The Chemical Content of Fermented Black Soldier Fly Larvae with *Trichoderma viride* as an Alternative Protein Source Feed for Native Chickens

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Abstract. *Black soldier fly* larvae or maggot flour contains high crude fibre which makes this raw material difficult to digest. High crude fibre content so that the fermentation process can be carried out as an effort to improve the quality of maggot flour using the fungus *Trichoderma viride* microorganism. This study was conducted to determine the quality of maggot flour fermented with *Trichoderma viride fungus* as an alternative protein source feed for native chickens. The research method used a completely randomized design consisting of 2 factors and 4 replications so that there were 16 analyzes of the experimental unit. Factor I is the level of *Trichoderma viride* (2 and 4%) and the second factor is the time of fermentation (7 and 14 days). The research variables included moisture, ash, crude fibre, crude protein, and extract ether. The results obtained indicate that the use of maggot fermented has a significant effect ($P < 0.05$) on the analysis of moisture, and extract ether, and in the analysis of ash, crude fibre, and crude protein there is no effect ($P > 0.05$). In conclusion, the use of fermented maggot *Black soldier fly flour* can be used as a protein source raw material.

Keywords: Black soldier fly · Fermented · native chicken · Trichoderma viride

1 Introduction

Feed ingredients are one of the determinants of the success of an animal's growth so that it meets its nutritional needs because it can affect the productivity or growth of livestock. Several feed ingredients are quite good so that they can be used as potential raw materials for poultry feed, one of which is maggot flour. The high nutrient content contained in maggots is the crude protein potential of around 41–42% [1]. Black Soldier Fly (BSF) (*Hermetia illucens*, Diptera: *Stratiomyidae*), is one of the various livestock that has begun to cultivate Indonesia widely. These flies originate from South America and then spread to subtropical and tropical regions [2]. Indonesia's tropical climatic

E. Widodo et al. (Eds.): ICESAI 2022, ABSR 28, pp. 438–445, 2023.
https://doi.org/10.2991/978-94-6463-116-6_54

conditions are ideal for black soldier fly cultivation. There are many types of insects that can be exploited as poultry feed ingredients, one of which is black soldier fly. The larvae of black soldier fly contains 44% dry matter, 42% crude protein, and 35% fat, including essential amino acids and fatty acids [3]. Black soldier fly is also straightforward to develop and does not require special equipment. Not only that, but the black soldier fly cycle is too swift. According to [4], black soldier fly life cycle from egg to adult fly lasts around 40–43 days, depending on environmental conditions and the feed media given.

The Larvae of black soldier fly contain chitin polysaccharide of about 8.7%, which can negatively affect the digestibility and utilization of nutrients [5]. The crude fibre is high enough (exceeding the standard for poultry needs) which is 8.53% and crude fat is around 27.30% [6] so that the fermentation process can be carried out as an effort to improve the quality of maggot flour. Reducing the chitin content on organic materials can be performed by fermentation using specific microorganism that are able to degrade chitin [7]. The occurrence of fermentation can cause changes in the properties of feed ingredients due to the breakdown of feed components, The length of fermentation is one of the factors that greatly affect the length of life and the number of microorganisms that proliferate. Trichoderma is one of the fungi that can produce chitinase enzymes that can degrade chitin [8]. *Trichoderma spp,* is known to produce chitinases, β-13-glucanases, and proteases. [9] stated that the fungus *Trichoderma viride* is a producer of complete cellulose enzymes to hydrolyze cellulose and crystals which causes a decrease in crude fibre content. [10] who states that *Trichoderma viride* can increase crude protein and reduce fibre content. [11] stated that crude fat in fermented products using *Trichoderma viride* can also reduce crude fat levels. Based on the quality of post-fermented black soldier fly maggot flour with BSF, it is suspected that it can be used to improve the quality of poultry feed ingredients.

2 Materials and Methods

2.1 Making Potato Destro Agar (PDA) Media [12]

Prepare tools and materials to be used. PDA media of as much as 19.5 g was put into an Erlemmener and dissolved with 500 mL of sterile distilled water, then homogenized using a centrifuge. Erlenmeyer was closed using a tube cover and then sterilized in an autoclave at 121ºC for ± 1 h. Then the media was poured into a sterile petri dish and allowed to solidify. The cup is wrapped so as not to be contaminated and wait for the media to solidify.

2.2 Transfer of Inoculants to Potato Destro Agar (PDA) Media [13]

Prepare bunsen, ose, and *Trichoderma viride* isolates. *Trichoderma viride* isolates were taken using an ossicle or borer and then transferred to a petri dish containing PDA media. The petri dish containing the isolate was wrapped so as not to be contaminated and waited for 1 week until the fungal mycelium grew on the cup.

2.3 Propagation of Inoculants in Organic Media (Maize) [14]

Soak the glutinous corn for 1 day. Weigh every 100 g of corn into a heat-resistant plastic bag. Sterilization of media (corn) by autoclave for ± 1 h. Prepare bunsen, ose and isolate *Trichoderma viride*. Take the *Trichoderma viride* to isolate using ose and then transfer it to organic media. Re-cover the organic media, and incubate for 1 week until the fungus grows on the organic media.

2.4 Cellulotic Microbial Activation Using *Aerator* [9]

Prepare tools and materials to be used, namely Trichoderma viride microbes, molasses, water, and buckets (containers). Molasses was dissolved in water and then mixed with Trichoderma viride in container 1 with as much as 2% and container 2 with as much as 4%. Microbes are activated by using an aerator for 24 h and after that, they are ready to be applied to the maggot flour to be fermented. Fermentation of maggot flour with *Trichoderma viride* [15] which maggot flour weighed as much as 1 kg for each treatment unit. Maggot flour is mixed with activated microbes evenly. Fermentation for treatment 1 for 7 days and fermentation for treatment 2 for 14 days.

2.5 Research Variables

The parameter observed in this study was the content in the proximate analysis of maggot Black soldier fly flour which was fermented using *Trichoderma viride* microbes.

2.6 Statistical Analysis

The study used a factorial design based on a completely randomized design (CRD) consisting of 2 factors and 4 replications, so there were 16 experimental units. The data obtained were analyzed using analysis of variance by the Factorial Design based on a Completely Randomized Design (CRD) [16].

3 Results and Discussion

The results of research on the nutrient content with the addition of inoculants with fermentation time copra meal are presented in Table 1. Table 1 shows the water content of fermented BSF, at the 2% inoculant level of 9.73 ± 0.66 level and the 4% inoculant 9.73 ± 0.58 and 7 days of fermentation 9.19 ± 0.29 and 14 days of 10.27 ± 0.15. Analysis of variance showed that the administration of different inoculant levels (2% and 4%) had no significant effect ($P > 0.05$) on the water content of fermented maggot flour, while with different fermentation times (7 days and 14 days) it showed a significant effect. ($P > 0.05$) on the water content of fermented maggot flour. Duncan's test results showed that the average 7-day fermentation time with a value of 9.19 ± 0.29 was significantly lower than the 14-day fermentation time with a value of 10.27 ± 0.15, which indicated that there was an increase in water content of 1.08%. This is because, at the time of fermentation, microorganisms produce water in their activities, so the

Table 1. The average nutritional value of fermented maggot larvae of *Trichoderma viride* with different fermentation times

Parameters (%)	Number of inoculants (%)	Fermentation time (days)		Average
		7	14	
Moisture	2	9.14 ± 0.28	10.32 ± 0.18	9.73 ± 0.66
	4	9.23 ± 0.33	10.23 ± 0.12	9.73 ± 0.58
	Average	9.19 ± 0.29[a]	10.27 ± 0.15[b]	9.73 ± 0.60
Crude fibre	2	9.77 ± 0.39	9.54 ± 0.64	9.65 ± 0.50
	4	9.87 ± 0.49	9.40 ± 0.33	9.64 ± 0.46
	Average	9.82 ± 0.42	9.47 ± 0.47	9.65 ± 0.47
Crude protein	2%	45.10 ± 1.01	45.13 ± 1.71	45.12 ± 1.31
	4%	43.97 ± 1.83	46.83 ± 1.83	45.40 ± 2.14
	Average	44.54 ± 1.49	45.98 ± 1.70	45.26 ± 1.72
Crude fat	2%	8.20 ± 0.28[a]	8.59 ± 0.25[a]	8.39 ± 0.32[a]
	4%	8.40 ± 0.07[a]	7.85 ± 0.17[b]	8.12 ± 0.31[b]
	Average	8.30 ± 0.21	8.22 ± 0.44	8.26 ± 0.34*
Ash	2%	11.68 ± 0.14	11.45 ± 0.11	11.57 ± 0.17
	4%	11.58 ± 0.14	11.60 ± 0.09	11.59 ± 0.11
	Average	11.63 ± 0.14	11.53 ± 0.12	11.58 ± 0.14

[a, b]: Different superscripts in the same row and column show significant differences ($P<0.05$)

longer the fermentation, the more water is produced. In line with the opinion of [17] who states that the increase in water content in the fermentation process is caused by the increasing number of mushrooms so the water content increases. This is because in the fermentation process there is a breakdown of glucose into carbon dioxide, thereby increasing the water content of the fermented material.

Table 1 shows the crude fiber test values with the average fiber content of fermented BSF larvae, at the 2% inoculant level of 9.65 ± 0.50, the 4% inoculant level of 9.64 ± 0.46% and the 7-day fermentation time of 9.82 ± 0.42% and 14 days 9.47 ± 0.47%. Analysis of variance showed that the application of different inoculant levels and fermentation time gave different mean values of crude fiber. The 2% inoculant level gave a higher value than the 4% level so there was an increase of about 0.01% the 7-day fermentation period was higher than 14 days and there was a decrease of 0.35%. Statistically, inoculant level and fermentation time had no significant effect ($P > 0.05$) on the crude fiber value of fermented maggot flour. According to [18], the higher the microbial population in fermented feed, the lower the crude fiber, this is because the inoculant activity of lignocellulolytic bacteria degrades the fiber faster.

Table 1 shows the average value of crude protein content of fermented BSF larvae at the 2% inoculant level of 45.12 ± 1.3% and the 4% inoculant level of 45.40 ± 2.14a and at the 7-day fermentation time of 44.54 ± 1.49% and at the 14-day fermentation

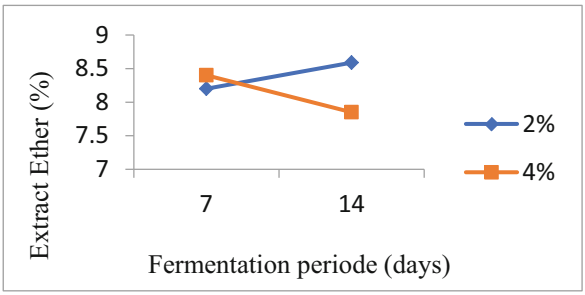

Fig. 1. Decreasing crude fat content with different levels of inoculants and fermentation time

time of 45.98 ± 1.70%. Analysis of variance showed that the administration of different levels of inoculants showed different levels of crude protein. As seen in the addition of a 2% inoculant level, crude protein content was lower than the 4% level and there was an increase of about 1.44%. Apart from the inoculant level, the 7-day fermentation time was lower than 14 days and there was an increase of 0.28%. However, statistically inoculant level and fermentation time did not significantly affect ($P > 0.05$) the crude protein value of fermented maggot flour. Research conducted by [19] shows that maggot's protein content is relatively high. Maggot in dry form contains 41–42% crude protein [20] in their research on the effect of the fermentation time of a mixture of *Trichoderma reesei* and *Aspergillus niger* on the protein and crude fiber content of year dregs that the longer the incubation time, the higher the protein content, the increase in protein content was due to the addition of protein given by microbial cells derived from their growth to produce single cell products. The statement [21] in their research is that *Trichoderma viride* microbes can increase crude fiber protein, this is because *Trichoderma viride* can utilize organic materials contained in the substrate to be broken down and convert them into increased protein content.

Table 1 shows the proximate test values for the crude fat content of fermented BSF larvae at the 2% inoculant level of 8.39 ± 0.32% and the 4% inoculant level of 8.12 ± 0.31%, while the 7-day fermentation period was 8.30 ± 0.21% and the fermentation time was 14 days. of 8.22 ± 0.44%. Analysis of variance showed that the administration of the 2% inoculant level was higher than the 4% level so there was a decrease of about 0.27% and the 7-day fermentation period was higher than the 14-day fermentation period and there was an increase of 0.08%. The results of Duncan's test showed that the inoculant level and fermentation time had a significant effect ($P > 0.05$) on the crude fat value of fermented maggot flour. [22] states that the longer fermentation time can reduce crude fat content because the microbial activity is more active in degrading complex compounds into simple compounds to facilitate fat degradation from fermented materials. Analysis of variance showed that the interaction between the inoculant level and different fermentation times had a significant effect ($P < 0.01$) on the crude fat value of BSF maggot larvae flour. In addition, there is an interaction between the administration of the inoculant level and the duration of fermentation. Table 1 shows the proximate test values for the ash of fermented BSF larvae at the 2% inoculant level of 11.57 ± 0.17% and the 4% inoculant level of 11.59 ± 0.11%, while the 7-day fermentation period was

11.63 ± 0.14% and the fermentation time was 14 days. of 11.53 ± 0.12%. It increases ash content due to the decreased organic matter after fermentation. This is presumably due to the activity of *Trichoderma viride* extracellular enzymes that degrade organic matter of BSF larvae especially fat content that causes shrinkage of organic matter. The decrease in organic matter resulted in an increase in the proportion of ash content. This is in line with the studies of [23] that fermentation increases the ash content. In Fig. 1, it was found that the administration of 2% and 4% inoculant levels resulted in a decrease in crude fat values. At 7 days of fermentation, the level of inoculant administration of 2% approached the same point while the level of salt administration of 4% was at a higher point which indicated that the level of inoculant administration of 2% did not reduce fat content. The decrease in crude fat value occurred along with the addition of inoculant levels and fermentation time.

4 Conclusions

Concluded that *Trichoderma viride* fermented BSF maggot flour decreased crude fat content by 0.27% in 2% and 4% inoculants and an increase in water content at 7 and 14 days of fermentation by 1.08% and can be used as raw material for protein source feed.

Acknowledgment. The author would like to express my sincere thanks to the Ministry of Research, Technology and Higher Education in providing BPPDN scholarships in our studies and research, as well as the staff of the biotechnology and waste valorization laboratory at Hasanuddin University who provided laboratory equipment and research facilities during the experiment.

References

1. Fauzi, R.U.A., Sari, E.R.N.: Analysis of maggot cultivation as an alternative to catfish feed. Ind. J. Agroindustrial Technol. Manage. **7**(I), 39–46 (2018)
2. Cickova, H., Newton, G.L., Lacy, R.C. & Kozanek, M.: The Use of Fly Larvae for Organic Waste Treatment. Waste Management. Elsevier Ltd. 35:68-80. (2015).
3. Sheppard, D.C., Tomberlin, J.K., Joyce, J.A., Kiser, B.C. and Sumner, S.M.: Rearing methods for the *black soldier fly* (Diptera : Stratiomyidae). Journal of Medical Entomology, 39(4): 695–698. (2002).
4. Tomberlin, J. K., Sheppard, D.C., Joyce, J.A.: Selected life history traits of Black Soldier Flies Diptera (2002)
5. Shiau, S.Y. and Yu, Y.P.: Dietary supplementation of chitin and chitosan depress growth in tilapia *Oreochromis niloticus* X O aureus. Aquaculture, 179(1): 439–446 (1999).
6. Wahyudi, N.I.N., Resky, S.R.P., Daruslam, A.M., Azhar, M.: Palatability of maggot as a protein source for poultry. Faculty of animal husbandry Polbangtan Gowa. J. Agrisystems. **16**(1), 1858–4330 (2020)
7. Beier, S., Bertilsson, S.: Bacterial chitin degradation-mechanisms and ecophysiological strategies. Front. Microbiol. **4**, 149 (2013)
8. Elad, Y., Chet, I. and Henis, Y.: Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Canadian Journal Microbiology 28(7): 719–725 (1982).

9. Tilawati.: Crude protein, crude fat and crude fiber content of coffee husk waste fermented using Aspergillus niger and Trichoderma viride fungi. Thesis Faculty of Animal Husbandry. Hasanuddin University. Makassar (2016)
10. Iskandar, B.: Study of differences in levels and duration of ripening of bagasse with Trichoderma rzianum on crude protein and crude fiber content. National Seminar on Animal Husbandry, University in Ponegoro (2009)
11. Azwar and Melati.: Quality improvement cassava peels flour through fermentation using Trichoderma viride. Proceedings of the National Symposium Aquaculture Biotechnology III. Faculty Fisheries and Marine Science, Institute Bogor Agriculture, pp. 64–72 (2010)
12. Azzahra, N., Jamilatun, M., & Aminah, A.: Aspergillus Growth Comparison fumigatus on Instant Media Modified Carrot Sucrose Agar and Potato Dextrose Agar. Indonesian Journal of Mycology, 4(1), 168–174 (2020).
13. Prasetyawati and Dania. Stages of Mushrooms Trichoderma viride propagation with brand media and its applications on plants murbei (*Morus. Sp*). Makassar Environmental and Forestry Research and Development Center (2017)
14. Rizal, S., Novianti, D., Mutiara, D.: Media effectiveness of corn, green beans, rice and bran for Trichoderma sp. mushrooms propagation. Prosiding Seminar Nasional Sains dan Teknologi Terapan 1, 53–57 (2018).
15. Daning and Karunia. Fermentation technology using molds trichoderma sp to improve the quality of nutrition of coffee skin as a ruminant feed. Faculty of animal husbandry Polbangtan Malang. J. Agriexstensia 17, 1 (2018)
16. Gasperszs, V.: Experimental Design Methods. CV. ARMICO. Bandung (1991)
17. Fardiaz, S.: Food Microbiology 1. Gramedia Main Library (1992)
18. Karo, E.K.: Nutrient content of fermented corn straw silage inoculum of Lignocellulolytic Bacteria. Thesis. Bachelor of Animal Science, Faculty of Animal Husbandry, Udayana University, Denpasar (2021)
19. Sheppard, D.C., Newton, G.L.: Valuable by-products of a manure management system using the black soldier fly - a literature review with some current results. In: International symposium; 8th, Animal, Agricultural and Food Processing Wastes, pp. 35–39 (2000)
20. Fransiska, C.S.: Effect of Ph and Inoculum in Utilization of Leather Waste Pineapple for Cellulase Enzyme Production. Major Chemical Engineering, Faculty of Engineering University Riau (2012)
21. Riadi and Purwanti.: Changes in temperature, pH, protein gauze, and crude fiber in fermented winged bean (*Psophocarpus tetragonolobus*) and corn flour with different levels of the fungus *Trichoderma viride*. Faculty of Agriculture, University of Veterans Bangun Nusantara. Journal of tropical livestock science and technology (2018)
22. Ginting, S.: Effect of fermentation time and roasting time of cocoa beans on the quality of cocoa powder, Food science and technology study program, faculty of agriculture. Quality University Medan. J. Stevia **1**(1), 6–11 (2011)
23. Oboh, G.: Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp. Solid media fermentation techniques. Electron. J. Biotechnol. **9**(1), 46–49 (2006)