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Research article

Impact of *Pichia manshurica* UNJCC Y-123 and *Pichia cecembensis* UNJCC Y-157 on Fermentation of Maggot (*Hermetia illucens*) Growth Media for Enhanced Broiler Chicken Carcass Quality

Dalia Sukmawati[1]*, Sarah Dewi Wardah[1], Mohamad Isnin Noer[1], Atin Supiyani[1], Roro Anggraini[1] and Hesham El Enshasy[2]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Jakarta, Building KH. Hasjim Asj'ari, 9th Floor, Rawamangun Muka Street, East Jakarta, 13220, Indonesia

[2]Innovation Centre of Agritechnology in Advanced Bioprocessing (ICA), Universiti Teknologi Malayisa (UTM), Pagoh Campus, Johor, Malaysia

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Abstract

Broiler chickens are widely used as livestock due to their relatively short growth period, enabling rapid turnover and efficient meat production. Black soldier fly (BSF) maggot flour is a potential protein source that can be used to enhance broiler productivity. This study aimed to evaluate the effects of fermentation using a yeast combination (*Pichia manshurica* UNJCC Y-123 and *Pichia cecembensis* UNJCC Y-157) on BSF maggot biomass, waste reduction index (WRI), feed conversion efficiency, and nutritional quality, as well as its impact on broiler carcass percentage during the starter period. The experiment employed a completely randomized design (CRD) and data was analyzed using descriptive statistics, ANOVA, and Duncan Multiple Range Test (DMRT) at the 95% confidence level. Toxicity testing showed both yeasts were non-toxic with LC_{50} > 1000. Synergism tests revealed that the two yeasts had a synergistic effect. Fermentation of BSF maggot media with these yeasts significantly influenced ($P<0.05$) maggot biomass and feed conversion efficiency but did not significantly affect the waste reduction index. The fermentation also had a significant effect ($P<0.05$) on the nutritional content of BSF maggot flour, improving parameters such as moisture, ash, crude fat, crude fiber, and crude protein content. Furthermore, supplementation of broiler feed with fermented BSF maggot flour significantly increased ($P<0.05$) the carcass percentage, reaching up to 60%. These findings indicate that fermentation using *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 enhances the quality of BSF maggot flour and supports its use as a sustainable protein source in broiler feed formulations.

Keywords: BSF maggot flour; broiler chicken carcass; fermentation; *P. manshurica*; *P. cecembensis*

*Corresponding author: E-mail: Dalia-Sukmawati@unj.ac.id
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1. Introduction

Broiler chickens are widely used as livestock because they have the advantage of relatively short maintenance time, able to grow quickly, and produce good quality meat (Tiwari et al., 2024). According to Hertiningsih et al. (2022), broiler meat contains amino acids that can easily digested by the body and has a nutritional composition of 18.6% protein, 15% fat, 65.95% water, and 0.79% ash. These advantages make the high value of broiler chicken consumption among the public.

To achieve optimal broiler chicken performance, strategic feed management efforts are needed to ensure maximum productivity. This can be achieved by paying attention to the nutritional quality of the feed and implementing appropriate feeding strategies (Utama et al., 2024). Quality chicken feed should contain the main nutritional components, high protein and energy. Feed is one of the factors that determine broiler meat quality (Prastyo & Kartika, 2017).

Broiler feed requirements vary depending on the period of rearing. The broiler rearing period is divided into starter period (0-3 weeks) and finisher period (4-6 weeks) (Budiansyah et al., 2023). Broilers in the starter period require feed of 40-70 g with a protein requirement of 23% (Wantulla et al., 2023). Feed that can optimize broiler growth in the starter phase is feed that has a high protein content (Utama et al., 2023).

The problem with broiler farmers is the high price of feed. In broiler farming, feed costs account for 70% of production costs (Hidayat et al., 2020). To overcome this problem, it is necessary to substitute feed with other ingredients. One of the substitute feed ingredients with high protein content in feed is fishmeal. However, the quality of fishmeal is uncertain, its availability is limited, and its price is unstable (Rambet et al., 2016). Therefore, alternative feed ingredients are needed as a source of protein for broiler chickens.

Maggot flour is made from BSF maggots that are dried and pulverized until they have a fine texture like flour (Sukmawati et al., 2023). BSF maggot has a high nutritional content, including 45-50% protein and 24-30% fat (Muhayyat et al., 2016). The use of BSF maggot flour can be combined with natural additives such as probiotic microorganisms to produce healthy broiler meat with low fat and high protein (Zhang et al., 2023). The nutritional content of BSF (black soldier fly) maggots is influenced by the composition of nutrients in the growth media (Sukmawati et al., 2023). The types of growing media commonly used for BSF maggots are vegetable and fruit waste (Tripathi et al., 2020). Tofu pulp can also be used as a growing medium for BSF maggots (Surianti et al., 2020). In addition to the type of growth medium used, the fermentation process in the growth medium can also affect the protein content in dried BSF maggots (Mumtaz et al., 2022).

Fermentation with microorganisms increases the nutritional value of the growth medium, providing BSF maggots with enough organic matter to support their growth (Li et al., 2023). Research by Mumtaz et al. (2022) showed that the growth medium of tofu pulp fermented with probiotic microorganisms had a significant effect on the protein content of BSF maggots. *Pichia cecembensis* is reported as a yeast that has potential as a probiotic agent.

This study aimed to determine the effects of the addition of yeast *P. manshurica* Y-123 and *P. cecembensis* Y-157 in the fermentation process of maggot BSF (black soldier fly) on the wet biomass, dry biomass, waste reduction index and feed digestibility efficiency, and the nutritional content of BSF maggot flour, particularly crude protein, crude fiber, crude fat, moisture content, and ash content. The BSF maggot flour that had the most nutritional content in accordance with the needs of broiler chickens was used as a

substitute feed. BSF maggot meal was given to broiler chickens to determine its effect on broiler carcass percentage during the starter period.

2. Materials and Methods

2.1 Study site and experimental design

The research was conducted at the Microbiology Laboratory Campus A, 9th floor, Biology Study Program, Faculty of Mathematics and Natural Sciences, State University of Jakarta and Global Food Laboratory, Bekasi from July to December 2023. An experimental method with a CRD was used in this research. The fermentation process of the BSF growing media was carried out using a completely randomized design (CRD) with 5 treatments and 5 replications based on Federer's calculation (n-1)(t-1) ≥ 15; therefore, 25 experimental units were obtained. Feeding of BSF maggot meal substitution to broiler chickens was carried out using a CRD consisting of 3 treatment groups and 9 replications based on Federer's calculation (n-1)(t-1) ≥ 15, resulting in 27 experimental broiler chickens.

2.2 Toxicity activity test

Toxicity activity testing of the yeast isolates was carried out based on Wulansari et al. (2022) in three stages, namely rejuvenation of yeast cells, making of yeast cell suspensions, and feeding of yeast cell suspensions to shrimp larvae. The method used was the Brine Shrimp Lethality Test using shrimp larvae (*Artemia salina* Leach). The number of shrimp larvae used was 250 which were divided into several groups with different concentrations of yeast (1000, 750, 500, 250, and 0 ppm). Each test group used as many as 10 shrimp larvae with 5 replicates. The toxicity activity of yeast isolates was observed after 24 h of exposure. If the LC50 value is less than 1000 ppm, it indicates the nature of toxicity or toxic activity. Vial tubes containing shrimp larvae that were still alive and dead after 24 h of exposure to the yeast were counted. The proportion of shrimp larvae mortality was calculated using the following formula:

$$\% \text{ Mortality} = \frac{Dead\ larvae\ total}{Larvae\ total}\ x\ 100\% \quad (1)$$

2.3 Synergism test

A yeast synergism test was conducted based on the method of Syamsia et al. (2021). The two yeasts, *P.* manshurica UNJCC Y-123 and *P. cecembensis* UNJCC Y-157, were streaked in close proximity on agar media using the scratch method to allow direct interaction at the point of intersection. Incubated for 24 h and observed a clear zone or zone of inhibition between the two isolates at their point of intersection.

2.4 Black soldier fly egg hatching

BSF egg hatching was carried out based on Fajri and Hamid (2021) by using a hatching medium in the form of rice bran mixed with water. A total of 12.5 g BSF eggs were used in this study. The hatching medium used was 400 g of rice bran with the addition of 400 mL of water with thorough mixing. Then, as much as 0.5 g of BSF eggs was hatched on the media. The BSF maggot were ready to be transferred to the grow-out medium at the age of 10 days.

2.5 Fermentation and harvesting of BSF maggots

Yeast propagation was initiated using yeast malt broth (YMB) composed of 3 g yeast extract, 3 g malt extract, 5 g peptone, and 10 g glucose per liter. The yeast was incubated at 30°C with continuous stirring at 35 rpm for 48 h. After the pre-culture period, the suspension was adjusted to an optical density (OD_{600}) of 2.0, measured with a spectrophotometer. A 10% (v/v) inoculum was then transferred into a sterilized pineapple extract medium and incubated at room temperature (27-30°C) without agitation for another 48 h to allow yeast adaptation. In parallel, four agro-industrial wastes—cabbage (*Brassica oleracea*), chicory (*Cichorium intybus*), papaya (*Carica papaya*), and tofu pulp—were selected, thoroughly washed, and chopped using a mechanical chopper. Each waste was weighed equally (1:1:1:1 ratio) to form a composite substrate of 2 kg (500 g each). The homogenized waste mixture was subjected to four fermentation treatments based on substrate-to-yeast inoculum ratios: (T1) 1:3, (T2) 3:1, (T3) 2:3, and (T4) 3:2. For example, the 1:3 treatment consisted of 500 g waste and 1500 mL inoculum. Each treatment was mixed thoroughly and incubated for 72 h at room temperature without agitation.

Following fermentation, the treated substrates were used as feed for black soldier fly (BSF, *Hermetia illucens*) larvae previously reared on standard feed until day 16. The fermented substrate was applied from day 16 to day 19 of the larvae's development—prior to their pre-pupal stage. On day 19, maggots were harvested, manually separated from the residual substrate, and washed. The growth performance of BSF larvae was assessed using three parameters: larval biomass (g), waste reduction index (WRI), and efficiency of conversion of digested feed (ECD). WRI was calculated as the difference between the initial substrate and the residual waste divided by the number of feeding days. ECD was determined as the ratio of biomass gain to the total amount of digested substrate, expressed as a percentage. These indicators were used to evaluate the efficiency of organic waste fermentation and its effect on larval development and bioconversion. Measurement of growth performance in the BSF maggots included biomass, waste reduction index (WRI), and efficiency of conversion digested feed (ECD) with formulas below:

$$\text{Maggot Biomass} = \text{Maggot final biomass} - \text{Maggot initial biomass} \quad (2)$$

$$\text{WRI} = \frac{Total\ growth\ media\ decrease}{Total\ time\ maggot\ eating\ the\ growth\ medium}\ x\ 100 \quad (3)$$

$$\text{ECD} = \frac{\text{Total increase in maggot weight}}{(\text{Amount of growth medium} - \text{Total residue})}\ \text{x}\ 100\% \quad (4)$$

2.6 Proximate analysis

The BSF maggots were killed by soaking in hot water for 1 min. Then, the BSF maggots were put into an oven at 50ºC for 12 h. Next, the dried BSF maggots were pulverized until a texture was like flour. The BSF maggot flour was subjected to proximate analysis. Proximate analysis was conducted using the analytical services of the Pusat Bioteknologi (Biotech Center), Institut Pertanian Bogor. The proximate analysis included crude protein, crude fiber, crude fat, ash content, and moisture content.

2.7 Carcass percentage of starter period broiler chicken

Each broiler chicken was given a marker bracelet on its leg to facilitate the calculation of the weight of each individual. When the chickens arrived, they were given 5% sugar water to recover the energy lost during the trip. Broiler chickens were kept in 130 L container boxes measuring 76 x 53.2 x 45 cm with a cardboard floor covered with plastic, which was replaced every day. Each cage unit contains 9 broilers. Acclimatization was conducted until the chickens were 7 days old at 32-33°C and fed commercial feed only. Commercial feed was given based on chicken rearing guidelines from PT Charoen Pokphand Indonesia according to the daily weight of broilers. Drinking water was given ad libitum. Vitachick was given every three days. Substitution feeding in the form of 5% BSF maggot flour from the total feed was done by mixing it in commercial feed. The 48-hour-old yeast suspension grown on physiological NaCl media was given to the broiler chickens by force feeding using a syringe every two days. Every morning before feeding, the daily body weight was weighed on each individual broiler. Then, the remaining feces were separated and dried in an oven for 12 h at 60°C. Starter period broilers were harvested and slaughtered at 21 days of age. Calculation of broiler carcass weight includes measurement of broiler slaughter weight without the weight of feathers, head, feet, and offal. The percentage of carcass weight was obtained by calculating based on Muchlis et al. (2021) as follows:

$$\textit{Carcass Weight Percentage} = \frac{Carcass\ weight}{Live\ weight}\ x\ 100\% \quad (5)$$

2.8 Data analysis

The toxicity activity data of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 were analyzed using SPSS Probit to obtain LC50 values. Synergism activity data of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 were analyzed descriptively. The growth data of BSF maggots, namely wet and dry biomass, waste reduction index, feed digestibility efficiency, and nutritional content consisting of crude protein, crude fiber, crude fat, moisture content, and ash content obtained were analyzed using analysis of variance at the 5% level and the results of the analysis that showed a significant effect were continued with Duncan's Multiple Range Test (Smets et al., 2020). The growth data of broiler starter period, namely feed consumption and dry matter digestibility, were analyzed descriptively. Meanwhile, carcass percentage was analyzed using analysis of variance at the 5% level and the results of the analysis that showed a significant effect were continued with DMRT (Smets et al., 2020). Data analysis was performed using the IBM SPSS V27 program.

3. Results and Discussion

3.1 Toxicity activity

The toxicity activity test results can be used as a preliminary test of a potentially toxic substance. Mortality percentage shows the mortality rate obtained from summing the dead shrimp larvae from each treatment (Jelita et al., 2020). Based on Table 1, there was 0% larval mortality in the control treatment (0 ppm), and an increase in the percentage of shrimp larval mortality as the concentration increased. The highest percentage level of shrimp larvae mortality occurred at a concentration of 1000 ppm. The higher the

concentration of yeast, the more chemical compounds it contains. The control treatment (0 ppm) had no dead shrimp larvae.

The results of toxicity testing are expressed in the form of LC50 (lethal concentration 50), where the LC50 value is the concentration that can kill 50% of all test organisms after 24 h of exposure. The toxicity test results of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 showed that both yeasts were non-toxic because their LC50 values were higher than 1000 ppm. The pellet fraction of *P. manshurica* UNJCC Y-123 had an LC50 value of 2702.54 ppm and the supernatant of 4621.68 ppm. Meanwhile, the pellets of *P. cecembensis* UNJCC Y-157 had an LC50 value of 5366.87 ppm and the supernatant 7100.81 ppm. From the LC50 value obtained, it can be concluded that the tested yeasts from both pellets and supernatant were in a non-toxic category because they had LC50 > 1000ppm (Smetana et al., 2021).

The LC50 values obtained from the pellets and supernatants of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-123 indicated that the two types of yeast were non-toxic and could be applied further for the fermentation of BSF maggot growing media and also as a natural additive for broiler starter period. The mechanism of action of the compounds (alkaloids, flavonoids, saponins, and tannins) contained in the supernatant and yeast pellets involves larval stomach poisoning. It is known that if these compounds enter the body of larvae, their digestive system gets disrupted. In addition, these compounds can also inhibit taste receptors in the larval mouth area which results in the larvae failing to get a taste stimulus and not being able to recognize their food so that the larvae die of starvation (Jelita et al., 2020).

Table 1. Results of the toxicity test of the yeasts *P. manshurica* and *P. cecembensis*

Yeast Combination	Yeast Concentration (Ppm)	Larvae Total	Larvae Dead Total	Mortality (%)	LC_{50} (ppm)
Pichia manshurica UNJCC Y-123 (*Pellet*)	1000	50	7	14	2702.54
	750	50	4	8	
	500	50	3	6	
	250	50	2	4	
	0	50	0	0	
Pichia manshurica UNJCC Y-123 (*Supernatant*)	1000	50	6	12	4621.68
	750	50	4	8	
	500	50	2	4	
	250	50	2	4	
	0	50	0	0	
Pichia cecembensis UNJCC Y-157 (*Pellet*)	1000	50	6	12	5366.87
	750	50	4	8	
	500	50	3	6	
	250	50	2	4	
	0	50	0	0	
Pichia cecembensis UNJCC Y-157 (*Supernatant*)	1000	50	6	12	7100.81
	750	50	5	10	
	500	50	4	8	
	250	50	1	2	
	0	50	0	0	

3.2 Synergism activity

Synergism activity testing was carried out on isolates of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 with the scratch method. The yeasts were scratched tangentially aseptically on YMA media so that the scratches between the yeasts met each other. After the inoculation process was carried out, the yeasts were then incubated for 48 h. The results showed that *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 were compatible, as no inhibition was observed at the intersection of the streaks on YMA medium. This indicates that the two yeast strains can grow in proximity without antagonistic interaction (Figure 1).

Figure 1. Test of synergistic activity of *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 by the scratch method

The synergism activity test is important because the basis of this research is the development of a consortium-based yeast starter. A consortium is synergistic (compatible) if there is no inhibition zone (clear) at the meeting of the scratches between the two isolates (Silitonga et al., 2022). The absence of an inhibition zone indicates that the yeasts in the consortium can grow together without negating each other.

The results of the yeast synergism test between *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 showed synergy because no inhibition zone was found. This shows that the work of enzymes in each yeast supported and complements each other to survive with nutritional sources in the media (Siahaan & Mullo, 2021).

According to Wulansari et al. (2022), the consortium of an isolate that associates synergistically is able to produce a better product than a single isolate. Based on the results of this study, the yeast isolates *P. manshurica* and *P. cecembensis* establish a synergistic relationship. This can support the potential of *P. manshurica* and *P. cecembensis* originally isolated from cocoa bean fermentation, suggesting that their natural compatibility may be used as a waste fermentation inoculum starter for preparing the growing medium of black soldier fly (BSF) maggots.

3.3 BSF maggot growth

The wet biomass or harvest weight of BSF (black soldier fly) maggots was obtained from the weighing of fresh maggot that had been separated from the growth medium on the 19th day of maintenance. Based on the data in Table 2, the analysis of variance showed that fermentation of BSF maggots in the growth medium had a significant effect ($P < 0.05$) on the biomass of BSF maggots.

Table 2. Wet biomass, dry biomass, WRI, and ECD of BSF maggots on different growing media

Fermentation Treatment	Wet Biomass (g)	Dry Biomass (g)	WRI (%)	ECD (%)
Control (Without fermentation)	958±26.31[a]	228.2±15.77[a]	6.44±0.14[a]	23.96±0.85[a]
P. manshurica UNJCC Y-123 (75%) + *P. cecembensis* UNJCC Y-157 (25%)	1087.2±43.88[b]	283.8±3.15[b]	6.09±0.10[a]	27.71±1.13[b]
P. manshurica UNJCC Y-123 (25%) + *P. cecembensis* UNJCC Y-157 (75%)	1139.4±37.61[bc]	268±7.85[b]	6.63±0.09[a]	28.03±1.43[b]
P. manshurica UNJCC Y-123 (60%) + *P. cecembensis* UNJCC Y-157 (40%)	1169.2±21.84[bc]	277.6±7.87[b]	6.76±0.26[a]	29.2±0.92[b]
P. manshurica UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%)	1192.8±18.76[c]	299.6±12.61[b]	6.6±0.25[a]	28.71±1.13[b]
Sig.	0.001	0.001	0.163	0.026

The wet biomass of BSF maggot was influenced by the nutrients in the growth media. According to Mokolensang et al. (2018), maggot growth is supported by providing growth media with sufficient nutrients. The average wet biomass of BSF maggots in the treatment of yeast fermented growth media was higher than that of BSF maggots on growth media without fermentation. This proves that the fermentation process with yeast inoculum was able to increase nutrients in the growth medium. In line with research by Sukmawati et al. (2022) which stated that fermentation of vegetable waste as a growing medium for BSF maggot increased the nutrient content of waste.

The highest average value of BSF maggot biomass was from the treatment of fermented growth media with *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%). The composition of the yeast inoculum was the best composition in producing BSF biomass. This was probably due to the fermentation process that helped the process of breaking down organic waste into simpler forms so that the fermented growth medium was more easily digested by the BSF maggots.

The enzymes activity produced by yeast during the fermentation process helps break down complex compounds into simpler ones. According to Sukmawati et al. (2023), *P. manshurica* UNJCC Y-123 had the ability to produce cellulase enzymes that could break down cellulose. Cellulase enzymes can help the process of decomposing crude fiber in organic waste. In addition, *P. cecembensis* UNJCC Y-157 acts as a probiotic yeast that can improve the nutritional quality of BSF maggot growth media. Probiotics can improve performance by increasing endurance. The increased endurance was utilized by the BSF maggots to digest the growth media well. Based on the value, *P. cecembensis* UNJCC Y-157 60% provided more wet biomass than *P. cecembensis* UNJCC Y-157 40% because its concentration can meet the nutritional needs of the maggots. Feed that is high in nutrients and easy to digest leads to the production of biomass in a process called bioconversion (Permana et al., 2021).

The fresh BSF maggots for which the wet biomass value had been calculated were then dried to obtain their dry biomass value. The dry biomass of BSF maggots obtained

from drying the fresh BSF maggot that had been harvested and dried in an oven for 12 h at 50°C. The average results of dry biomass of BSF maggots are presented in Table 2. Based on the data in Table 2, the results of the analysis of variance showed that the effect of fermentation on BSF maggot growth media in terms of dry biomass was significantly different ($P < 0.05$). The results of Duncan's test showed that dry biomass in all fermentation treatments of BSF maggot growth media was significantly different from dry biomass of BSF maggot on growth media without fermentation (control). However, differences among the fermentation treatments with various concentrations of growth media were not statistically significant ($P > 0.05$)

Dry biomass was obtained from wet biomass that had undergone drying. The average value of dry biomass that differs between treatments is due to differences in nutrient content and feed utilization efficiency of each treatment. Therefore, the food substances digested by BSF maggots to form body tissues are also different, thus affecting their dry biomass (Utama et al., 2023).

The waste reduction index (WRI) is an indicator of the extent to which BSF maggots reduce organic waste used as growth medium, calculated at the end of cultivation (harvesting) period. The value of the WRI is directly proportional to the ability of BSF maggots to reduce growth media. Based on the data in Table 2, the results of the analysis of variance showed that the fermentation treatment of the BSF maggots growth media gave a different effect that was not significant ($P>0.05$) on the waste reduction index. These results indicate that the fermentation treatment had no statistically significant impact on the waste reduction ability of BSF maggots.

The growth medium in this study consisted of a mixture of cabbage, chicory, tofu pulp and papaya waste in the same ratio. The waste contained high fiber, so it needed a fermentation process to break down cellulose and lignin. The yeast *P. manshurica* and *P. cecembensis* were reported to be able to produce cellulase enzymes that played a role in the breakdown of fiber in waste. According to Permana et al. (2021), the fermentation process broke down waste so that it was more easily digested by BSF maggots.

Although the various fermentation treatments did not have a significantly different effect, fermentation produced a different WRI value when compared to the control treatment. This was probably because BSF maggots that were given fermented growth media were able to more easily digest waste and absorb nutrients so that the reduced waste was higher. Meanwhile, unfermented growth media made it more difficult for BSF maggots to digest waste, so the reduction value was lower.

The fermentation process of the growth media with *P. manshurica* and *P. cecembensis* was able to reduce crude fiber in the growth media with the help of cellulase enzymes produced so that the growth media could be more easily digested by the BSF maggots. The decrease in fiber content in the substrate that is degraded by enzymes produced by microbes causes the substrate to be more easily digested by BSF maggots (Liu et al., 2015). A high waste reduction index value implies a high ability of the larvae to reduce the substrate.

The efficiency of conversion of digested feed (ECD) is a measure of the efficiency of the growth medium consumed and digested by BSF maggots during the rearing period. This value illustrates the level of efficiency of BSF maggots in converting the growth medium into biomass. Based on the data in Table 2, the results of the analysis of variance showed that the effect of fermentation on maggot BSF growth media was significantly different ($P < 0.05$) on the digestibility efficiency of BSF maggot feed. Duncan's test results showed that the efficiency values in all fermentation treatments were significantly different from those without fermentation (control).

The efficiency of conversion digested feed value reflects the quality and amount of growth media consumed by BSF maggots for growth. Based on the results in Table 2, it can be seen that the fermentation treatment gave significantly different results from the treatment without fermentation. This shows that the fermentation process was able to increase the digestibility of waste as a growth medium for the BSF maggots so that the available growing medium could be digested properly by BSF maggots. The fermentation process is able to break down the crude fiber contained in waste so that the digestibility of BSF maggots can increase (Damanik et al., 2024).

Growth media consumption can affect the digestibility efficiency of BSF maggot feed. A low consumption of growth media results in the amount of growth media consumed and converted into BSF maggot biomass decreasing, causing the digestibility efficiency value of BSF maggot feed to be low. According to Cimini & Moresi (2021), a low value of efficiency conversion of digested feed in BSF maggot is related to the quality of nutrients in the growth media. A low quality of nutrients in the growth medium canl result in a low efficiency of conversion digested feed value.

The fermentation process of growth media using *P. manshurica* and *P. cecembensis* yeasts was able to improve the nutritional quality of the growth media resulting in efficiency of conversion of digested feed values that were significantly different from BSF maggot growth media without fermentation. The ability of *P. cecembensis* as a probiotic yeast can improve the nutritional quality of the growth media so that the digestibility of BSF maggots becomes higher.

3.4 Nutritional content of BSF maggot flour

The dried BSF maggot flour was produced from the pulverization of dried maggots. The BSF maggot flour was analyzed for its nutritional content through the proximate test. The nutritional content parameters measured in this study were crude protein, crude fiber, crude fat, moisture content, and ash content. Crude protein is an important component in animal feed. The results of the analysis of variance in Table 3 showed that each treatment had a significantly different effect ($P < 0.05$) on the crude protein content of BSF maggots.

The results of Duncan's test showed that crude protein levels in the control were significantly different from all fermentation treatments, except for the treatment of *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%). Based on the results in Table 3, the highest crude protein content was obtained in maggots grown on media fermented by *P. manshurica* UNJCC Y-123 40% + *P. cecembensis* UNJCC Y-157 60%, with a crude protein content value of 44.30%. The high protein content was caused by good nutritional quality, which led to the BSF maggots being able to easily digest amino acids.

The highest crude protein content in this study was BSF maggot cultivated on fermented media with *P. manshurica* UNJCC Y-123 40% + *P. cecembensis* UNJCC Y-157 60%, which amounted to 44.30%. The high protein content in this study makes maggots potential as an alternative feed source of protein for livestock, especially poultry. Protein is needed by poultry for the formation of important parts in the body such as muscles, bones, eggs and beaks in chickens. The results of the analysis of variance showed that each treatment gave a significantly different effect ($P < 0.05$) on the crude fiber content of BSF maggot. The results of Duncan's test showed that the crude fiber content in the control was significantly different from all fermentation treatments. Based on the results in Table 3, the highest crude fiber content was obtained in maggots grown on growth media without fermentation with a crude fiber value of 12.24%.

Table 3. Nutritional content of BSF maggot flour on different growing media

Fermentation Treatment	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Water Content (%)	Ash Content (%)
Control (Without fermentation)	38.80±1.55^{d}	12.24±0.43^{c}	28.12±0.005^{e}	11.46±0.12^{c}	5.88±0.020^{a}
P. manshurica UNJCC Y-123 (75%) + *P. cecembensis* UNJCC Y-157 (25%)	38.22±0.01^{c}	8.34±0.27^{b}	24.60±0.33^{c}	10.81±0.09^{b}	12.07±0.06^{d}
P. manshurica UNJCC Y-123 (25%) + *P. cecembensis* UNJCC Y-157 (75%)	37.19±0.13^{b}	3.22±0.45^{a}	22.98±0.095^{b}	10.39±0.05^{a}	10.13±0.20^{c}
P. manshurica UNJCC Y-123 (60%) + *P. cecembensis* UNJCC Y-157 (40%)	36.37±0.05^{a}	3.43±0.31^{a}	20.47±0.030^{a}	11.09±0.16^{c}	21.17±0.19^{e}
P. manshurica UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%)	44.30±0.07^{e}	3.74±0.005^{a}	25.28±0.045^{d}	11.18±0.04^{c}	7.09±0.17^{b}
Sig.	0.000	0.000	0.000	0.006	0.000

From Table 3, the comparison of crude fiber among each treatment was significantly different. The fermentation process in the growth medium assisted by cellulase enzymes from *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 made crude fiber content in maggots lower when compared to maggots cultivated on growth media without fermentation.

The results of the analysis of variance in Table 3 showed that each treatment gives a significantly different effect ($P < 0.05$) on the crude fat content of BSF maggot. The results of Duncan's test showed that the crude fat content in the control was significantly different from all fermentation treatments. Based on the results in Table 3, the highest crude fat content was obtained in maggots grown on growth media without fermentation, with a crude fat value of 28.12%.

The average value of crude fat of BSF maggot cultured on unfermented growth media was significantly higher than that of maggots cultured on fermented growth media. The fat content of maggots in the treatment was influenced by the fat content in the feed media given. The high fat content of the fresh maggots in this study can be utilized by livestock as an energy source.

Based on the results in Table 3, the results of the analysis of variance showed that each treatment had a significantly different effect ($P < 0.05$) on the water content of BSF maggots. Duncan's test results indicated that fermentation treatments with higher proportion differences (75%:25% and 25%:75%) significantly reduced water content compared to control, while treatments with more balanced proportions (60%:40% and 40%:60%) showed water content similar to the control.

The average value of the water content of BSF maggots cultured on growth media without fermentation was higher than the water content of fermented BSF maggots, while

the lowest water content was in the fermentation treatment with *P. manshurica* UNJCC Y-123 (25%) + *P. cecembensis* UNJCC Y-157 (75%). The difference in the average water content of the treatments was influenced by the water content contained in the growth media used. The highest content result was from BSF maggots grown in media in the form of fresh waste without going through the fermentation process.

Based on the data in Table 3, the highest ash content was obtained in maggots grown on media fermented with *P. manshurica* UNJCC Y-123 60% + *P. cecembensis* UNJCC Y-157 40%, with an ash content value of 21.17±0.19, which was significantly different from the control. Based on the results of Duncan's test analysis, all growth media fermentation treatments on ash content were significantly different from the control.

The highest ash content in this study was BSF maggots cultured on *P. manshurica* UNJCC Y-123 (60%) + *P. cecembensis* UNJCC Y-157 (40%) media, which amounted to 21.17%. The average ash content of the control was significantly lower than that of BSF maggots on *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%) fermentation growth media. The ash content reflects the mineral content in the feed, and the higher the ash content, the higher the mineral content. Basically, minerals are inorganic substances that the body needs in small amounts (Purnamasari et al., 2023). The results of the growth of BSF maggots and the nutritional content of BSF flour in this research study were selected and applied for the growth of broiler chickens in the starter period. Based on the SNI 8173-2:2022 standards, broiler chickens in the starter period require feed with nutritional content consisting of 13% moisture content, 9% ash content, 20% crude protein, 5% crude fiber, and 4% crude fat. The BSF maggot flour fermented with *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%) had nutritional content that was quite in accordance with the standard and was 11.18% moisture content, 7.06% ash content, 44.3% crude protein, 3.74% crude fiber, and 25.28% crude fat. In addition, the fermentation treatment of BSF maggot growth media using *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%) also had a significantly different effect on BSF maggot wet biomass, therefore the production of large quantities of BSF maggot flour could be achieved.

3.5 Broiler chicken carcass

Carcass percentage was obtained as the percentage of live chicken weight without head, feet, and offal. The results of the analysis of variance in Table 4 showed that the use of treatments had a significant effect ($P<0.05$) on carcass percentage. The factors that affect carcass percentage are sex, age, body weight, hormones and food.

Table 4. Percentage of starter period broiler chicken carcasses with different feeding treatments

Feeding Treatment	Carcass Percentage (%)
100% commercial feed (control)	56.57±1.85[a]
95% commercial feed + 5% maggot flour	61.69±1.34[b]
95% commercial feed + 5% maggot flour + 10% yeast suspension	66.01±1.05[c]
Sig.	0.001

The treatment with 5% BSF maggot flour and 10% yeast suspension in the feed caused broiler chickens to experience an increase in carcass percentage results compared to the control. This was due to an increase in feed consumption which made the food substances in the feed absorbed properly by the chickens.

Feed consumption affects the nutritional substances needed for broiler growth, thus influencing growth rate and carcass weight percentage. Final live weight is determined by cumulative weight gain throughout the rearing period, with studies showing that higher live weights result in improved carcass percentages (Rumondor et al., 2016). The use of probiotic yeast *P. cecembensis* as a natural additive contributes to increasing the effectiveness of digestive enzymes so that nutritional substances consumed by broiler chickens can be converted properly. Probiotic yeast works by increasing the production of lactic acid and digestive enzymes to increase the absorption of feed nutrients. The absorption of nutrients in the feed can affect the carcass produced (Wang et al., 2019).

4. Conclusions

The toxicity activity of yeast *Pichia manshurica* UNJCC Y-123 and *Pichia cecembensis* UNJCC Y-157 had LC50 value>1000 ppm, so the yeasts were not classified as toxic. Synergism activity showed that *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 were synergistic. Fermentation process with the yeast *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 on the growing medium of black soldier fly maggot had a significant effect ($P<0.05$) on maggot biomass and efficiency of conversion digested feed (ECD) but had no significant effect on the waste reduction index. The fermentation process with yeast *P. manshurica* UNJCC Y-123 and *P. cecembensis* UNJCC Y-157 on the black soldier fly maggot growth media had a significant effect ($P < 0.05$) on the nutritional content of black soldier fly maggot flour including the moisture content, ash content, crude fat content, crude fiber content, and crude protein content. The fermentation treatment of BSF maggot growth media using *P. manshurica* UNJCC Y-123 (40%) + *P. cecembensis* UNJCC Y-157 (60%) also had a significantly different effect on BSF maggot wet biomass and achieved the highest crude protein, so that the production of large quantities of BSF maggot flour could be achieved. Furthermore, feeding black soldier fly maggot flour and yeast suspension combination of *P. manshurica* UNJCC Y-123 + *P. cecembensis* UNJCC Y-157 had a significant effect ($P <0.05$) on carcass percentage.

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6. Authors’ Contributions

D. S. designed and wrote the manuscrip; S. D. W.conduced experiment and analysed data; M. I. N.desaigned the research; A. S. coordinated reseach; H. El E. supervised the experiment; and R.A.i revised the manuscript.

7. Conflicts of Interest

The authors and the institutes where the work was carried out declare that there are no conflicts of interest regarding the publication of this article.

ORCID

Dalia Sukmawati https://orcid.org/0000-0001-9641-9321
Sarah Dewi Wardah https://orcid.org/0009-0005-0160-9760
Mohamad Isnin Noer https://orcid.org/0000-0002-5024-9109
Atin Supiyani https://orcid.org/0000-0002-2279-568X
Roro Anggraini https://orcid.org/0009-0003-3022-5710
Hesham El Enshasy https://orcid.org/0000-0002-9712-2033

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