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The Effect of Chitinase Enzyme on Black Soldier Fly Larvae Meal to Optimise Its Use as a Sustainable Protein Source in Ruminants: An In Vitro Study

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ABSTRACT

Chitin, a major component of the insect exoskeleton, serves as a source of dietary fibre but also acts as an anti-nutritional factor, limiting the bioavailability of protein and minerals in black soldier fly (BSF) larvae. Chitinase enzymes, typically secreted by chitinolytic bacteria such as Actinomycetes, can hydrolyse chitin into simpler, digestible forms. This study aimed to evaluate the nutrient profile and in vitro digestibility of BSF larval meal hydrolysed with chitinase enzyme derived from *Streptomyces* BLH 5-14. This study was set up with the following treatments: soybean meal; BSF meal without chitinase (EC0); and BSF larval meal treated with chitinase at levels of 2, 4, and 6 units per 100 grams (EC2, EC4, and EC6), each replicated six times in a randomised complete design. The results indicated that chitinase treatment reduced chitin and acid detergent fibre (ADF) content, while crude protein and neutral detergent fibre (NDF) showed varying trends. ADF content in the EC2 treatment declined from 25.51% to 17.28%, and chitin content dropped from 8.47% to 4.59%. Although bacterial rumen populations rose when the dose was increased to 6 U/100 g, the chitinase enzyme administration up to the level 6 U/100 g to BSF larval meal was insufficient to improve overall digestibility in both the rumen and post-rumen phases.

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Protein digestibility in the rumen and post-rumen was numerically increased by 12.30% and 5.51%, respectively, in comparison to soybean meal. In conclusion, the application of chitinase enzyme up to level 6 U/100 g to BSF larval meal was inadequate to enhance the overall digestibility in both the rumen and post-rumen stages.

Keywords: Black soldier fly meal, chitinase, digestibility, protein, *Streptomyces*

INTRODUCTION

Insect meals have recently gained notoriety as a sustainable source of protein for animal feed (Jayanegara et al. 2017a; Linh et al. 2024; Vargas-Serna et al. 2025; Zou et al. 2024). Supported by its simple production system and fast growth rate, Black Soldier Flies (*Hermetia illucens*) have been especially noted for their capacity to effectively transform low-quality organic material into high-quality nutrients (Liland et al., 2017; Lu et al., 2022; Wang and Shelomi, 2017). Food waste, farm waste, animal manure, and other organic wastes can all be broken down by larvae of this species to provide high-protein biomass (Meneguz et al., 2018; Msangi et al., 2022; Siddiqui et al., 2022). It has been explained that the addition of BSF larval meal to animal feed reduces reliance on unsustainable fishmeal in addition to promoting waste utilisation, and thus supporting circular economy principles (Linh et al., 2024; Vargas-Serna et al., 2025). BSF larval meal thus offers a sustainable and promising substitute for conventional protein sources, with a lower environmental footprint (van Huis, 2022; Veldkamp et al., 2022).

Nutritionally, BSF larval meal contains 45.20-58.10% crude protein (CP), 19-20.70% ether extract (EE), 7.44-9.85% acid detergent fibre (ADF), 12.40-32.70% neutral detergent fibre (NDF), 1.34-3.65% calcium, 0.85-1.11% phosphorus, and 5,325-5,159 kcal kg^{-1} dry matter (DM) gross energy (Matin et al., 2021). The true protein of BSF is, nevertheless, overestimated due to the nonprotein nitrogen from compounds like chitin, which binds protein. Chitin is present in BSF throughout its life cycle, and its concentrations vary in the different stages: larvae (7.8-9.5%), prepupae (9.1-10.9%), pupae (10.3-10.7%), adult flies (23.7-31.1%), shed exoskeletons (22.4-23.8%), and cocoons (5.6-8.4%) (Soetemans et al., 2020). Chitin, which is a N-acetyl-β-D-glucosamine polymer, provides the insect exoskeleton with its rigidity. However, its high content in BSF larvae meal can limit its nutritional value by reducing *in vitro* digestibility and volatile fatty acid production (Jayanegara et al., 2017a; 2017b). In order to optimise the protein value of BSF larval meal, effective treatments are needed to eliminate the negative effect of chitin as well as mto improve digestibility. Arbia et al. (2013) reported that chitin extraction typically involves two key steps, demineralisation and deproteinization, that can be carried out by chemical or biological means. In addition, exogenous protease supplementation has been shown to significantly improve protein digestibility and growth

performance in fish, showing its potential for the optimisation of BSF larval meal as a sustainable protein supplement for animal diets (Haider et al., 2024).

Chitinases have been extensively studied for their ability to hydrolyse colloidal chitin and chitin from black soldier fly larvae into water-soluble N-acetylchitooligosaccharides. Gebele et al. (2024) reported that when 1% endochitinase Chit36-TA from *Trichoderma asperellum* was applied for 24 hours, it led to a hydrolysis degree of 32% for colloidal shrimp chitin and 12% for insect larvae chitin. In addition to its application, chitinase has also been used as a feed additive in growing pigs (Yang et al., 2025) and in *Nile tilapia* (Agbohessou et al., 2024), and it has been shown to positively affect feed efficiency, protein digestibility, and increase beneficial bacteria in the intestines. Moreover, the addition of persimmon peel, which had strong chitinase activity (approximately 50% activity at pH 3.0 and pH 8.0 conditions) to layer diets improved overall *in vitro* digestibility, including protein digestibility, at levels less than 6% (Sangkaew & Koh, 2021). However, previous studies primarily focused on its direct supplementation in monogastric and aquaculture. To the best of our knowledge, this is the first study to conduct the use of chitinase from *Streptomyces* BLH 5-14 to hydrolyse chitin of black soldier flies (BSF) and assess its digestibility in a ruminal *in vitro* assay. We hypothesised that the chitinase enzyme would improve the availability of nutrients, especially crude protein, and subsequently increase ruminal digestibility. Thus, this study aimed to evaluate the nutrient profile and *in vitro* digestibility of BSF larval meal that was hydrolysed with a chitinase derived from *Streptomyces*.

MATERIAL AND METHODS

Ethical Approval

This study has obtained ethical clearance approval in the chemical field from the National Research and Innovation Agency (Approval Number: 006/KE.04/SK/12/2022).

Feedstuffs and Experimental Design

The black soldier fly larval meal and soybean meal are obtained from a poultry shop located in Bandung, West Java, Indonesia. These feedstuffs were ground to pass through a 1.0 mm aperture for further processing. The nutrient composition of these feedstuffs used in this *in vitro* study is presented in Table 1.

The experiment was based on a completely randomised design using five treatments with six replications for each treatment. The treatments consisted of

SBM : soybean meal
EC0 : BSF larval meal (without chitinase)

EC2 : BSF larval meal incubated with chitinase at levels of 2 U/100 g
EC4 : BSF larval meal incubated with chitinase at levels of 4 U/100 g
EC6 : BSF larval meal incubated with chitinase at levels of 6 U/100 g

Table 1
Nutrient content of soybean meal and black soldier fly (BSF) larval meal

Nutrient Contents (% DM)	Feedstuff	
	Soybean Meal	BSF-Larval Meal
Dry Matter	91.07	96.66
Organic Matter	86.77	85.31
Ash	13.23	14.69
Crude Protein	28.39	33.16
Ether Extract	3.58	29.15
Crude Fibre	13.82	12.15
Nitrogen Free Extract (NFE) [a]	40.97	10.85
Total Digestibility Nutrient (TDN)	66.75	84.09
Neutral Detergent Fibre (NDF)	43.41	33.45
Acid Detergent Fibre (ADF)	13.24	25.51
Hemicellulose	30.17	7.93

Note. [a]NFE = 100 − (Ash % + Crude protein % + Crude fat % + Crude fibre %)

Production of Chitinase

The isolate used to produce chitinase is Streptomyces BLH 5-14 from the Indonesian cultured collection (InaCC), obtained from the Sulawesi marines, Indonesia. The pre-cultured isolate of Streptomyces BLH 5-14 is prepared in a baffled Erlenmeyer flask with a spiral, consisting of 0.4% yeast extract, 1% malt extract, 0.4% glucose (ISP-2) medium, and supplemented with 3% artificial sea water (ASW). The medium is shaken in an incubator shaker for three days at 28°C and 190 rpm.

Chitinase was then produced using a medium composition derived from Narayana & Vijayalakshmi (2009), which is composed of chitin-yeast extract-salts (CYS) medium (yeast extract, 0.5%; $MgSO_4.7H_2O$, 1.0%; K_2HPO_4, 2.0%); $FeSO_4.7H_2O$, 0.1%; and colloidal chitin, 5.0%; supplemented with 3% artificial sea water (ASW). The final pH of the medium was adjusted to 7. Colloidal chitin preparation used the method by Sandhya et al. (2004). Production was carried out for seven days in an incubator shaker at 28 °C and 190 rpm. The enzyme was harvested using a centrifuge at 12,000 rpm and 4 °C for 20 minutes, then stored in a refrigerator at 4 °C.

Chitinase Activity Assay

Chitinase activity was measured as described by Akeed et al. (2020). Each reaction contained 0.4 mL of the enzyme solution (diluted 5 times with acetate buffer at pH 5) and 0.5 mL of 1 % (w/v) colloidal chitin in 0.1 M sodium acetate buffer (pH 5.0). Subsequently, the mixture was incubated at 60 °C for 30 minutes, and then the reaction was terminated by 1mL DNS (NaOH 10 g/L, dinitrosalicylic acid $C_7H_4N_2O_7$ 10 g/L, phenol C_6H_6O 2 g/L, Na_2SO_3 0.05g/100mL and sodium potassium tartrate $C_4H_4KNaO_6.4H_2O$ 20 g/100mL when using). Each mixture was immediately incubated at 100 °C for 10 min, cooled in an ice bath, and centrifuged at 8,000×g for 5 min at 4°C. The supernatant is measured using a visible spectrophotometer at a wavelength of 540 nm. A standard curve was plotted using N-acetyl glucosamine (NAG, Sigma). One unit of chitinase activity was defined as the amount of enzyme that produced 1 μmol of GlcNAc per min under reaction conditions.

Incubation of BSF Larval Meal with Chitinase

The hydrolysis method was carried out following Wang et al. (2001), who conducted the enzyme xylanase on the feed, with a slight modification in the hydrolysis temperature. The main enzyme activity detected was chitinase (0.1 units/ml). The BSF larval meal was sprayed using a single-nozzle spray bottle. A total of 100 grams of BSF larval meal was sprayed with a medium solution of enzymes, with volumes of 20, 60, and 60 ml for treatments of 2, 4, and 6 U/100 g, respectively. Subsequently, 40 ml of distilled water was added to the BSF larval meal treated with 2 U/100 grams, and 20 ml of distilled water was added to the BSF larval meal treated with 4 U/100 grams. The treated feeds were then incubated at 29°C for 24 hours, followed by drying for 2 days at 60°C. Prior to being used in an *in vitro* study, the treated feeds were kept in the freezer.

In vitro Procedure

The *in vitro* fermentation was conducted using a modified version of the technique by Goering and van Soest (1970). A 0.5 g sample, 40 mL of McDougall buffer, and 10 mL of cattle rumen fluid were added to an anaerobic bottle (100 mL). The bottles were placed in a shaker water bath and incubated for 24 hours at 39 °C. After 24 hours, the pH was measured, the supernatant was collected, and the residue was dried at 60°C for 48 hours. The residue was collected to analyse nitrogen. The supernatant was analysed for NH_3 (Conway & O'Malley, 1942) and the total population of bacteria and protozoa. For determining in vitro pepsin digestibility, a modified version of the procedure by Palmer and Jones (2000) was followed. After 24 hours of anaerobic incubation, the residues in the bottles were washed with distilled water on a vortex mixer, then centrifuged at 2,500 rpm for 10 minutes. The supernatant was discarded, and the procedure was repeated before adding 40 ml of acid

pepsin, which was prepared by dissolving 2 g of pepsin (1:10,000) in 1 L of 0.1 M HCl. Each tube was then thoroughly vortexed and incubated at 39 °C for 24 hours. The tubes were centrifuged, the remaining supernatant was discarded, and the residues were dried at 60 °C for 48 h. Residues were analysed for nitrogen.

Chemical Analyses

AOAC (2002) procedures were carried out to analyse dry matter, ash, ether extract, crude protein, and crude fibre of feed samples, while the determination of neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents followed the methods described by van Soest et al. (1991). Chitin content was determined using the procedure by Black and Schwartz (Black & Schwartz, 1950; Borić et al., 2020). Total digestible nutrient (TDN) content was estimated in accordance with Hartadi et al. (1980).

Statistical Analysis

Data were analysed by ANOVA, and differences were declared significant at $p < 0.05$, tested by Duncan's Multiple Range test, with SAS software version 9.0. Correlation and principal component analyses among parameters were conducted using RStudio software (version 2024, Post Software, PBC).

RESULTS AND DISCUSSION

Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and chitin content of BSF larval meal hydrolysed using different levels of chitinase enzyme are presented in Table 2. Protein content of the non-treated BSF larval meal of the present study was 33.16%, which was similar to the results of Renna et al. (2022) and Pedrazzani et al. (2024). However, the protein content observed was less than in other studies, in which the protein had a value greater than 44% (Jayanegara et al. 2017a; Matin et al. 2021). Moreover, the chitin content was 8.47%, as reported in Soetemans et al. (2020) and Renna et al. (2022). Hydrolysis of BSF larval meal by chitinase 2 U/100 g in this study was the optimal treatment to alter its nutrient content, particularly by reducing ADF and chitin content ($p < 0.0001$). ADF content decreased from 25.51% to 17.28%, and chitin content decreased from 8.47% to 4.59% at this level of enzyme. However, the breakdown of ADF and chitin was not as effectively enhanced by raising the enzyme concentration to 4 and 6 U/100 g. Instead, the crude protein content reduced slightly at these concentrations, showing that chitinase degrades structural carbohydrates and chitin effectively at a level of optimal enzyme level, while excessive application of enzymes could not provide any further advantage.

These findings suggest that chitinase treatment can modify the nutritional profile of BSF larval meal, specifically by reducing chitin and fibre content while potentially enhancing

crude protein to a certain extent. The incomplete hydrolysis of chitin could be attributed to the complex matrix of proteins, chitin, and minerals in BSF larval meal, which may hinder the enzyme's access to chitin. Furthermore, it seems that a one-stage biological procedure, such as hydrolysis with enzymes (Pedrazzani et al., 2024) or fermentation with chitinolytic bacteria (Mulyono et al., 2019), was not sufficient to fully break down chitin complexes. A multi-stage approach combining enzymatic hydrolysis with other methods may be required for more complete chitin breakdown. As reported by Pedrazzani et al. (2024), there is an enhanced ability to remove proteins directly from biomass for the extraction of chitin using enzymatic, chemical, and mechanochemical milling, ultrasonication methods. Pedrazzani et al. (2024) demonstrated that the total protein retained in the BSF larval chitin extract using enzymatic, chemical-enzymatic, and mechanochemical milling, ultrasonication methods was 46.7%, 15.5%, 15.7%, and 13.0%, respectively.

The changes in fermentative characteristics in the rumen of BSF larval meal with the addition of chitinase are presented in Table 3. Significant differences in rumen digestibility of dry matter, total gas production, and total populations of bacteria and protozoa ($p<0.01$), and rumen digestibility of protein ($p<0.05$) were observed among the treatments. Our study has shown that the use of 6 U/100 g of chitinase (EC6) was insufficient to enhance overall digestibility in both the rumen and post-rumen phases, despite the fact that bacterial rumen populations increased when the dose was increased to 6 U/100 g. Digestibility of protein in the rumen and post-rumen for the BSF larval meal treatment at 6 U/100 g chitinase (EC6) was higher by 12.30% and 5.51%, respectively, compared to soybean meal. This suggests that although chitinase hydrolysed the nutrient profile of the BSF larval meal, digestibility was not always improved beyond a certain point. This could be due to a number of factors, such as enzyme saturation, the structural resistance of the remaining chitin and fibre, or possible interactions between the hydrolysed components and microbial digestion.

Table 2
Nutrient content of BSF larval meal incubated with chitinase enzyme

Nutrient Contents (% DM)	**Incubate BSF-larval Meal with Chitinase Enzyme (U/100 g)**				**SEM**	**p-value**
	EC0	**EC2**	**EC4**	**EC6**		
Crude Protein	33.17b	34.05a	32.36d	32.62c	0.20	<.0001
Neutral Detergent Fibre (NDF)	33.45	29.71	35.83	29.27	1.26	0.2021
Acid Detergent Fibre (ADF)	25.51a	17.28d	21.92b	19.63c	0.94	<.0001
Hemicellulose	7.93	12.43	13.91	9.64	1.14	0.2565
Chitin	8.47a	4.59b	5.04b	7.65a	0.51	<.0001

Note. Means in the same row with different letters differ significantly; SEM: standard error of the means; EC0 - BSF larval meal; EC2 - BSF larval meal incubated with chitinase enzyme (2 U/100 g); EC4 - BSF larval meal incubated with chitinase enzyme (4 U/100 g); EC6 - BSF larval meal incubated with chitinase enzyme (6 U/100 g)

Table 3
In vitro *rumen fermentation and post-ruminal digestibility of soybean meal and hydrolysed BSF larval meal*

Parameters	Treatments					SEM	p-value
	SBM	EC0	EC2	EC4	EC6		
Rumen Digestibility 24h							
Dry Matter (IVDMD24), %	56.97a	42.85b	47.19b	33.48c	47.52b	1.92	0.0005
Protein (IVCPD24), %	49.83ab	54.70a	58.69a	41.54ab	55.96a	1.77	0.0105
Pepsin Digestibility							
Dry matter (IVDMD48), %	71.74	70.17	68.78	68.76	72.71	1.72	0.94
Protein (IVCPD48), %	77.67	77.78	78.72	75.73	81.95	0.85	0.2202
NH_3 (mM)	19.20	31.67	24.00	28.40	25.50	3.12	0.7676
pH	7.00	6.92	6.92	7.00	7.00	0.02	0.56
Gas Production 24h, mL/200 mg DM	32.53a	11.49bc	10.27c	12.13b	10.88bc	1.14	<.0001
a	0.31	0.02	0.14	0.00	0.00	0.06	0.382
b	33.14a	15.57b	13.98c	14.79bc	14.53bc	1.04	<.0001
c	0.14a	0.07b	0.08b	0.09b	0.07b	0.01	<.0001
a + b	33.45a	15.59b	14.12c	14.79bc	14.53bc	1.05	<.0001
Total Population of Bacteria ($\times 10^9$cfu/mL)	3.54b	2.34c	2.55c	3.37b	4.30a	0.15	<.0001
Total Population of Protozoa ($\times 10^6$ cells/mL)	1.57e	8.95a	6.21b	4.61c	3.43d	0.48	<.0001

Note. Means in the same row with different letters differ significantly; SEM: standard error of the means. a - initial gas production (mL/200 mg DM), b - gas production during incubation (mL/200 mg DM); a + b - potential gas production (mL/200 mg DM); c - fractional rate of gas production per hour; SBM: Soybean meal; EC0 - BSF larval meal; EC2 - BSF larval meal incubated with chitinase enzyme (2 U/100 g); EC4 - BSF larval meal incubated with chitinase enzyme (4 U/100 g); EC6 - BSF larval meal incubated with chitinase enzyme (6 U/100 g)

On the other hand, BSF meal treatment had a lower rate of DM digestibility in the rumen than soybean meal (SBM). The lower dry matter digestibility of BSF larval meal might be attributed to its high ether extract content (approximately 29%), which is known to exert a toxic effect on cellulolytic bacteria, thereby reducing fibre digestibility (Behan et al., 2019; Lima et al., 2017). Additionally, the presence of chitin, which was not optimally hydrolysed by chitinase, may have further contributed to the reduced digestibility. Previous studies evaluating the addition of BSF larval meal instead of SBM to ruminant diets have shown that BSF larval meal generally has lower nutritional value due to reductions in IVDMD caused by their chitin and high fat content (Jayanegara et al. 2017b). However, inclusion of defatted BSF larval meal substitution up to 40% of SBM (6.4% in TMR) exhibited higher IVDMD and IVNDFD levels (Kahraman et al., 2023). Notably, in this study, the post-rumen digestibility of BSF larval meal treated with chitinase was similar to that of SBM.

In the present study, ruminal ammonia (NH_3) concentrations in vitro ranged from 19.20 to 31.67 mM, representing a state that is conducive to the development of robust rumen microbes. This range is broader than the 5 to 11 mM range established by Schwab and Broderick (2017) as being most optimal to sustaining microbial nitrogen flow at its optimal level, whose optimal ammonia content is determined by diet and fermentative dynamics. The increased NH_3 concentration in the presence of added black soldier fly meal might be attributed to the increased degradability of its crude protein in the rumen. The result coincided with that obtained by Renna et al. (2023), where BSF larval meal (2.49 mmol/g DM) showed higher ammonia production than SBM and rapeseed meal, 4.48 and 3.06 mmol/g DM, respectively. In contrast to previous research, wherein it was implied that the incorporation of 40% (Kahraman et al., 2023) and 50% (Jayanegara et al., 2017b) BSF larval meal into total mixed rations led to the reduction in NH_3-N levels, in the current study, no significant difference ($p>0.05$) was observed. The difference is due to particular protein fractions present in BSF larval meal, which could have varied degradation kinetics compared to soybean meal. Kahraman et al. (2023) explained that soybean meal's high proportion of rapidly (B1) and intermediately (B2) degradable protein fractions may be behind its impact on NH_3-N concentration.

Furthermore, ruminal pH value in the present work was 6.92 to 7.08, which falls within the ideal range of 6.8 to 7.2 for facilitating rumen digestion (Phesatcha et al., 2021; Totakul et al., 2021). The stability of ruminal pH suggests that the addition of the chitinase enzyme did not exert a significant effect ($p<0.05$) on this parameter, thereby maintaining an environment conducive to optimum microbial activity. In line with this, the results suggest that the addition of chitinase tends to alter the rumen microbial population. The use of 6 U/100 g chitinase (EC6) resulted in an increase in the total bacterial population from 2.34×10^9 cfu mL^{-1} to 4.30×10^9 cfu $mL^{-}1$, and a decrease in the protozoan population from 8.95×10^6 cells mL^{-1} to 3.43×10^6 cells mL^{-1}.

Interestingly, in the current study, the treatment of BSF larval meal with chitinase did not increase the total gas production (Table 3, Figure 1). The lower gas production in BSF larval meal (19.25-20.21 mL/200 mg DM), even with chitinase treatment, may be attributed to the composition of the substrate. In comparison, soybean meal, characterised by a higher fermentable fraction, demonstrated significantly greater gas production (31.26 mL/200 mg DM). Treatment SBM showed the highest values for both b (33.14 mL) and c (0.14 mL/h), indicating more extensive and rapid fermentation. Meanwhile, treatments with BSF larval meal (EC0-EC6) produced significantly less gas from the insoluble fraction, with b values ranging from 13.98 to 15.57 mL, and showed lower fermentation rates (c = 0.07-0.09 mL/h). Protein and fat, which are high in BSF larval meal, contribute little to gas and VFA production (Getachew et al., 1998).

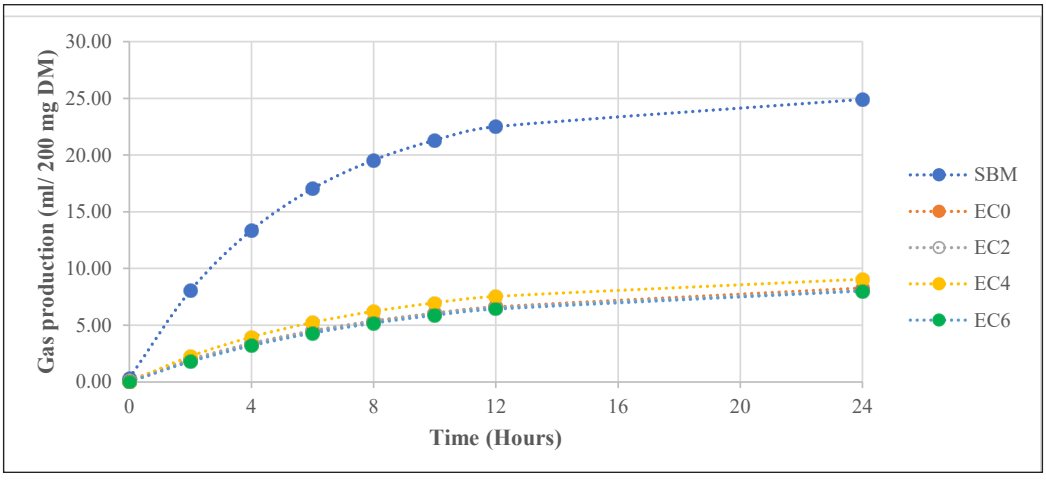

Figure 1. Cumulative total gas production of experimental diets during 24h incubation

Even the different sources of nitrogen, including NPN at similar concentrations of total ammonia nitrogen (0 - 128 mmol/L), as reported by Shen et al. (2023), could influence microbial populations and *in vitro* rumen fermentation profiles (gas production, dry matter digestibility, total volatile fatty acid, acetate, propionate). BSF larval meal, despite being treated with chitinase, has a higher fibre and ether extract (EE) content, which is less fermentable, thus explaining the lower gas production (Jayanegara et al. 2017a). The authors explained that BSF larval meals had lower total gas production at 24 hours than SBM, with recorded values of 214.5 and 93.8-115.8 mL g^{-1} DM, respectively, which is due to their higher fibre and EE content (Jayanegara et al., 2017a). This finding suggests that while chitinase may alter microbial populations, the fermentation indices and gas production are strongly influenced by the composition of the substrates, particularly their fermentable carbohydrate content and fibre levels.

Treatment EC6 was also reported to possess the ability to enhance fermentation efficiency and microbial balance, indicating a change in the nutritional profile of BSF larval meal. As illustrated in Figure 2, chitin was positively related to ADF and negatively to hemicellulose, which means that the higher the ADF content, the lower the digestibility and nutrient availability can be (Nurdianti et al., 2024; Sándor et al., 2022). Moreover, near interrelations were established among digestibility coefficients and parameters of fermentation, GP24 having positive correlations with IVDMD24 and negative with protozoa, while IVDMD values were highly correlated with N-NH_3 and IVCPD. These correlations were also supported by PCA analysis (Figure 3), which showed that treatment EC6, as well as SBM, sustained better fermentation and a favourable microbial population. On the other hand, treatments EC0, EC2, and EC4 were associated with greater protozoa and ammonia-N content, suggesting reduced nitrogen utilisation and fermentation indices.

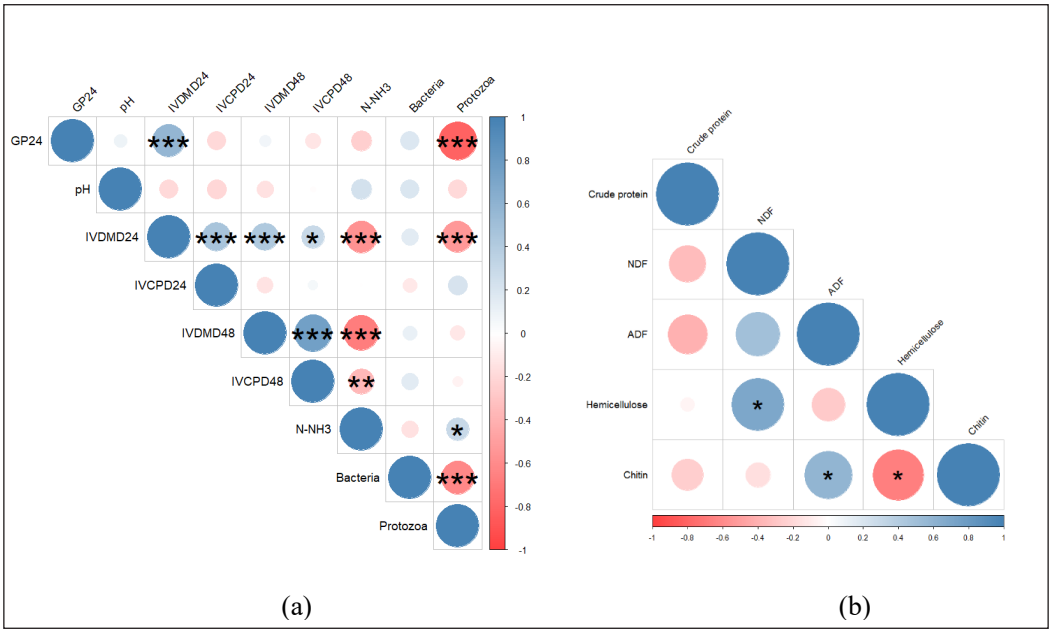

Figure 2. Correlation plot between (a) in vitro digestion and fermentation parameters (n = 60) and (b) chemical composition (n = 12). Positive and negative correlation coefficients are represented by blue and red colour scales, respectively. Significance levels: ***p < 0.001, **p < 0.01, *p < 0.05

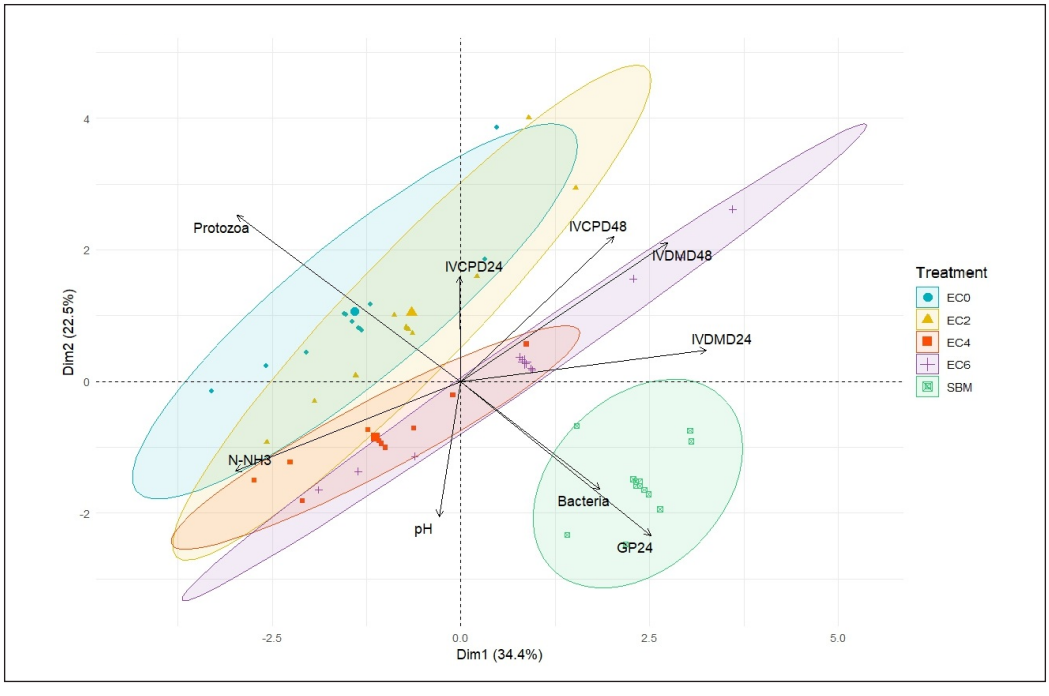

Figure 3. PCA of soybean meal and hydrolysed BSF larval meal

CONCLUSION

The application of chitinase enzyme up to level 6 U/100 g to BSF larval meal was insufficient to improve the overall *in vitro* ruminal and post-ruminal digestibility. However, chitin and acid detergent fibre contents were considerably decreased by treatment with 2 U/100 g, while bacterial rumen populations increased when the dose was raised to 6 U/100 g. Further research is needed to determine the optimal dosage of enzymes and complementary methods to optimise the digestibility and fermentation characteristics of BSF larval meal.

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REFERENCES

Agbohessou, P. S., Mandiki, R., Mes, W., Blanquer, A., Gérardy, M., Garigliany, M. M., Lambert, J., Cambier, P., Tokpon, N., Lalèyè, P. A., & Kestemont, P. (2024). Effect of fatty acid-enriched black soldier fly larvae meal combined with chitinase on the metabolic processes of Nile tilapia. *British Journal of Nutrition*, *131*(8), 1326-1341. https://doi.org/10.1017/S0007114523003008

Akeed, Y., Atrash, F., & Naffaa, W. (2020). Partial purification and characterisation of chitinase produced by *Bacillus licheniformis* B307. *Heliyon*, *6*(5), Article e03858. https://doi.org/10.1016/j.heliyon.2020.e03858

Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods: A review. *Food Technology and Biotechnology*, *51*(1), 12-25.

Association of Official Analytical Chemists. (2002). *Official methods of analysis* (17th ed., Vol. 1, pp. 120-155). AOAC International.

Behan, A. A., Loh, T. C., Fakurazi, S., Kaka, U., Kaka, A., & Samsudin, A. A. (2019). Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. *Animals*, *9*(7), Article 400. https://doi.org/10.3390/ani9070400

Black, M. M., & Schwartz, H. M. (1950). The estimation of chitin and chitin nitrogen in crawfish waste and derived products. *Analyst*, *75*(889), 185-189. https://doi.org/10.1039/an9507500185

Borić, M., Vicente, F. A., Jurković, D. L., Novak, U., & Likozar, B. (2020). Chitin isolation from crustacean waste using a hybrid demineralisation/DBD plasma process. *Carbohydrate Polymers*, *246*, Article 116648. https://doi.org/10.1016/j.carbpol.2020.116648

Conway, E. J., & O'Malley, E. (1942). Microdiffusion methods: Ammonia and urea using buffered absorbents (revised methods for ranges greater than 10 µg. N). *Biochemical Journal*, *36*(7-9), 655-661. https://doi.org/10.1042/bj0360655

Gebele, L., Wilke, A., Salliou, A., Schneider, L., Heid, D., Stadelmann, T., Henninger, C., Ahmed, U., Broszat, M., Müller, P., Dusel, G., Krzyżaniak, M., Ochsenreither, K., & Eisele, T. (2024). Recombinant expression and characterisation of the endochitinase Chit36-TA from *Trichoderma asperellum* in *Komagataella phaffii* for chitin degradation of black soldier fly exuviae. *Bioprocess and Biosystems Engineering*, *47*(10), 1751-1766. https://doi.org/10.1007/s00449-024-03067-4

Getachew, G., Blümmel, M., Makkar, H. P. S., & Becker, K. (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. *Animal Feed Science and Technology*, *72*(3-4), 261-281. https://doi.org/10.1016/S0377-8401(97)00189-2

Goering, H. K., & van Soest, P. J. (1970). *Forage fibre analyses (apparatus, reagent, procedures and some applications)* (Agriculture Handbook No. 379). United States Department of Agriculture.

Haider, R., Khan, N., Aihetasham, A., Shakir, H. A., Fatima, M., Tanveer, A., Bano, S., Ali, W., Tahir, M., Asghar, M., Farooq, A., Aftab, S., Haq, A. U., & Sarwar, M. (2024). Dietary inclusion of black soldier fly (*Hermetia illucens*) larvae meal, with exogenous protease supplementation, in practical diets for striped catfish (*Pangasius hypophthalmus*, Sauvage 1878). *PLoS ONE, 19*(12), Article e0313960. https://doi.org/10.1371/journal.pone.0313960

Hartadi, H., Reksohadiprodjo, S., Lebdosukojo, S., Tilman, A. D., Kearl, L. C., & Harris, L. E. (1980). *Tables of feed composition for Indonesia*. International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University.

Jayanegara, A., Yantina, N., Novandri, B., Laconi, E. B., Nahrowi, N., & Ridla, M. (2017a). Evaluation of some insects as potential feed ingredients for ruminants: Chemical composition, in vitro rumen fermentation and methane emissions. *Journal of the Indonesian Tropical Animal Agriculture*, *42*(4), 247-254. https://doi.org/10.14710/jitaa.42.4.247-254

Jayanegara, A., Novandri, B., Yantina, N., & Ridla, M. (2017b). Use of black soldier fly larvae (*Hermetia illucens*) to substitute soybean meal in ruminant diet: An in vitro rumen fermentation study. *Veterinary World*, *10*(12), 1436-1446. https://doi.org/10.14202/vetworld.2017.1439-1446

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023). Comparative analysis of in vitro fermentation parameters in total mixed rations of dairy cows with varied levels of defatted black soldier fly larvae (*Hermetia illucens*) as a substitute for soybean meal. *Fermentation*, *9*(7), Article 652. https://doi.org/10.3390/fermentation9070652

Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbø, R., Torstensen, B. E., & Lock, E.-J. (2017). Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS One*, *12*(8), Article e0183188. https://doi.org/10.1371/journal.pone.0183188

Lima, E. D. S., Valente, T. N. P., Roca, R. D. O., Cezario, A. S., Santos, W. B. R dos, Deminicis, B. B., & Ribeiro, J. C. (2017). Effect of whole cottonseed or protected fat dietary additives on carcass characteristics and meat quality of beef cattle: A review. *Journal of Agricultural Science*, *9*(5), 175-183. https://doi.org/10.5539/jas.v9n5p175

Linh, N. V., Wannavijit, S., Tayyamath, K., Dinh-Hung, N., Nititanarapee, T., Sumon, M. A. A., Srinual, O., Permpoonpattana, P., Doan, H., & Brown, C. L. (2024). Black soldier fly (*Hermetia illucens*) larvae meal: A sustainable alternative to fish meal proven to promote growth and immunity in koi carp (*Cyprinus carpio* var. *koi*). *Fishes*, *9*(2), Article 53. https://doi.org/10.3390/fishes9020053

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A. P., & Paengkoum, P. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A review. *Insects*, *13*(9), Article 831. https://doi.org/10.3390/insects13090831

Matin, N., Utterback, P., & Parsons, C. M. (2021). True metabolizable energy and amino acid digestibility in black soldier fly larvae meals, cricket meal, and mealworms using a precision-fed rooster assay. *Poultry Science*, *100*(7), Article 101146. https://doi.org/10.1016/j.psj.2021.101146

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of the Science of Food and Agriculture*, *98*(15), 5776-5784. https://doi.org/10.1002/jsfa.9127

Msangi, J. W., Mweresa, C. K., & Ndong'a, M. F. O. (2022). Using organic wastes as feed substrate for black soldier fly larvae. *Journal of Insects as Food and Feed*, *8*(4), 441-451. https://doi.org/10.3920/JIFF2021.0047

Mulyono, M., Yunianto, V. D., Suthama, N., & Sunarti, D. (2019). The effect of fermentation time and *Trichoderma* levels on digestibility and chemical components of black soldier fly (*Hermetia illucens*) larvae. *Livestock Research for Rural Development*, *31*(10), Article 150. https://www.lrrd.org/lrrd31/10/mulyo31150.html

Narayana, K. J. P., & Vijayalakshmi, M. (2009). Chitinase production by *Streptomyces* sp. Anu 6277. *Brazilian Journal of Microbiology*, *40*(4), 725-733. https://doi.org/10.1590/S1517-83822009000400002

Nurdianti, R. R., Dickhoefer, U., & Castro-Montoya, J. M. (2024). Relationship between nutritional composition and fibre digestibility in tropical forages compared to temperate forages. *Italian Journal of Animal Science*, *23*(1), 1839-1853. https://doi.org/10.1080/1828051X.2024.2434697

Palmer, B., & Jones, R. J. (2000). The effect of PEG addition in vitro on dry matter and nitrogen digestibility of *Calliandra calothyrsus* and *Leucaena leucocephala* leaf. *Animal Feed Science and Technology*, *85*(3-4), 259-268. https://doi.org/10.1016/S0377-8401(00)00125-5

Pedrazzani, C., Righi, L., Vescovi, F., Maistrello, L., & Caligiani, A. (2024). Black soldier fly as a new chitin source: Extraction, purification and molecular/structural characterisation. *LWT - Food Science and Technology*, *191*, Article 115618. https://doi.org/10.1016/j.lwt.2023.115618

Phesatcha, B., Phesatcha, K., Viennaxay, B., Thao, N. T., & Wanapat, M. (2021). Feed intake and nutrient digestibility, rumen fermentation profiles, milk yield and compositions of lactating dairy cows supplemented by *Flemingia macrophylla* pellet. *Tropical Animal Science Journal*, *44*(3), 288-296. https://doi.org/10.5398/tasj.2021.44.3.288

Renna, M., Coppa, M., Lussiana, C., le Morvan, A., Gasco, L., & Maxin, G. (2022). Full-fat insect meals in ruminant nutrition: In vitro rumen fermentation characteristics and lipid biohydrogenation. *Journal of Animal Science and Biotechnology*, *13*, Article 138. https://doi.org/10.1186/s40104-022-00792-2

Sandhya, C., Krishna, L. A., Nampoothri, K. M., Binod, P., Szakacs, G., & Pandey, A. (2004). Extracellular chitinase production by *Trichoderma harzianum* in submerged fermentation. *Journal of Basic Microbiology*, *44*(1), 49-58.

Sándor, Z. J., Banjac, V., Vidosavljević, S., Káldy, J., Egessa, R., Lengyel-Kónya, É., Tömösközi-Farkas, R., Zsolt, Z., Adányi, N., Libisch, B., & Biró, J. (2022). Apparent digestibility coefficients of black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), and blue bottle fly (*Calliphora vicina*) insects for juvenile African catfish hybrids (*Clarias gariepinus* × *Heterobranchus longifilis*). *Aquaculture Nutrition*, *2022*, Article 4717014. https://doi.org/10.1155/2022/4717014

Sangkaew, M., & Koh, K. (2021). Improvement in the in vitro digestibility of shrimp meal by the addition of persimmon peel. *The Journal of Poultry Science*, *58*(1), 51-57. https://doi.org/10.2141/jpsa.0200024

Schwab, C. G., & Broderick, G. A. (2017). A 100-year review: Protein and amino acid nutrition in dairy cows. *Journal of Dairy Science*, *100*(12), 10094-10112. https://doi.org/10.3168/jds.2017-13320

Shen, J., Zheng, W., Xu, Y., & Yu, Z. (2023). The inhibition of high ammonia to in vitro rumen fermentation is pH dependent. *Frontiers in Veterinary Science*, *10*, Article 1163021. https://doi.org/10.3389/fvets.2023.1163021

Siddiqui, S. A., Ristow, B., Rahayu, T., Putra, N. S., Widya Yuwono, N., Nisa', K., Mategeko, B., Smetana, S., Saki, M., Nawaz, A., & Nagdalian, A. (2022). Black soldier fly larvae (BSFL) and their affinity for organic waste processing. *Waste Management*, *140*, 1-13. https://doi.org/10.1016/j.wasman.2021.12.044

Soetemans, L., Uyttebroek, M., & Bastiaens, L. (2020). Characteristics of chitin extracted from black soldier fly in different life stages. *International Journal of Biological Macromolecules*, *165*, 3206-3214. https://doi.org/10.1016/j.ijbiomac.2020.11.041

Totakul, P., Matra, M., Sommai, S., & Wanapat, M. (2021). *Cnidoscolus aconitifolius* leaf pellet can manipulate rumen fermentation characteristics and nutrient degradability. *Animal Bioscience*, *34*(10), 1607-1615. https://doi.org/10.5713/ab.20.0833

van Huis, A. (2022). Edible insects: Challenges and prospects. *Entomological Research*, *52*(4), 161-177. https://doi.org/10.1111/1748-5967.12582

van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vargas-Serna, C. L., Pineda-Osorio, A. N., Gallego-Ocampo, H. L., Plaza-Dorado, J. L., & Ochoa-Martínez, C. I. (2025). Transforming coffee and meat by-products into protein-rich meal via black soldier fly larvae (*Hermetia illucens*). *Sustainability*, *17*(2), Article 460. https://doi.org/10.3390/su17020460

Veldkamp, T., Meijer, N., Alleweldt, F., Deruytter, D., van Campenhout, L., Gasco, L., Roos, N., Smetana, S., Fernandes, A., & van der Fels-Klerx, H. J. (2022). Overcoming technical and market barriers to enable sustainable large-scale production and consumption of insect proteins in Europe: A SUSINCHAIN perspective. *Insects*, *13*(3), Article 281. https://doi.org/10.3390/insects13030281

Wang, Y., McAllister, T. A., Rode, L. M., Beauchemin, K. A., Morgavi, D. P., Nsereko, V. L., Iwaasa, A. D., & Yang, W. (2001). Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). *British Journal of Nutrition*, *85*(3), 325-332. https://doi.org/10.1079/BJN2000277

Wang, Y. S., & Shelomi, M. (2017). Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods*, *6*(10), Article 91. https://doi.org/10.3390/foods6100091

Yang, Y., Shenrui, X., Wenjun, G., Yu, W., Lu, W., & Changhua, L. (2025). Chitinase improves the available energy, amino acids digestibility of black soldier fly and fecal microbiota of growing pigs. *Animal Bioscience*, *38*(8), 1733-1745. https://doi.org/10.5713/ab.24.0920

Zou, X., Liu, M., Li, X., Pan, F., Wu, X., Fang, X., Zhou, F., Peng, W., & Tian, W. (2024). Applications of insect nutrition resources in animal production. *Journal of Agriculture and Food Research*, *15 backstage*, Article 100966. https://doi.org/10.1016/j.jafr.2024.100966