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Hybrid treatment of black soldier fly larvae (*Hermetia illucens*) as a sustainable and efficient protein source in poultry diets

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Abstract. The purpose of this study was to investigate the potential of BSFL meal protein hydrolysate as a sustainable alternative source of protein in poultry diets. Protein hydrolysis of BSFL meal was carried out by using sodium chloride (NaCl), potassium chloride (KCl), and Thermostable Alkaline Protease enzyme (TAPzyme) under mild conditions: moderate temperature of 50 °C and pH 9. A total of 8 treatments: 10% NaCl (T1); 10% KCl (T2); 10% TAPzyme (T3); 20% TAPzyme (T4); 10% NaCl and 10% TAPzyme (T5); 10% NaCl and 20% TAPzyme (T6); 10% KCl and 10% TAPzyme (T7); and, 10% KCl and 20% TAPzyme (T8) were performed to determine protein hydrolysate concentration (µg/mL), and percentage of protein concentration decreased (%) at the end of this research. The hybrid treatment, i.e., Treatment 6 (10% NaCl and 20% TAPzyme), had the lowest protein concentration and highest protein concentration decreased at 280.782 µg/mL and 49.20%, respectively. This result shows that salt and protease's synergistic effect managed to hydrolysed BSFL protein into smaller peptides efficiently than salt and protease alone.

1. Introduction

Poultry used amino acids derived from dietary protein to perform various functions. The protein requirement for broiler chickens differ in each life cycle stages, i.e., starter stage (22-25%); grower stage (21-23%); and, finisher stage (19-21%) [1]. *Hermetia illucens* black soldier fly larvae (BSFL) is known to convert organic waste into protein and fat-rich biomass that ideal for processing animal feed, biodiesel, and chitin. [2,3]. Rearing BSF was an effective way to dispose of organic waste by turning it into biomass with high protein and fat. [4]. Increasing expensive sources of protein and amino acids used in the



formulation of compound diets for poultry, aquaculture, and livestock, such as fish meal and soybean meal, perhaps are supplemented by BSFL that minimises the potential of food and feed insecurity [5,6]. However, protein hydrolysis is still necessary to maximise protein absorption in animals' bodies and improve growth performance.

Plant hydrolysate protein had become an attractive means of producing high-quality, small, or large peptides for both dietary and physiological or regulatory functions in livestock, poultry, and fish. There were also antimicrobial, antioxidant, antihypertensive, and immunomodulatory activities in some plant or animal peptides [13,14]. Several studies on protein hydrolysate synthesis from different sources had been carried out. Protein hydrolysate from animal processing by-products has been identified as a good source of amino acids for newly weaned animals [15]. A protein hydrolysate from an oilseed flour mixture improved the nutritional profile and protein quality [16]. An enzyme was recently introduced in animal feed formulation to carry out protein hydrolysis and improve animal digestibility. Proteases were added to feed to increase dietary protein hydrolysis and minimise pollutions in the environment [7,8]. Previously, TAPzyme was applicable as a stain remover [9]. TAPzyme seems to be a potential enzyme to hydrolyse protein from BSFL as it belongs to the serine protease group [10]. Serine protease was incorporated into broiler feed formulation and contributed to significant improvements in broiler efficiency as it improves protein and digestibility of energy [11].

The exogenous protease may enhance the nutritional value of diets based on maize/soy and boost the energy and amino acid values for broiler starters [12]. In a previous study, the supplementation of fish protein hydrolysate (FPH) in compound diets for feeding newly hatched sea bass larvae shows the significance of peptides' level and molecular weight distribution [17]. It indicates that FPH may influence sea bass larvae's growth performance and immunological status. Feed supplementation with protease enhanced the nutritional value of feed and thereby increased the potential for digestion. The proper use of protease in an animal feed allowed the animals and their ecosystem to retain optimum nutrients [18]. Recently, dietary supplementation with a mixture of organic acid salt and protease complex in a fish meal improved tilapia nutrients' growth and digestibility compared to those fed with fish meal products [19]. Protease helps in accelerating the growth efficiency and increases protein digestibility in poultry diets [7,18]. Nevertheless, studies based on BSFL hydrolysis have remained very limited. Therefore, this study explores BSFL protein hydrolysis's ability as an alternate protein source in poultry feed by consecutively combining protein denaturation and protein hydrolysis.

2. Materials and Methods

2.1. Protein hydrolysis of BSFL

Protein hydrolysis of BSFL meal was carried out using TAPzyme with a specific activity of 2205 U/mg [20]. The dried BSFL meal used in this study was obtained from a local BSFL producer, Ori Biotechnology Sdn. Bhd. (Selangor, Malaysia). A total of 5 grams of BSFL meal was used in each treatments, i.e., 10% NaCl (T1); 10% KCl (T2); 10% TAPzyme (T3); 20% TAPzyme (T4); 10% NaCl and 10% TAPzyme (T5); 10% NaCl and 20% TAPzyme (T6); 10% KCl and 10% TAPzyme (T7); and, 10% KCl and 20% TAPzyme (T8). The BSFL meal in T1, T2, T5, T6, T7, and T8 were dissolved in distilled water, while T3 and T4 were dissolved in Tris-HCl with pH 9 according to their respective treatments. The hydrolysis reaction was carried out in an incubator shaker at 25 °C (T1, T2, T5, T6, T7, and T8) for 48 hours and 50 °C (T3, and T4) for 24 hours, with pH 9 and a rotation speed of 150 rpm [13]. The different temperatures were applied depending on the stability of salts and TAPzyme. The hydrolysis reaction of protease was terminated by heating the sample and separating the supernatant and pellet by centrifugation [21].

2.2. Determination of protein concentration

Using the Bradford method, the protein concentrations of T1, T2, T3, T4, T5, T6, T7, and T8 were determined [22]. At a wavelength of 595 nm, the absorbance of the samples was measured using a UV-Vis spectrophotometer. Based on a standard bovine serum albumin (BSA) curve, protein concentration was determined.

2.3. Determination of the percentage of protein concentration decreased

The protein concentrations of untreated BSFL (*a*) and protein concentrations of treated BSFL (*b*) were determined to calculate the percentage of protein concentration decreased, as shown in equation Eq. (1).

$$\text{Protein concentration decreased \%} = \frac{a-b}{a} \times 100\% \quad (1)$$

2.4. Statistical analysis

The statistical analysis was conducted by SPSS 23 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate the effects of the protein concentration and percentage of protein concentrations decreased, followed by Tukey's HSD (real significant differences) for post-hoc testing to compare the significance (*p*) between the means of different BSFL meal treatments. The significant difference between the mean and standard error of the mean was declared at (*p* < 0.05) (SEM).

3. Results and Discussion

Table 1 summarises the effect of the different treatments on BSFL on protein concentration and the percentage of protein concentration decreased. There were statistically significant differences between the treatments as a whole. The treated BSFL using 10% NaCl in Treatment 2 showed the highest protein concentration ($534.500 \pm 3.490 \mu\text{g/mL}$). On the other hand, the treated BSFL in Treatment 6 had the lowest protein concentration ($280.782 \pm 3.236 \mu\text{g/mL}$) using 10% NaCl and 20% TAPzyme. Treatment 2 was significantly different from all the treatments (*p* < 0.05) while Treatment 6 did not differ (*p* > 0.05) to Treatment 8. The different percentages of TAPzyme were used in Treatment 3 and Treatment 4 had almost similar in protein concentration of BSFL meal ($352.705 \pm 6.09 \mu\text{g/mL}$ and $361.167 \pm 3.541 \mu\text{g/mL}$) and did not significantly different to each other (*p* = 0.926). The combination of salts and TAPzyme was known as hybrid treatments of BSFL (Treatment 5, 6, 7, and 8). A significant difference only showed in the same combination of salt and TAPzyme (*p* = 0.961 and *p* = 0.906, respectively).

Table 1. Effect of the different treatments on BSFL meal

Treatment of BSFL meal	Protein concentration of treated BSFL meal ($\mu\text{g/mL}$)	Protein concentration decreased (%)	<i>p</i> -value	
			$\mu\text{g/mL}$	%
T1	495.397 ± 2.741^d	10.36 ± 0.84^b	0.000	0.001
T2	$534.500 \pm 3.490^{e,f}$	3.29 ± 0.30^a	0.000	0.001
T3	352.705 ± 6.091^c	32.08 ± 1.92^c	0.926	0.958
T4	361.167 ± 3.541^c	30.46 ± 1.53^c	0.926	0.958
T5	321.423 ± 1.175^b	41.84 ± 0.37^d	0.961	0.985
T6	280.782 ± 3.236^a	49.20 ± 0.34^f	0.906	0.960
T7	313.860 ± 6.867^b	$43.22 \pm 0.98^{d,e}$	0.961	0.985, 0.078
T8	289.628 ± 3.880^a	$47.59 \pm 0.79^{e,f}$	0.906	0.078, 0.960

Means with different superscript letters (a-f) within the same column differ significantly (Turkey test, *p* < 0.05), 10% NaCl (T1); 10% KCl (T2); 10% TAPzyme (T3); 20% TAPzyme (T4); 10% NaCl and 10% TAPzyme (T5); 10% NaCl and 20% TAPzyme (T6); 10% KCl and 10% TAPzyme (T7); and, 10% KCl and 20% TAPzyme (T8)

Meanwhile, the percentage of protein decreased was evaluated, as shown in Table 1 and Figure 1. The hybrid treatments of BSFL meal (Treatment 5, 6, 7, and 8) reduced more in protein concentration, ranging from 40 to 50%. The combination of 10% salts and 10% TAPzyme (Treatment 5 and 7) demonstrated increases in the percentage of protein concentration decreased ($41.84 \pm 0.37\%$ and $43.22 \pm 0.98\%$) but did not significantly different to each other ($p = 0.985$). Treatments 6 and 8 (10% salts and 20% TAPzyme) had almost similar percentage of protein decreased ($49.20 \pm 0.34\%$ and $47.59 \pm 0.79\%$) and did not significantly different to each other ($p = 0.960$). Treatment 6 demonstrated as the best among hybrid treatments due to the higher percentage of protein concentration decreased. Treatment 2 (10% KCl) showed the lowest percentage of protein decreased ($3.29 \pm 0.30\%$) but significantly difference ($p < 0.05$) to all treatments. TAPzyme that were used alone in Treatments 3 and 4 did not show any significant difference to each other ($p > 0.05$) but significantly different to other treatments with an almost similar percentage of protein concentration decreased ($32.08 \pm 1.92\%$ and $30.46 \pm 1.53\%$, respectively). The protein concentration of the treated BSFL meal was ranged from 280 to 534 $\mu\text{g/mL}$ while their percentage of protein decreased, 3 to 50%.

As referred to Figure 1, Treatment 1 showed a higher percentage of protein concentration decreased (495.397 $\mu\text{g/mL}$; 10.36%) than Treatment 2 (534.500 $\mu\text{g/mL}$; 3.29%) in salt treatment. At first, the neutral salts were tested (Treatments 1 and 2) on BSFL. It was found that NaCl was able to denature BSL protein better than KCl. Due to its larger size, KCl denatured less BSFL protein than NaCl, possibly unable to partially unfold quaternary protein structure [23]. This phenomenon was observed as Na and K atoms are in the same group in the Periodic Table, which raises atomic radius as it moves downwards. Previous studies have shown that Na^+ has a much greater affinity for side-chain carboxylates and backbone carbonyls than K^+ , thus weakening salt bridges and hydrogen bonds of the secondary protein structure [24]. A previous study also argued that Na^+ would bind to protein surfaces containing COO^- groups better than K^+ . [25].

Meanwhile, Treatments 3 and 4 were not significantly different from each other except for the other treatments. In Treatment 3, enzymatic hydrolysis showed a significantly different protein concentration when employing 10 % TAPzyme (352.705 $\mu\text{g/mL}$; 32.08%). Double the TAPzyme amount (Treatment 4) did not hydrolyse the protein concentration any further (361.167 $\mu\text{g/mL}$; 30.46%). This result indicates that TAPzyme efficiency depends on its concentrations and enzymatic hydrolysis conditions. These findings are consistent with the enzymatic hydrolysis performed by previous studies [13,26]. The key benefits of enzymatic protein hydrolysis were preferred, i.e., mild hydrolysis conditions and minimal loss of amino acids; proteases are more accurate and efficient in regulating the degree of peptide bond hydrolysis; and after hydrolysis, small amounts of enzymes can be easily deactivated to enable protein hydrolysate isolation [14].

Treatments 5, 6, 7 and 8 were recognised as hybrid treatment had a higher percentage of protein concentration decreased than salts and TAPzyme treatment alone. Interestingly, better protein hydrolysis was demonstrated when 10% salts and 10% TAPzyme (Treatments 5 and 7) were combined. This observation shows that the combination of either NaCl or KCl and TAPzyme caused BSFL protein to hydrolyse efficiently. The monovalent Na^+ and K^+ have been reported to control enzymatic activity and assist in folding the enzyme [27]. As expected, increasing twice the amount of TAPzyme in the hybrid treatment (Treatments 6 and 8) slightly reduced the protein concentration. This synergistic effect has improved growth, nutrient retention, and digestibility in animal feed production, as reported in previous studies [19,28].

However, different factors, including enzyme concentration, pH, and hydrolysis time, need to be optimised to evaluate high protein concentration [29]. This technique takes time, but it is noteworthy that smaller peptides could be produced by protein hydrolysis. These peptides have beneficial effects on the

improvement of intestinal morphology, function and resistance of animals, including swine and poultry, to infectious diseases, thereby improving their health, well-being, growth and feeding efficiency [14].

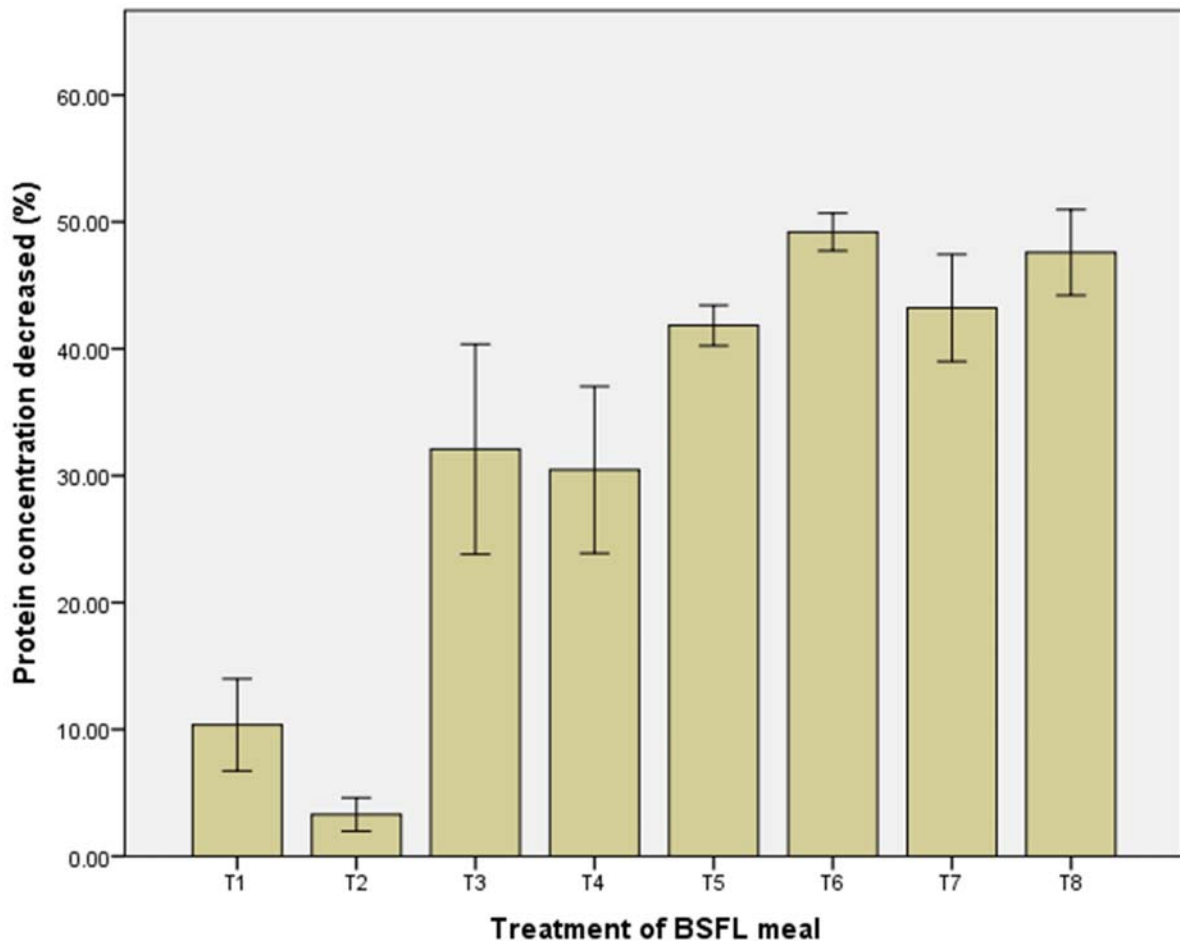


Figure 1. Percentage of protein concentration decreased on treated BSFL meal under different treatments.

Note: Points and different superscript letters (a-f) show mean for triplicate samples, and error bars represent standard deviations with significant difference ($p < 0.05$).

4. Conclusion

In conclusion, a substantial amount of BSFL protein has been hydrolysed due to the synergistic effect between salt and protease. In the future, optimising treatment conditions such as pH, hydrolysis time, hydrolysis temperature, salts, and protease concentration should and would be studied. The protein hydrolysate from treated BSFL could be included in the feed formulation as an efficient protein source in poultry diets. This practice could enhance digestibility efficiency and growth performance.

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References

- [1] NRC 1994 *Nutrient Requirements of Poultry: Ninth Revised Edition*, 1994
- [2] Putra S N K M, Ishak N H and Sarbon N M 2018 Preparation and characterisation of physicochemical properties of golden apple snail (*Pomacea canaliculata*) protein hydrolysate as affected by different proteases *Biocatal. Agric. Biotechnol.* **13** 123–28
- [3] Barroso F G, de Haro C, Sánchez-Muros M J, Venegas E, Martínez-Sánchez A and Pérez-Bañón C 2014 The potential of various insect species for use as food for fish *Aquaculture* **422–423** 193–201
- [4] Huis A Van and Itterbeeck J Van 2013 Edible insects: Future prospects for food and feed security *Food Agric. Organ. United Nations* **171** 2–20
- [5] Liu X, Chen X, Wang H, Yang Q, Ur Rehman K, Li W, Cai M, Li Q, Mazza L, Zhang J, Yu Z and Zheng L 2017 Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly *PLoS One* **12**(8): e0182601
- [6] Abd El-Hack M E, Shafi M E, Alghamdi W Y, Abdelnour S A, Shehata A M, Noreldin A E, Ashour E A, Swelum A A, Al-sagan A A, Alkhateeb M, Taha A E, Abdel-moneim A M E, Tufarelli V and Ragni M 2020 Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: A comprehensive review *Agric.* **10** 1–31
- [7] Oxenboll K M, Pontoppidan K and Fru-Nji F 2011 Use of a protease in poultry feed offers promising environmental benefits *Int. J. Poult. Sci.* **10** 842–48
- [8] Ibrahim N A, Azid S Z A and Al-Amsyar S M 2019 Eco-friendly enzymatic dehairing on animal hides *AIP Conf. Proc.* **2068**
- [9] Ibrahim N A, Ibrahim N, Lizawardi N S R, Fauzi N F I and Al-Amsyar S M 2020 Production and application of thermostable protease 50a as liquid protein stain remover *IOP Conf. Ser. Earth Environ. Sci.* **596**
- [10] Mohd Zuki N A, Mohamad Kamal S S, Nik Qalbee N K, Ibrahim N A, Mat K, Rusli N D, Harun H C, Mahmud M, Samat N and Al-Amsyar S M 2020 Optimisation of Protease-Treated Black Soldier Fly Larvae (BSFL) using Response Surface Methodology (RSM) for Broiler Feed *IOP Conf. Ser. Earth Environ. Sci.* **596** 012086
- [11] Fru-nji F, Klunter A, Fischer M and Pontoppidan K 2011 A feed serine protease improves broiler performance and increases protein and energy digestibility *J. Poult. Sci.* **48** 239–46
- [12] Taylor P, Cowieson A J and Ravindran V 2008 Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids *British Poultry Science* **49** 37–41
- [13] Firmansyah M and Abduh M Y 2019 Production of protein hydrolysate containing antioxidant activity from *Hermetia illucens* *Heliyon* **5**(6): e02005
- [14] Hou Y, Wu Z, Dai Z, Wang G and Wu G 2017 Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance *J. Anim. Sci. Biotechnol.* **8** 1–13
- [15] Martínez-Alvarez O, Chamorro S and Brenes A 2015 Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review *Food Res. Int.* **73** 204–12
- [16] Radha C, Ramesh Kumar P and Prakash V 2008 Preparation and characterisation of a protein hydrolysate from an oilseed flour mixture *Food Chem.* **106** 1166–74

- [17] Kotzamanis Y P, Gisbert E, Gatesoupe F J, Zambonino Infante J and Cahu C 2007 Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **147** 205–14
- [18] Novelli P K, Barros M M, Pezzato L E, de Araujo E P, de Mattos Botelho R and Fleuri L F 2017 Enzymes produced by agro-industrial co-products enhance digestible values for Nile tilapia (*Oreochromis niloticus*): A significant animal feeding alternative *Aquaculture* **481** 1–7
- [19] Huan D, Li X, Chowdhury M A K, Yang H, Liang G and Leng X 2018 Organic acid salts, protease and their combination in fish meal-free diets improved growth, nutrient retention and digestibility of tilapia (*Oreochromis niloticus* × *O. aureus*) *Aquac. Nutr.* **24** 1813–21
- [20] Ibrahim Noor Azlina N Y 2013 Thermostable Alkaline Serine Protease *African J. Biotechnol.* **12** 844–56
- [21] Caligiani A, Marseglia A, Leni G, Maistrello L, Dossena A and Sforza S 2017 Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin *Food Res. Int.* **105** 812–20
- [22] Bradford M M 1976 A Rapid and Sensitive Method for the Quantitation Microgram Quantities of Protein Utilising the Principle of Protein-Dye Binding **254** 248–54
- [23] Nightingale E R 1959 Phenomenological theory of ion solvation. Effective radii of hydrated ions *J. Phys. Chem.* **63** 1381–87
- [24] Vrbka L, Vondrášek J, Jagoda-Cwiklik B, Vácha R and Jungwirth P 2006 Quantification and rationalisation of the higher affinity of sodium over potassium to protein surfaces *Proc. Natl. Acad. Sci. U. S. A.* **103** 15440–44
- [25] Collins K D 1997 Charge density-dependent strength of hydration and biological structure *Biophys. J.* **72** 65–76
- [26] Rafi N M, Halim N R A, Amin A M and Sarbon N M 2015 Response surface optimisation of enzymatic hydrolysis conditions of lead tree (*Leucaena leucocephala*) seed hydrolysate *Int. Food Res. J.* **22** 1015–23
- [27] Sinha R and Khare S K 2013 Characterisation of detergent compatible protease of a halophilic *Bacillus* sp. EMB9: Differential role of metal ions in stability and activity *Bioresour. Technol.* **145** 357–61
- [28] Khodambashi Emami N, Zafari Naeni S and Ruiz-Feria C A 2013 Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids *Livest. Sci.* **157** 506–13
- [29] Pasupuleti V K and Demain A L 2010 Protein hydrolysates in biotechnology 1-229