: Ration containing 30% cricket; R4: Ration containing 30% CER, NH₃: Ammonia, VFA: Volatile fatty acid, DM: Dry matter, CFU: Colony forming unit, IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility

to be negligible due to substantial CF reduction following the chemical extraction. In the previous study, chitin in some insect species was recovered in nitrogenous fiber fractions namely neutral detergent insoluble crude protein and acid detergent insoluble crude protein⁴.

Quality of protein depends very much on amino acid composition. High proportion of glutamic acid in cricket protein, which is a non-essential amino acid, was in agreement with other studies that characterized amino acid profiles of cricket^{20,23,24}. Low proportion of methionine in cricket was also observed in these studies^{23,24}. Jozefiak *et al.*²⁵ described that, in comparison to fish meal, insects including cricket generally contain lower concentration of methionine that need to be considered when formulating ration based on insect protein. In contrast to other studies, histidine was in high proportion although it decreased considerably after exoskeleton removal or chemical extraction. Such difference in chemical composition within an insect species might be caused by a number of factors such as developmental stage, rearing condition and composition of growth media for insect production^{1,3,25}. An appropriate balance between essential and non-essential amino acids is required for effective utilization of dietary protein. Ratio between essential and non-essential amino acids in C, CER and CCE was 1.11, 0.87 and 1.03, respectively. These cricket preparations thus meet high-value protein sources in which at least 40% of their amino acids should be essential²⁰.

High proportion of linoleic acid, palmitic acid and oleic acid in cricket was also observed by other authors^{19,20,23,24}. These fatty acids contributed to almost 90% of total fatty acid present in cricket. Supporting present study finding, in the study of Oonincx *et al.*¹⁹, linoleic acid, palmitic acid and oleic acid made up more than 75% of total fatty acid. Apparently such pattern is not only belong to cricket but also to some other insect species. House cricket, locust and mealworm larvae were reported to contain linoleic acid, palmitic acid and oleic acid 68.35-86.14% from total fatty acid²⁰. High proportion

of linoleic acid found in cricket is apparently due to 12 desaturase activity in the insect that uses oleoyl-CoA as a substrate to produce linoleic acid, thus converting oleic acid to linoleic acid²⁶. Fatty acid profile of insects may vary greatly since such composition is influenced by dietary fatty acid profile although there are few exceptions like in yellow mealworm¹⁹. In the context of animal nutrition, insect lipid contributes to animal by supplying energy and essential fatty acids. Polyunsaturated fatty acid (PUFA) like linoleic acid is favorable since PUFA may be deposited in animal product, particularly in monogastric animal and contribute to human health. For ruminant, PUFA undergoes massive lipolysis and stepwise biohydrogenation processes to form various isomers of fatty acids with higher degree of saturation and therefore, dietary fatty acid profile could not directly be represented in the product²⁷.

***In vitro* fermentative and digestibility characteristics of cricket:** Values of IVDMD and IVOMD of cricket observed in the present study were higher than those of researchers previous study⁴. In that study, IVDMD and IVOMD values of cricket were 64.2 and 64.7%, respectively. Such differences may be attributed to different chemical composition of cricket in the two studies. Content of CF of cricket in researchers previous study was 14.6% DM whereas, it was much lower, i.e., 6.9% DM in this study. Negative influence of fiber, particularly lignocellulose fraction on digestibility has been widely recognized²⁸. It is interesting to observe that exoskeleton removal and chemical extraction did not increase IVDMD and IVOMD of cricket although the treatments were successful in (partially) removing chitin. Apparently chitin is degraded in the rumen since some rumen microbial species possess chitinolytic activity such as endochitinase, exochitinase, N-acetyl-glucosaminidase, chitosanase and chitin deacetylase and these microbes included bacteria, anaerobic fungi and protozoa species²⁹⁻³¹.

An increase of ruminal ammonia concentration following substitution of soybean meal by cricket meal or CER was apparently due to higher CP content of R2-R4 in comparison to R1. Ammonia in the rumen is originated from protein after transformation through proteolysis and deamination processes by proteolytic microbes³² and this was confirmed by an increase of proteolytic bacteria population in R2. Ammonia itself is further utilized by rumen microbes for microbial protein synthesis. Type of protein present in feed material may also determine ammonia concentration in the rumen. Protein in soybean meal is considered as ruminally degradable¹⁵ and thus potentially contribute to high concentration of ammonia. It seems that cricket contains high proportion of rumen degradable protein as well based on its ruminal ammonia concentration, however, this requires further study either by employing *in sacco* method or by using protein fractionation method of Cornell³³. Partial replacement of soybean meal by cricket meal did not decrease *in vitro* gas production and digestibility. However, its full replacement led to lower values of both parameters. Despite cricket contains higher CP content than that of soybean meal, it also contains higher fiber thus limiting its use to completely replace soybean meal in ruminant diet. Another factor that may explain such decrease of *in vitro* gas production, IVDMD and IVOMD is an increase of dietary EE with cricket substitution. Dietary EE has been known to cause negative effect on carbohydrate degradation by rumen microbes, particularly fiber degradation³⁴.

Results from this study imply that exoskeleton removal and chemical extraction are effective methods to reduce chitin present in cricket without adversely affecting other nutrients such as amino acid and fatty acid profiles. However, chitin decrease is not accompanied with an increase in cricket's digestibility *in vitro*. In application, therefore, such chitin removal may not be necessary when cricket is used in ruminant feed, but it would be useful for those of monogastrics like poultry and swine. With regard to the use of cricket in ruminant diet, we recommend that the insect may be used up to 50% in replacing soybean meal. Further studies involving *in sacco* and *in vivo* experiments are required in order to confirm the results obtained since this study is limited to *in vitro* rumen fermentation experiments.

CONCLUSION

Cricket contains high CP, EE and chitin contents and treatment with either exoskeleton removal or chemical extraction is effective in decreasing chitin content of the insect. Cricket contains high proportion of glutamic acid and

histidine but low in methionine. Linoleic acid, palmitic acid, oleic acid and stearic acid are major fatty acids present in cricket. Exoskeleton removal or chemical extraction does not alter amino acid and fatty acid profiles of cricket. Cricket is relatively highly digestible and may be used to substitute soybean meal up to 50% in concentrate for ruminant.

SIGNIFICANCE STATEMENT

This study discovers that treatment with either exoskeleton removal or chemical extraction is effective in decreasing chitin content of cricket; this can be beneficial for improving nutritive value and nutrient utilization of cricket to be used as an animal feed. The present study also discovers that cricket meal may be used to substitute soybean meal up to 50% in concentrate for ruminant. This study will help researchers to uncover the critical areas of insect utilization for ruminant feed in which such study presently is still limited.

ACKNOWLEDGMENT

This study was funded by Indonesian Ministry of Research, Technology and Higher Education through "Penelitian Berbasis Kompetensi" research grant, contract number 011/SP2H/LT/DRPM/IV/2017.

REFERENCES

1. Makkar, H.P.S., G. Tran, V. Heuze and P. Ankers, 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.*, 197: 1-33.
2. Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. *Ann. Rev. Entomol.*, 58: 563-583.
3. Sanchez-Muros, M.J., F.G. Barroso and F. Manzano-Agugliaro, 2014. Insect meal as renewable source of food for animal feeding: A review. *J. Clean. Prod.*, 65: 16-27.
4. Jayanegara, A., N. Yantina, B. Novandri and E.B. Laconi, 2017. Evaluation of some insects as potential feed ingredients for ruminants: Chemical composition, *in vitro* rumen fermentation and methane emissions. *J. Indonesian Trop. Anim. Agric.*, (In Press).
5. Wang, D., W.Z. Shao, X.Z. Chuan, Y.B. Yao, H.A. Shi and N.X. Ying, 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian-Aust. J. Anim. Sci.*, 18: 667-670.
6. Chaudhari, S.S., Y. Arakane, C.A. Specht, B. Moussian and D.L. Boyle *et al.*, 2011. Knickkopf protein protects and organizes chitin in the newly synthesized insect exoskeleton. *Proc. Natl. Acad. Sci. USA.*, 108: 17028-17033.

7. Khempaka, S., M. Mochizuki, K. Koh and Y. Karasawa, 2006. Effect of chitin in shrimp meal on growth performance and digestibility in growing broilers. *J. Poult. Sci.*, 43: 339-343.
8. Karlsen, O., H. Amlund, A. Berg and R.E. Olsen, 2017. The effect of dietary chitin on growth and nutrient digestibility in farmed Atlantic cod, Atlantic salmon and Atlantic halibut. *Aquacult. Res.*, 48: 123-133.
9. Agboola, S., D. Ng and D. Mills, 2005. Characterisation and functional properties of Australian rice protein isolates. *J. Cereal Sci.*, 41: 283-290.
10. De Souza, D., A.F. Sbardelotto, D.R. Ziegler, L.D.F. Marczak and I.C. Tessaro, 2016. Characterization of rice starch and protein obtained by a fast alkaline extraction method. *Food Chem.*, 191: 36-44.
11. AOAC., 2005. Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 978-0935584752.
12. Wardeh, M.F., 1981. Models for estimating energy and protein utilization for feeds. Ph.D Thesis, Utah State University Logan.
13. No, H.K., S.P. Meyers and K.S. Lee, 1989. Isolation and characterization of chitin from crawfish shell waste. *J. Agric. Food Chem.*, 37: 575-579.
14. Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.*, 18: 104-111.
15. Jayanegara, A., S.P. Dewi, N. Laylli, E.B. Laconi, Nahrowi and M. Ridla, 2016. Determination of cell wall protein from selected feedstuffs and its relationship with ruminal protein digestibility *in vitro*. *Med. Pet.*, 39: 134-140.
16. Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.*, 28: 7-55.
17. Ogimoto, K. and S. Imai, 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo.
18. Brock, F.M., C.W. Forsberg and J.G. Buchanan-Smith, 1982. Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. *Applied Environ. Microbiol.*, 44: 561-569.
19. Oonincx, D.G.A.B., S. van Broekhoven, A. van Huis and J.J.A. van Loon, 2015. Feed conversion, survival and development and composition of four insect species on diets composed of food by-products. *PLoS ONE*, Vol. 10, No. 2. 10.1371/journal.pone.0144601.
20. Osimani, A., C. Garofalo, V. Milanovic, M. Taccari and F. Cardinali *et al.*, 2017. Insight into the proximate composition and microbial diversity of edible insects marketed in the European Union. *Eur. Food Res. Technol.*, 243: 1157-1171.
21. Wasiko, A., P. Bulak, M. Polak-Berecka, K. Nowak, C. Polakowski and A. Bieganski, 2016. The first report of the physicochemical structure of chitin isolated from *Hermetia illucens*. *Int. J. Biol. Macromol.*, 92: 316-320.
22. Finke, M.D., 2007. Estimate of chitin in raw whole insects. *Zoo Biol.*, 26: 105-115.
23. Zielińska, E., B. Baraniak, M. Karas, K. Rybczynska and A. Jakubczyk, 2015. Selected species of edible insects as a source of nutrient composition. *Food Res. Int.*, 77: 460-466.
24. Ghosh, S., S.M. Lee, C. Jung and V.B. Meyer-Rochow, 2017. Nutritional composition of five commercial edible insects in South Korea. *J. Asia-Pac. Entomol.*, 20: 686-694.
25. Jozefiak, D., A. Jozefiak, B. Kieronczyk, M. Rawski, S. Swiatkiewicz, J. Dlugosz and R.M. Engberg, 2016. Insects-A natural nutrient source for poultry-A review. *Ann. Anim. Sci.*, 16: 297-313.
26. Starcevic, K., A. Gavrilovic, Z. Gottstein and T. Masek, 2017. Influence of substitution of sunflower oil by different oils on the growth, survival rate and fatty acid composition of Jamaican field cricket (*Gryllus assimilis*). *Anim. Feed Sci. Technol.*, 228: 66-71.
27. Jayanegara, A., M. Kreuzer and F. Leiber, 2012. Ruminal disappearance of polyunsaturated fatty acids and appearance of biohydrogenation products when incubating linseed oil with alpine forage plant species *in vitro*. *Livest. Sci.*, 147: 104-112.
28. Van Soest, P.J., 2006. Rice straw, the role of silica and treatments to improve quality. *Anim. Feed Sci. Technol.*, 130: 137-171.
29. Sakurada, M., D.P. Morgavi, Y. Tomita and R. Onodera, 1995. Chitinolytic activity of the anaerobic rumen fungus *Piromyces communis* OTS1. *Curr. Microbiol.*, 31: 206-209.
30. Kopečný, J., B. Hodrova and C.S. Stewart, 1996. The isolation and characterization of a rumen chitinolytic bacterium. *Lett. Applied Microbiol.*, 23: 195-198.
31. Miltko, R., G. Belzecki and T. Michalowski, 2012. Chitinolytic enzymes of the rumen ciliate *Eudiplodinium maggii*. *Folia Microbiol.*, 57: 317-319.
32. Owens, F.N., S. Qi and D.A. Sapienza, 2014. Applied protein nutrition of ruminants-current status and future directions. *Prof. Anim. Scient.*, 30: 150-179.
33. Licitra, G., T.M. Hernandez and P.J. van Soest, 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.*, 57: 347-358.
34. Bettero, V.P., T.A. Del Valle, R.V. Barletta, C.E. de Araújo and E.F. de Jesus *et al.*, 2017. Use of protected fat sources to reduce fatty acid biohydrogenation and improve abomasal flow in dry dairy cows. *Anim. Feed Sci. Technol.*, 224: 30-38.