Journal of Animal & Plant Sciences, 34(6): 2024, Page: 1606-1615
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.6.0836

EFFECT OF ORGANIC ACID AND STEAMING TREATMENTS ON CHITIN CONTENT AND NUTRITIONAL QUALITY OF *Hermetia illucens* LARVAE AS BROILER FEED

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ABSTRACT

Chitin, an anti-nutritional factor, hinders nutrient absorption in animal feed. Its presence in Black Soldier Fly (*Hermetia illucens* L.) larvae limits feed utilization in broiler chickens due to its indigestibility and nutrient-binding properties that may deter growth of animals. This study aimed to modify the chitin content by applying physical and chemical treatments to raw *Hermetia illucens* L. (BSF) larvae, addressing the nutritional aspects of the feed. The research design employed was a complete randomized factorial design with three factors and three replications. Factor 1 included Control (C) and Steaming (S) as physical treatments, Factor 2 involved the use of three organic acids, i.e. acetic acid (A), citric acid (C), and propionic acid (P), and Factor 3 included three concentrations, namely 2%, 4%, and 6%. Broiler chickens (n = 54) were exposed to feed resulting from the physicochemical treatment of BSF larvae. Chemical treatment significantly influenced most parameters and was not heavily dependent on concentration. Acetic acid emerged as the best organic acid in enhancing the nutritional profile of the feed product while simultaneously yielding the lowest chitin content. Confirmation through biological parameters indicated that treatment with acetic acid resulted in improved digestibility, including crude protein and organic matter. Correlation results further affirmed thatchitin content influences feed digestibility and remains a challenge to be addressed. The best physicochemical treatment for post-processed BSF larvae was the control (non-steamed) treatment with 2% acetic acid with reduced chitin content and improved digestibility and other nutritional parameters.

Keywords: Acetic acid, Chitin, Digestibility, Spearman's correlation coefficients.

Published first online November 30, 2024 Published final December 22, 2024

INTRODUCTION

The consumption of insects as a protein source has been extensively discussed and promoted by researchers worldwide (Kim *et al.,* 2019). Protein derived from insects is more economical, environmentally friendly, and plays a crucial role in nature. Insects exhibit high feed conversion efficiency, making them a sustainable and cost-effective protein source for animal feed. They require less feed, water, and land, and can be mass-produced (Rumpold and Schluter, 2013). Additionally, insect farming can reduce organic waste that may otherwise pollute the environment. The Black Soldier Fly (BSF) or *Hermetia illucens* L., originally from America, has spread to subtropical and tropical regions worldwide (Surendra *et al.,* 2020). The tropical climate conditions in Indonesia are highly conducive to BSF cultivation (Shelomi, 2020). From a cultivation perspective, BSF is easily scalable for mass production. BSF meal has the potential to replace fish meal up to 100% in broiler feed without negative effects on digestibility (Rambet *et al.*, 2016).

One inhibiting factor for nutrient absorption in animal feed is the presence of anti-nutritional substances (Tadele, 2015). Therefore, before being used as feed, the content of anti-nutritional substances should be reduced through various processing methods, including physical, mechanical, chemical, and biological approaches. BSF larvae, as a protein source for feed, also contain an anti-nutritional substance, namely chitin. The chitin content in BSF larvae increases with the larval age (Eggink and Dalsgaard, 2023). Chitin is a widely distributed biopolymer abundant on Earth. It is a major component of the exoskeleton or shell of crustaceans (such as shrimp) and insects, as well as the cell walls of yeast, algae, and fungi (Iber *et al.,* 2022). Chitin possesses unique properties, including biodegradability, bioactivity, non-toxicity, and flexibility (Joseph *et al.,* 2021). The ultrastructure of chitin in the larval phase exhibits greater complexity and organization resembling a honeycomb, as

opposed to the adult phase, which appears more fibrous and parallel in arrangement (Triunfo *et al.,* 2022; Wasko *et al.*, 2016). The honeycomb structure, in theory, embodies a robust and resilient geometric configuration within the biological system, which may pose challenges in terms of modification or elimination for specific purposes (Zhang *et al*., 2015). Chitin binds to nitrogen from the amino acids forming proteins, making proteins difficult to digest. It is a coarse fiber that is hard to digest and can bind essential nutrients required for animal growth. If not broken down, these nutrients cannot be optimally absorbed by the animal's body. Chitin herein reduces the performance of digestive enzymes on fats and proteins (Attia *et al.,* 2023; Shah *et al.,* 2022).

Chitin can be degraded enzymatically and non-enzymatically under controlled conditions. It is a solid, amorphous substance, white in color, and highly resistant to bacterial influence (Zhang *et al.,* 2022). Chitin is soluble in concentrated nitric acid, concentrated hydrochloric acid, and concentrated sulfuric acid (Marono *et al.,* 2016). Organic acids, including acetic acid, butyric acid, citric acid, fumaric acid, and lactic acid have no negative impact on the ileum villi but can increase the length and weight of the intestines, optimizing nutrient digestion in the intestines (Andreopoulou *et al.,* 2014; Attia *et al.,* 2013; Attia, 2018). This study aims to improve the quality of BSF meal through hydrolysis using acetic, citric, and propionic acids. Efforts to overcome limitations in BSF meal processing involve chemical treatment using organic acids, as these acids are safe and readily available in the market.

MATERIALS AND METHODS

Insect rearing: BSF or *H. illucens* eggs were purchased from a farmer in Medan City, North Sumatra, Indonesia. Newly hatched BSF larvae were collected as they dropped from the wire mesh into trays containing tofu dregs, using a ratio of 10 g of eggs per 200 g of substrate. The larvae were then transferred the next day to a standard rearing medium consisting of fruit and vegetable (F&V) waste at a 1:1 ratio, with quantities adjusted according to container size. The larvae were reared in this medium until 18 days of age, then were harvested and prepared for experimentation.

Study design: The research design applied was a complete randomized factorial design with three factors and three replications. Factor 1 consisted of Control (C) and Steaming (S) as physical treatments, Factor 2 involved the use of three organic acids: acetic acid (A), citric acid (C), and propionic acid (P), and Factor 3 included three concentrations: 2%, 4%, and 6%, as illustrated in Figure 1. The graphical illustration was generated using BioRender (https://app.biorender.com/). The concentration range was selected to represent the minimum and maximum thresholds, with the midpoint at 3%, based on the optimal chitosan degradation concentration reported in the study by Sikorski *et al.* (2021). This design was employed for *in vivo* digestibility using broiler chickens to obtain biological parameters.

BSF larva meal preparation: Eighteen-day-old BSF larvae were divided into two groups for physical treatment, namely control and steaming. The BSF larvae were steamed at 60°C for 8 hours before being dried in an oven at 60°C for 1–2 hours. The dried results were ground to homogeneity and reacted with specific types and concentrations of organic acids. The measured parameters included chitin content (%), crude protein (%), crude lipid (%), gross energy (kcal/kg), and organic matter (%).

***In vivo* digestibility on broiler chickens:** The broiler chickens were subjected to the feed resulting from the physicochemical treatment of BSF larvae. A total of 54 chickens, adjusted to match the number of BSF larva samples, were housed in 54 metabolic cages. These chickens underwent a 2-day acclimation period under the conditions of the cages, familiarizing them with the experimental feed. Following a 12-hour fasting period, the chickens were provided with the treatment feed, supplemented with 2% titanium dioxide (TiO_2) as an indicator. Four hours post-feeding, the chickens were euthanized, and digesta from the ileum was harvested. Then, the digesta was subjected to drying, grinding, and chemical analysis to determine parameters such as crude protein digestibility (%), digestible energy (%), and digestible organic matter (%).

Data analysis: Data were presented in tabular and graphical formats using GraphPad Prism version 8.0. A three-way ANOVA was conducted with Minitab version 19.0, followed by Tukey's post-hoc test ($P<0.05$) for multiple comparisons and mean grouping. The experimental unit for the analysis was individual broiler chickens. The data were checked for normality using the Shapiro-Wilk test, and power analysis was performed to ensure adequate sample size and statistical power. A non-parametric Spearman's correlation analysis was performed using GraphPad Prism version 8.0 to assess relationships between proximate composition and biological performance, including feed digestibility in broiler chickens.

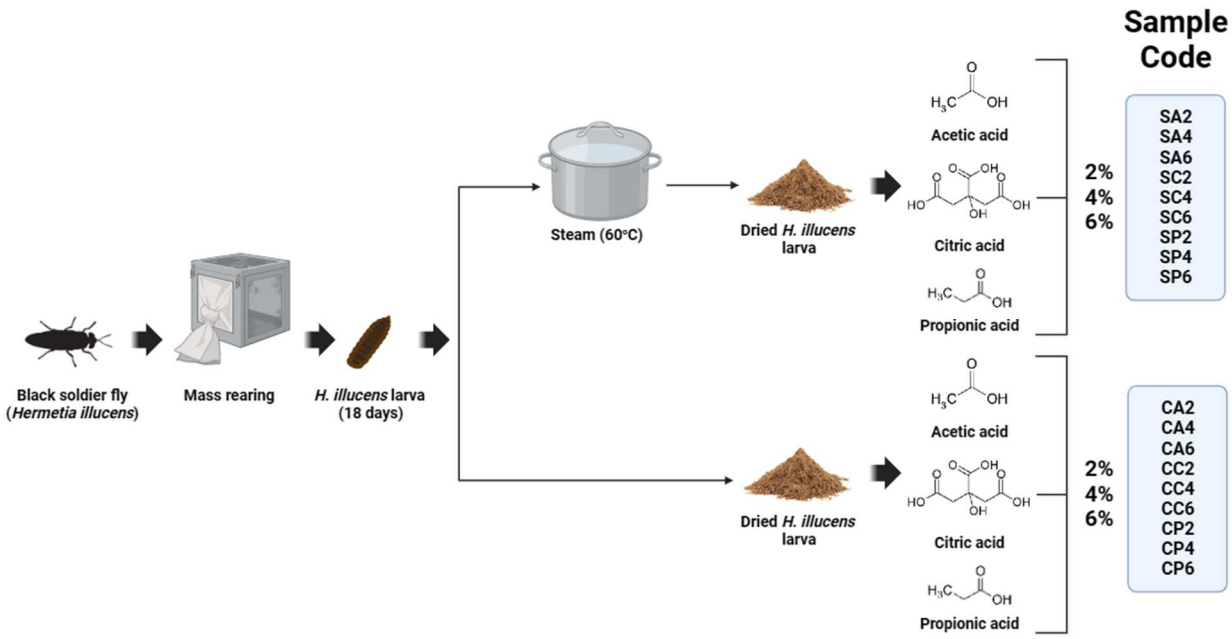

Figure 1. Illustrated framework of study design and factorial treatments.

RESULTS AND DISCUSSION

The incorporation of BSF into animal feed faces challenges due to the existence of an anti-nutrient compound, specifically chitin, within its external body structure. Similar to the indigestible characteristics of coarse fiber in plants, chitin imposes constraints on avian digestion (Karasov and Douglas, 2013). Efforts to improve the digestibility of *H. illucens* larva-based feed by reducing chitin component were investigated through physical treatments, namely steaming and non-steaming (control), coupled with the utilization of various concentrations of organic acids, such as acetic acid, citric acid, and propionic acid. Mean chitin contents significantly differed among physical treatments (control, steaming), organic acid treatments (acetic acid, citric acid, propionic acid), and tested concentrations (2%, 4%, 6%). However, the interactions between physical and chemical treatments, as well as the three-way interactions, exhibited non-significant values (Table 1). The physicochemical treatments yielded varied results contingent upon the parameters investigated. The highest chitin content in the control sample was obtained from CC4, measuring 7.4%, while in the steamed sample, it was observed in SC2. The lowest chitin content in the control treatment was recorded in CA2 at 4.2%, whereas in the steaming treatment, CA6 exhibited a chitin content of 4.6% (Table 2).

Table 1. Statistical test results on proximate composition of *H. illucens* larvae under different physicochemical treatments

Source of Variation	Chitin (%)		Crude Protein (%)		Crude Lipid (%)		Gross energy (kcal/kg)		Organic matter (%)	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
P	17.90	*	28.00	*	0.95	0.34	192.68	*	0.04	0.84
Ch	330.27	*	1166.81	*	0.36	0.70	376.39	*	12.47	*
Co	7.69	*	35.42	*	0.96	0.39	5.76	*	0.70	0.50
P×Ch	1.98	0.15	45.32	*	0.43	0.65	24.34	*	11.75	*
P×Co	89.87	*	10.83	*	0.87	0.43	33.16	*	14.84	*
Ch×Co	10.60	*	13.00	*	0.10	0.98	4.16	*	1.57	0.20
P×Ch×Co	2.53	0.06	24.98	*	0.47	0.76	8.02	*	0.42	0.79

Data compare effects of physical (*P*), chemical (*Ch*) and concentration (*Co*) treatments on treated *H. illucens* larval powder performance. Significant effects were tested using three-way ANOVA followed by multiple comparisons using Tukey's test. * $P \leq 0.05$.

Table 2. Proximate compositions of *H. illucens* larvae under different physicochemical treatments

Group(s)*	Chitin (%)	Crude Protein (%)	Crude Lipid (%)	Gross energy (kcal/kg)	Organic matter (%)
CA2	4.2±0.15^{h}	44.2±0.13^{a}	38.1±0.87^{a}	4,303±0.7^{hi}	86.4±0.78^{abc}
CA4	4.9±0.23^{efg}	**44.3±0.22^{a}**	38.1±0.69^{a}	4,308±2.0^{hi}	87.1±0.42^{ab}
CA6	4.9±0.27^{efg}	44.0±0.01^{a}	38.0±0.413^{a}	4,333±21.6^{ghi}	86.9±0.21^{ab}
CC2	5.3±0.09^{def}	42.4±0.10^{de}	38.4±0.49^{a}	4,350±48.8^{efg}	86.2±0.72^{abc}
CC4	**7.4±0.13^{a}**	42.0±0.03^{ef}	38.2±0.62^{a}	4,433±8.1^{c}	87.4±0.56^{a}
CC6	7.2±0.22^{ab}	41.9±0.04^{f}	**38.5±0.52^{a}**	4,421±17.9^{cd}	87.5±0.32^{a}
CP2	5.0±0.17^{efg}	42.0±0.01^{def}	38.2±0.22^{a}	4,305±3.5^{hi}	85.1±0.25^{c}
CP4	5.5±0.18^{def}	42.2±0.17^{ef}	38.1±0.86^{a}	4,334±3.8^{ghi}	85.7±0.46^{bc}
CP6	5.7±0.15^{d}	43.1±0.03^{def}	38.0±0.21^{a}	4,297±27.5^{i}	86.3±0.50^{abc}
SA2	5.4±0.21^{def}	43.9±0.12^{b}	38.2±0.77^{a}	4,344±3.0^{fgh}	**87.4±0.65^{a}**
SA4	4.8±0.26^{fgh}	43.9±0.07^{a}	38.0±0.89^{a}	4,331±5.1^{ghi}	86.7±0.45^{ab}
SA6	4.6±0.28^{gh}	44.1±0.27^{a}	37.9±0.27^{a}	4,317±3.9^{ghi}	86.7±0.56^{ab}
SC2	7.0±0.26^{abc}	41.0±0.01^{g}	38.4±0.51^{a}	**4,507±1.7^{a}**	86.3±0.47^{abc}
SC4	6.7±0.30^{bc}	41.3±0.30^{g}	38.3±0.51^{a}	4,481±6.1^{ab}	86.3±0.56^{abc}
SC6	6.6±0.29^{bc}	42.0±0.04^{ef}	37.4±0.53^{a}	4,449±3.4^{bc}	86.1±0.30^{abc}
SP2	6.3±0.22^{c}	42.5±0.30^{cd}	38.1±0.47^{a}	4,388±1.8^{de}	87.1±0.25^{ab}
SP4	5.5±0.31^{de}	42.5±0.09^{d}	38.0±0.69^{a}	4,377±246^{ef}	86.1±0.17^{abc}
SP6	5.4±0.14^{def}	43.0±0.08^{bc}	37.9±0.23^{a}	4,356±3.4^{efg}	86.2±0.44^{abc}

The values are Mean±Standard deviation

*Control+Acetic acid 2% = CA2, Control+Acetic acid 4% = CA4, Control+Acetic acid 6% = CA6, Control+Citric acid 2% = CC2, Control+Citric acid 4% = CC4, Control+Citric acid 6% = CC6, Control+Propionic acid 2% = CP2, Control+Propionic acid 4% = CP4, Control+Propionic acid 6% = CP6, Steam+Acetic acid 2% = SA2, Steam+Acetic acid 4% = SA4, Steam+Acetic acid 6% = SA6, Steam+Citric acid 2% = SC2, Steam+Citric acid 4% = SC4, Steam+Citric acid 6% = SC6, Steam+Propionic acid 2% = SP2, Steam+Propionic acid 4% = SP4, Steam+Propionic acid 6% = SP6. Values with different letters denote a statistical significant at 5% within the same column or parameter. Bold numbers show the highest value in each parameter.

Decreasing chitin content in the samples indicates their suitability as animal feed (Bach and Babayan, 1982). A contradictory trend was observed where the control treatment, using higher concentrations of acetic acid, led to an increase in chitin content. Conversely, in the steaming treatment, the use of high concentrations of acetic acid (6%) resulted in lower chitin content. In general, acetic acid treatments, both in the control and steaming, demonstrated superior nutritional aspects compared to other treatments. Zhang *et al.* (2022) recommended the use of a 7.5% acetic acid concentration for efficient demineralization and deproteinization of shrimp chitin, subsequently leading to a prompt depolymerization process at elevated temperatures. The influence of physical treatment, specifically steaming, may potentially affect the chitin characteristics present in *H. illucens* larvae. Steaming and boiling prior to drying potentially affected chitin reduction and alleviates the drying effects on larvae before processing them into flour for feed applications (Hahn *et al.,* 2020). Mirwandhono *et al.* (2022) reported that boiling treatment at 100°C increased the yield of chitin and chitosan for extraction purpose. In this study, the acetic acid treatment, whether at low or high concentrations, resulted in lower chitin levels compared to previous research that employed a combination of 30% propionic acid and 30% formic acid and fermentation technique (Yunilas *et al.*, 2023). Even though this study did not measure the chitin of raw *H. illucens* as baseline data, our results may still be highly favorable when referring to the chitin levels in normal BSF larvae, which can reach 37 to 40% dry matter according to the literature (Spranghers *et al.,* 2017). The compatibility of acetic acid as an organic solvent and hydrolyzing agent for chitin involves the release of acetic acid concurrently with acetonitrile and acetamide. The degree of acetylation is determined based on the liberation of N-acetyl groups from N-acetyl D-glucosamine units of chitin through acid hydrolysis (Kasaai, 2009). The influence of physicochemical treatments on crude protein content yielded the highest value in the CA4 treatment, reaching 44.3%, whereas the lowest was recorded in the CC2 treatment at 41% (Figure 3).

The increase in protein content correlates with the concentration levels of the organic acids employed, although a distinct trend was observed in the Control+Citric acid treatment, where the protein content decreased. Acid hydrolysis utilizing acetic acid, on the other hand, yields superior proximate performance, particularly in terms of crude protein. Yunilas *et al.* (2023) reported a range of crude protein from 32.3% to 34.7% using organic acids at concentrations of 3, 6, and 9%. Soetemans *et al.* (2019) utilized a variety of organic acids and obtained a crude protein of 56.2% using acetic

acid in pellet samples resulting from solvent extraction. Schiavone *et al.* (2017) achieved a crude protein of 67% during a defatting process of samples in their study. Defatting process was not conducted in this study, potentially influencing the yield of crude protein in the samples. Furthermore, the crude lipid parameter consistently ranged between 37.9% and 38.5%, resulting no significant differences across all treatments (Table 2).

Similar results were also reported by Yunilas *et al.* (2023), with lipid content ranging from 30.2% to 32.4% using acid hydrolysis treatment. Soetemans *et al.* (2019) reported that defatting process can yield high crude lipid content in the upper layer when reacted with any organic acid, with the highest obtained from lactic acid (75-85%). They added that after the separation between the upper layer, supernatant, and pellet, no high crude lipid was found in BSF samples except for the reaction between the pellet and oxalic acid (39.7%). Several studies have reported that BSF larvae naturally have a high content of fat or lipid, and the concentration of these molecules was dominated by free fatty acids. Loho and Lo (2023) reported that the crude lipid in BSF larva was around 35% which was higher than grasshopper but lower than termites. The killing method applied to BSF larvae also determines the lipid concentration, where blanching or steaming techniques in this study were expected to reduce crude lipid in the samples (Caligiani *et al.,* 2019; Ushakova *et al.,* 2016). However, it turned out that the lipid contents were still relatively high and were not influenced by physicochemical treatment. Lipases become active when larvae are mechanically killed, resulting in the formation of free fatty acids, which, when exposed to acidic conditions, tend to change the structure of proteins and decrease emulsifying properties, making lipid separation easier to quantify (Kim *et al.,* 2011). Once again, defatting step was not initiated in this study, which contribute to the consistency of crude lipid contents in all samples.

The highest organic matter content, reaching 87.53%, was recorded in the CC6 treatment, whereas the lowest value, measuring 85.13%, was observed in the CP2 treatment (Table 2). Statistical analyses revealed significant differences in means for treatments incorporating different organic acids, the interaction between physical treatment and the type of organic acid, as well as the interaction between physical treatment and the concentration of organic acid, as presented in Table 1. The observed differences resulting from physicochemical treatments on the organic matter content are evident. In the control treatment, all values tend to increase at high concentrations, whereas in the steaming treatment, all values decrease at the final concentration. Organic matter is one of the parameters in proximate analysis that relates to the overall proportion of carbon compounds, including carbohydrates, lipids, fibers, and proteins. The variations in organic matter in BSF larvae after processing with organic acids may be attributed to differences in the solubility of certain nutritional compounds during the treatment process.

The gross energy content of treated BSF larvae varies depending on the type of physical treatment, organic acids used, and their concentration levels (Table 2). The Steam+Citric acid treatment resulted in the highest gross energy content in this study, but the trend is inversely proportional to the concentration of acid used. The lowest gross energy content was obtained in the CP6 treatment, measuring 4,297 kcal/kg. Results from the three-way ANOVA indicate that all treatments and their interactions yield significantly different mean values (Table 1). Ordoñez *et al.* (2022) reported that the gross energy content of reared BSF larvae reached 23.7 MJ/kg or equivalent to 5,660 kcal/kg, a value higher than that obtained in this study. Meanwhile, Hu *et al.* (2017) reported that the formulation of BSF larvae as yellow catfish meal has a gross energy content ranging from 4,473 to 4,609 kcal/kg. In contrast, Wallace *et al.* (2017) obtained a formulation of feed based on BSF larvae in the white larval phase, assuming low chitin content, resulting in a gross energy content of 2,890 kcal/kg. Based on these studies, it can be observed that manipulation of gross energy parameters in the final product of BSF larvae as feed can be achieved by adding organic materials or harvesting larvae at specific developmental stages. Using the proximate composition data of BSF larvae, we developed the following equations to estimate the gross energy (GE) and metabolizable energy (ME) of BSF meal: For Gross Energy (GE):

$$GE(kcal/kg) = (5.65 \times Crude\ protein) + (9.45 \times Crude\ fat) + (4.1 \times Carbohydrates)$$

In addition, Metabolizable Energy (ME) was estimated using an empirical equation adapted from the literature (NRC, 1994): $ME(kcal/kg) = 0.96 \times GE - (4.31 \times Crude\ fiber)$. In order to consider insects as an alternative protein source, it is crucial to investigate the impact of digestion on their protein content. The bioavailability of protein relies on the digestibility of the protein source, predominantly influenced by the extent of protein hydrolysis post-digestion and the intestinal capacity for amino acid absorption. The digestibility of a feed ingredient reflects the extent of its nutritional value. A lower digestibility value indicates lower nutritional benefits, while higher digestibility corresponds to higher nutritional value. In this study, we assessed the crude protein digestibility (CPD) of both treated and untreated BSF larvae subjected to physical and chemical treatments through *in vivo* approach. Only chemical treatments using various organic acids yielded significant results, as indicated in Table 3. The CPD values for our products ranged from 64.1% (CP2) to 72.0% (CA2) (Table 4). Moreover, the highest CPD was observed in control BSF

larvae treated with acetic acid, followed by a similar trend in steamed samples. The results demonstrated that acetic acid hydrolysis led to superior CPD values, surpassing the effects of citric and propionic acids, with statistical significance. Other studies have reported diverse CPD parameters for *H. illucens* larva-based meals, with values of 67.3%, (Marono *et al.,* 2015), 81.6%, (Arango *et al.*, 2004), 87.7% (Bosch *et al.,* 2014), and 89.7% (Bosch *et al.,* 2016).

Table 3. Percent digestibility performance of *H. illucens* larvae after different physicochemical treatments as feeds to broiler chickens.

Source of variation	Digestible crude protein (%)		Digestible energy (%)		Digestible organic matter (%)	
	F	***P***	***F***	***P***	***F***	***P***
P	0.02	0.89	1.47	0.23	0.51	0.48
Ch	96.84	*	3.41	0.05	42.47	*
Co	1.41	0.26	0.16	0.86	0.50	0.61
P×Ch	1.96	0.16	0.65	0.53	1.78	0.18
P×Co	0.35	0.71	0.18	0.83	1.16	0.33
Ch×Co	0.54	0.71	0.19	0.94	0.88	0.48
P×Ch×Co	0.38	0.82	0.21	0.93	1.95	0.12

Data compare effects of physical (*P*), chemical (*Ch*) and concentration (*Co*) treatments on treated *H. illucens* larval powder performance. Significant effects were tested using three-way ANOVA followed by multiple comparisons using Tukey's test. * $P \leq 0.05$.

Table 4. Digestibility performances of *H. illucens* larvae after different physicochemical treatments as feeds to broiler chickens.

Group(s)	Digestible crude protein (%)	Digestible energy (%)	Digestible organic matter (%)
CA2	72.0±1.85^{a}	63.3±3.04^{a}	67.0±0.53^{a}
CA4	71.8±1.29^{a}	63.2±1.68^{a}	66.6±0.22^{ab}
CA6	71.6±1.05^{a}	63.4±1.77^{a}	66.4±0.88^{abc}
CC2	64.4±0.42^{d}	62.8±1.27^{a}	63.0±0.84^{e}
CC4	65.9±1.78^{d}	64.0±0.64^{a}	63.9±0.74^{cde}
CC6	66.5±1.69^{bcd}	63.1±1.11^{a}	64.8±1.10^{abcde}
CP2	64.1±0.55^{d}	61.4±1.25^{a}	64.3±1.11^{bcde}
CP4	65.3±0.69^{d}	61.7±1.54^{a}	65.4±0.74^{abcde}
CP6	65.4±0.55^{d}	61.6±1.23^{a}	64.7±0.78^{abcde}
SA2	70.4±0.23^{abc}	63.2±1.46^{a}	66.2±1.20^{abc}
SA4	70.9±1.51^{ab}	63.7±1.40^{a}	66.1±0.76^{abc}
SA6	70.6±1.48^{ab}	63.6±1.46^{a}	66.6±0.91^{ab}
SC2	65.4±1.69^{d}	63.6±1.19^{a}	64.5±1.27^{abcde}
SC4	66.9±1.72^{bcd}	63.5±2.09^{a}	64.1±0.68^{bcde}
SC6	66.1±1.86^{cd}	63.3±1.51^{a}	63.5±0.72^{de}
SP2	65.5±1.59^{d}	62.6±1.71^{a}	65.4±0.56^{abcde}
SP4	65.3±2.04^{d}	62.2±2.00^{a}	65.9±0.51^{abcd}
SP6	65.4±1.69^{d}	63.6±1.19^{a}	65.1±0.47^{abcde}

Note: Control+Acetic acid 2% = CA2, Control+Acetic acid 4% = CA4, Control+Acetic acid 6% = CA6, Control+Citric acid 2% = CC2, Control+Citric acid 4% = CC4, Control+Citric acid 6% = CC6, Control+Propionic acid 2% = CP2, Control+Propionic acid 4% = CP4, Control+Propionic acid 6% = CP6, Steam+Acetic acid 2% = SA2, Steam+Acetic acid 4% = SA4, Steam+Acetic acid 6% = SA6, Steam+Citric acid 2% = SC2, Steam+Citric acid 4% = SC4, Steam+Citric acid 6% = SC6, Steam+Propionic acid 2% = SP2, Steam+Propionic acid 4% = SP4, Steam+Propionic acid 6% = SP6. Values with different letters denote a statistical significant at 5% within the same column or parameter. Bold numbers show the highest value in each parameter.

In addition to the CPD parameters, we investigated the digestible energy (DE) and digestible organic matter (DOM) of our samples. The highest DE value was recorded in CC4 (64%), while the lowest was observed in CP2 (61.4%) as illustrated in Table 4. Statistical tests showed no significant differences among the treatments (Table 3). The DE values in this research are comparable to the findings of Rambet *et al.* (2016),

who reported maggot energy digestibility in broiler chicken diets as 62.03% to 64.77%, with the best results obtained from substituting fishmeal up to 25% in the feed. The slightly lower digestibility values in this study can be attributed to the use of a single feed, specifically post-treated BSF larvae, without the addition of other feed ingredients. There are numerous factors that can contribute to variations in digestibility values due to the diversity of feed ingredients and formulations in each treatment. Information and research data on the digestibility of BSF larvae in single diets are limited. The energy content and digestibility depend significantly on the fat and chitin content, with BSF larvae having a relatively high fat content, reaching up to 39%. Untreated BSF larvae meal has been used in broiler chicken diets as a substitute for conventional, often expensive feed sources such as fishmeal and soybean meal. However, optimal usage is typically less than 100% in feed substitution, possibly due to inadequate processing leading to reduced palatability, as the dark color of BSF larvae meal is less attractive to and disliked by poultry (Bamgbose, 1999).

Regarding DOM, the highest value was found in CA2 (67%), with the lowest in CC2 (63%) as illustrated in Table 4. Similar to the CPD parameter, significant differences were only noted among chemical interactions, and acetic acid treatments emerged as the most effective factor, whether applied to control or steamed samples, in yielding the best DOM value. Acetic acid, being a mild organic acid, has the potential to improve the breakdown of complex organic compounds present in BSF larvae, making them more easily digestible. This acid can aid in the hydrolysis of carbohydrates, fats, and proteins, thereby increasing the availability of organic matter that is readily digestible. For a more comprehensive understanding, a correlation analysis was conducted for each parameter.

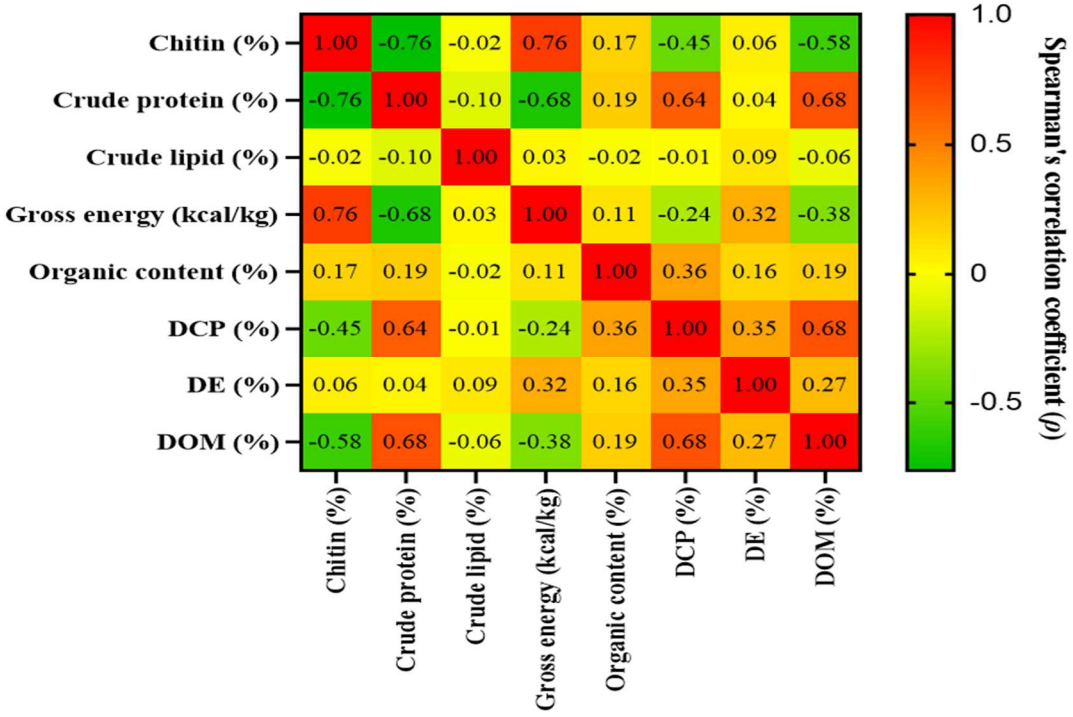

Figure 2. Spearman's correlation heatmap showing the correlation between proximate composition of *H. illucens* larvae after treatments and digestibility in broiler chickens

Digestible crude protein = DCP, Digestible energy = DE, Digestible organic matter = DOM.

Figure 2 illustrates the Spearman's correlation coefficients between the measured chemical characteristics and the biological performance or *in vitro* digestibility of *H. illucens* larva meals. Chitin exhibited a strong negative correlation with crude protein ($P<0.05$) and moderate negative correlations with DCP ($P<0.05$) and DOM ($P<0.05$), while showing a strong positive correlation with gross energy ($P<0.05$). The strong positive correlation between chitin and gross energy is due to the contribution of chitin to caloric density, despite its indigestibility in monogastric animals, for example broiler chickens. The effect of chitin on energy utilization and growth performance in chicken feed is reported in mixed results. Despite an apparent metabolizable energy (AME) value of 8.97 MJ/kg, chitin inclusion showed no significant effect on weight gain or feed efficiency,

though improvements in feed conversion and nitrogen retention were observed at 0.5–1.0 g/kg in some studies (Hossain and Blair, 2007; Shi *et al.,* 2005). Crude protein demonstrated a strong and significant positive correlation (P<0.05) with DCP and DOM, but a negative correlation with gross energy. In this study, crude lipid and organic matter did not exhibit any strong or significant correlations with all variables. Regarding biological parameters, the DE values indicated a lack of significant association with all variables obtained from proximate characteristics. Average digestibility of food from insects ranges from 45% to 66%, which is generally lower than plant-based protein due to chitin interfering with protein adsorption in the digestive system of livestock (Longvah *et al.*, 2011). This emphasizes the significance of estimating chitin content when using insect meals in animal nutrition. The fiber content of insects, measured as acid detergent fiber (ADF), primarily consists of chitin with a significant amount of associated cuticular proteins (Merzendorfer, 2014). Chitin is not broken down and absorbed in the small intestine, potentially impacting protein digestibility (Schiavone *et al.*, 2017). This aligns with our results, highlighting chitin as the primary factor influencing the digestibility properties of our products. The correlation between chitin content and crude protein reveals a strong and negative association, suggesting that higher chitin content is linked to a reduction in crude protein levels, and vice versa. This phenomenon can be explained by the fact that chitin is a non-soluble polymer containing nitrogen, potentially leading to an underestimation of protein solubility. Such underestimation may occur if nitrogen measurements were not taken into account during the conversion to protein content (Soetemans *et al.*, 2019).

Conclusions: The physical treatments of steaming and acid hydrolysis using different concentrations of acetic, citric, and propionic acids produced distinct effects on the biological performance and proximate composition of BSF larva meal. Among these, chemical treatment significantly influenced most parameters, with acetic acid (2%) proving most effective in enhancing the nutritional quality of the feed and reducing chitin content combined with no physical treatment (control). Biological analyses confirmed that acetic acid treatment resulted in improved digestibility, particularly for crude protein and organic matter. Correlation analyses further supported that chitin negatively impacts feed digestibility. Overall, this study highlights BSF larvae, especially when treated with acetic acid, as a promising high-nutrition, low-chitin feed option for broiler chickens.

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