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THE ORGANOLEPTIC, CHEMICAL AND MICROBIOLOGICAL QUALITY OF MAGGOT'S FRASS AS ALTERNATIVE POULTRY FEED INGREDIENTS

Cahya Setya UTAMA, Bambang SULISTIYANTO, Binti MARIFAH, and Rona Indra CAHYA

Department of Animal Sciences, Faculty of Animal and Agricultural Sciences, Diponegoro University, Jl. Prof. H. Soedarto, S.H, Semarang City, Central Java 50275, Indonesia

Email: cahyasetyautama@gmail.com

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ABSTRACT: Maggot's frass is waste from cultivating maggots (insect larvae) which consists of media from maggot cultivation mixed with feces, skin and dead body of the maggots. The aim of the study was to examine the organoleptic quality, chemistry, worm eggs, lead (Pb) as heavy metal and microbiological profile of maggot's frass as an alternative ingredient of poultry feed. A completely randomized design (CRD) with 3 treatments (T1: frass media for household waste, T2: frass media for tofu dregs, and T3: frass media for vegetable and fruit waste) and 7 replications was used. The results showed that there was no effect of different types of media treatment on the organoleptic quality, chemistry and microbiological profile of maggot's frass. The results of chemical analysis of maggot's frass revealed moisture of 26.39 - 46.26%, crude protein of 10.92 - 16.37%, worm eggs in the dregs media tofu (16 EPG), vegetable and fruit waste (32 EPG), total bacteria of 1.91-4.95 x 10^8cfu/g, and no any *Escherichia coli* and *Salmonella isolates*. Maggot's frass which comes from fruit and vegetable waste was recommended. Therefore, maggot feed using fruit and vegetable waste treatment is recommended because of its high crude protein and metabolic energy and also without any *E.coli* and *Salmonella* contamination.

Keywords: Black Soldier fly; Feed; Maggot's frass; Larva; Waste.

INTRODUCTION

New feed resources are solution to farmers regarded to the problem of cost and efficacy of feed. Alternative feed ingredients usually come from waste, such as agricultural waste, market waste, household waste and industrial waste (Boumans et al., 2022; Siddiqui et al., 2022). Population growth causes an increase in organic waste which has a negative effect on the environment (Henault-Ethier et al., 2017). Maggot or larvae of the Black Soldier Fly (BSF; *Hermetia illucens*) is bioconversion agents that able to convert organic waste into protein biomass (Chiam et al., 2021). Maggot has been used as an alternative feed ingredient to replace fish meal, because maggot's crude protein content is high (Luthada-Raswiswi et al., 2022). Makkar et al. (2014) reported that the use of maggot as much as 19% in poultry feed showed increased the digestibility and had no negative effects. Furthermore, utilization 10-15% maggot flour into poultry feed can improve performance and quality of carcasses (Hwangbo et al., 2009). However, intensive maggot cultivation has resulted in the emergence of new problems, namely by-products in the form of frass which has a fairly good nutritional content, it has the potential to be used as a poultry feed ingredient.

Frass is leftover feed media from maggot cultivation mixed with fecal, skin and dead body of the maggot. Feed media directly affected the physical and chemical properties of frass, because the residue of undigested feed remained in the frass (Garttling and Schulz, 2022). The Maggot feed media is based on the organic waste, such as vegetable waste, fruit waste, restaurant waste, sausage industrial waste, tofu dregs, cassava and cassava peels. That was reported the quality of frass produced from different maggot feed media. Song et al. (2021) noted that frass from a mixture of tofu dregs and wheat flour contained 4.78% nitrogen (N), or 29.87% protein. Frass from a mixture of radish and carrot waste contains 2.04% N or 12.75% protein, from restaurant waste contains 3.15% N or 19.69% protein (Chiam et al., 2021), from a mixture of fruit and vegetable waste contains 1.83% N or 11.44% protein (Lopes et al., 2022). Klüber et al. (2022) stated that frass from maggot cultivation with Palm Kernel Meal (PKM) feed had a crude protein content of 19.32%, 0.36% crude fat and 21.11% crude fiber. Taking into the results of the research above, it appears that frass is worthy of consideration for use as poultry feed.

This study aimed to examine the quality of frass as an alternative feed ingredient in terms of organoleptic, chemical, and microbiological quality. The benefit of research is to become a reference for breeders to try alternative feed

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ingredients that are easy to get, inexpensive, of good quality, and meet the needs of animals. The research hypothesizes are the different types of maggot feed affect the organoleptic, chemical, and microbiological quality of maggot's frass.

MATERIALS AND METHODS

Materials

Materials used in the present study included frass, 0.3 N H_2SO_4, 1.5 N NaOH, aquadest, salt, lead colorimetric test kit, 0.85% physiological NaCl, crystal violet solution, 96% alchohol, Lugol's solution, pH paper test (Merck®, Germany), silica gel, filter paper and safranin O (C.I. 50240). The tools used were analytical balance kern ABJ-220 with an accuracy of 0.01 gram, porcelain cup, sistered glass, Universal Drying Oven UN 55, 50 ml glass beaker, desicator, vacuum pomp, soxhlet, thermo furnace F48010-26, glass jar, glass handle loop - W4, object class 25,4x76.2 mm, 22×22 ml cover glass, and an Olympus CX-33 microscope.

Methods

The research design used a completely randomized design (CRD) with 3 treatments and 7 replications. Treatment T1: using feed from household waste, T2: using feed from tofu dregs, T3: using feed from vegetable and fruit waste. Parameters observed included frass organoleptic tests, nutritional quality of frass, worm eggs, heavy metal (Pb) and maggot's frass microbiological profile.

Organoleptic test

Organoleptic tests measured include color, odor, texture and contamination. Data collection was carried out with 20 semi-trained panelists. The test is carried out using a scoring method with 7 levels of a comparison scale (Utama and Christiyanto, 2021).

Pollution assessment

Score 1: 0 level contamination; Score 2: 1 level contamination; Score 3: 2 level contamination; Score 4: 3 level contamination; Score 5: 4 level contamination; Score 6: 5 level contamination; Score 7: 6 level contamination.

Odor assessment

Score 1: The odor of ammonia is very strong; Score 2: A strong odor of ammonia; Score 3: Ammonia odor is a bit overpowering; Score 4: Typical odor of ammonia; Score 5: Slight odor of ammonia; Score 6: Very slight odor of ammonia; Score 7: Ammonia has no odor.

Texture assessment

Score 1: No lumps; Score 2: Very few lumps; Score 3: Few lumps; Score 4: Moderate; Score 5: more lumps; Score 6: Very many lumps; Score 7: Combine everything.

Color rating

Score 1: Deep Black	Score 2: Black	Score 3: Dark Dark Brown	Score 4: Dark Brown	Score 5: Chocolate	Score 6: Light Brown	Score 7: Yellow Brown.

Proximate test

Proximate analysis consisting of moisture, ash content, crude fat, crude protein and crude fiber was carried out using the AOAC (2005).

Worm contamination test

Worm egg contamination testing was carried out using the Mc Master method (Foka et al., 2021). The number of eggs observed in each column is calculated using the formula: EPG = 2n × 50

Which, n= The number of worm eggs counted in the counting chamber; EPG= Eggs per gram.

Lead (Pb) heavy metal contamination test

Testing for the Pb metal content in the samples was carried out using the atomic absorption spectrophotometer (AAS) method (Oloo and Awuor, 2019). Calculation of the concentration of lead (Pb) uses the equation for the concentration of Pb as follows (AOAC, 2005); (μg/g) = "C x VC x V" / "W W"

Which, C = concentration measured in mg/L (ppm) converted to μg/L (ppb); V = total sample volume (mL) converted to liters (L); W = sample weight (g).

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Microorganism contamination test

Determination of the number of bacterial colonies for each sample was measured using the total plate count (TPC) method. Bacterial population is calculated as follows: Bacterial population (cfu/g) = Number of Colonies × Dilution

Data analysis

The research data obtained was tested using analysis of variance (ANOVA) to determine the effect of treatment with a significant level of 5%. If there is a real effect, it be continued with the Duncan multiple range test.

RESULTS AND DISCUSSIONS

Organoleptic quality of Maggot's frass

Organoleptic tests including data on color, odor, texture and contamination of frass did not show any differences in the characteristics shown in table 1.

The results of organoleptic parameters showed that among the treatment of different types of maggot feed did not show a significant difference (P>0.05) to frass color. The average value of frass color was 1.34. The value of 1.34 showed that frass has a deep black color. Van Looveren et al., (2022) stated that frass has a black color. Frass color is influenced by moisture, contaminants in feed and maggot feed ingredients (Basri et al., 2022).

The results of showed that the treatment of different types of maggot feed did not affect the odor of frass (P>0.05). The average odor rating is a score of 4, which means it has a distinctive odor of ammonia. The odor of ammonia comes from the decomposition of organic matter by microorganisms. Jayanegara et al. (2017) stated that the odor of ammonia is due to the evaporation of gases resulting from the decomposition of bacteria. Factors that can affect the odor of frass are moisture, fat and protein content and the type of feed (Tschirner and Simon, 2015).

The average result of the texture value of frass is 2.70. The value of 2.70 indicates that the texture of the frass has a few lumps. A slightly lumpy texture indicates that the moisture of Maggot's frass is quite high. Jayanegara et al. (2017) stated that the moisture affected the texture, because high moisture facilitates the appearance of lumps and damage caused by the emergence of bacteria, mold and fungi. The texture of a feed ingredient may affected feed consumption and digestibility (Pazoki et al. 2017).

The results of frass contaminates showed that the treatment did not affect frass contamination (P>0.05). In the assessment of contamination, the average score is 2.53. This shows that there are 2 types of contamination in frass. Contaminants that are often found in frass are yarn and plastic. Beale et al. (2022) stated that contamination of frass in the form of plastic material could be minimized by separating it before giving it to maggot. Maggot's frass contamination comes from a mixture in feed that cannot be digested and will remain in frass. Lopes et al. (2022) stated that materials that cannot be digested by maggot will remain in frass.

The organoleptic conditions of maggot's frass in this study showed the appropriate color and odor. The presence of contaminants in maggot's frass needs to be cleaned before being used as a poultry feed ingredient.

Table 1 - Frass organoleptic test

Parameter \ Treatments	T1	T2	T3	P-Values
Color	1.25±0.26	1.37±0.31	1.64±0.45	NS
Odor	4.75±1.49	4.08±1.51	4.02±1.10	NS
Texture	2.53±0.79	2.56±0.92	3.02±0.77	NS
Contamination	2.35±0.29	2.60±0.40	2.64±0.53	NS

NS: non-significant (P>0.05).

Chemical quality of maggot's frass

The chemical quality of frass includes water content, organic matter, crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), pH, metabolic energy, the presence of worm eggs and heavy metals (Pb) shown in table 2. Different types of maggot feed influence characteristics and contents of maggot frass.

The types of maggot feed showed significant differences (P<0.05) in frass moisture. The highest moisture was at T3 with 46.29% followed by T1 35.41% and T2 26.39%. The difference in moisture in Frass is caused by the moisture of the type of feed given. Pham et al. (2015) stated that vegetable waste has a high moisture, namely around 91.56%. The moisture of maggot's frass from the three treatments is high because the moisture is more than 14%. Sharif et al. (2021) stated that moisture that is too high can reduce the quality of feed ingredients, because it will become a medium for growing microorganisms. Different treatments showed significant results (P<0.05) on frass ash content. Ash content showed the amount of inorganic material content in a material. Elechi et al. (2021) stated that during the maggot maintenance phase, frass mixed with maggot manure which has a lot of inorganic substances. The treatment 1 had a high ash content indicating that frass contained a lot of inorganic substances which affected the organic matter content. Yildirim-Aksoy et al. (2020) stated that the ash component in maggot's frass consists of mineral content, salts and inorganic compounds in the form of oxides in organic waste provided and not consumed by maggot.

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Table 2 - Chemical Quality of Maggot's frass

Parameter \ Treatment	T1	T2	T3	P-Values
Moisture (%)	35.41±13.27[a]	26.39±12.02[b]	46.26±9.13[c]	*
Dry matter (%)	95.70±1.61	96.79±1.46	95.20±1.31	NS
Ash (%)	23.50±9.67[a]	19.05±5.83[a]	13.16±5.43[b]	*
Crude protein (%)	10.92±3.01[b]	14.72±6.75[a]	16.37±3.98[a]	*
Extract ether (%)	2.04±0.97	3.40±3.63	2.70±1.87	NS
Crude fiber (%)	16.62±5.53	18.95±7.64	17.14±6.01	NS
Nitrogen free extract (NFE; %)	46.92±4.71[b]	43.88±4.88[b]	50.63±5.40[a]	*
Metabolic energy (Kkal/Kg)	2318.52±438.00[b]	2454.48±452.36[b]	2617.45±291.89[a]	*
pH	6.98±0.45	6.75±0.77	6.88±1.31	NS
Worm eggs (*Egg per Gram*)	0[c]	16.00±5.77[b]	32.00±3.47[a]	*
Pb (lead) (mg/ kg)	0	0	0	NS

Different superscripts in the same line show a significant difference (P<0.05), NS: non-significant (P>0.05); * : P<0,05.

The results of crude protein levels in table 2 show that there are differences in the type of treatment feed (P<0.05). Frass crude protein levels in the study were highest at T3, namely 16.37%. Arabzadeh et al. (2022) stated that frass has a crude protein content of around 19.32%. The T3 treatment has a higher protein content which is possible because fruit and vegetable waste has a better nutritional content. The protein content in maggot's frass comes from leftover feed, maggot droppings, loose maggot skin and dead maggot during the cultivation period (Chiam et al. 2021).

Treatment of did not show significant differences in extract ether content of frass. Maggot's frass extract ether content ranges from 2.04 – 3.40%. Arabzadeh et al. (2022) stated that maggot's frass contains extract ether of around 0.36%. Maggot frass's fat content comes from maggot's ability to convert fiber in organic waste into fat in maggot's body which comes out with feces. According to Zheng et al. (2012), the black soldier fly has cellulose bacteria in its digestion to help convert organic waste fiber into fat and protein in its body biomass.

The results of the statistical analysis showed that the treatment of different types of feed did not affect the crude fiber content (P>0.05). The crude fiber content in frass ranges from 16.62 - 18.95%. Arabzadeh et al. (2022) stated that maggot's frass has a crude fiber content of 21.11%. The low crude fiber content in frass is because the crude fiber will be hydrolyzed by microbes into an energy source in the form of carbon for BSF maggot. Frass crude fiber content is affected by the type of feed given during the cultivation period. Garttling and Schulz (2022) stated that the content of the type of feed given will affect frass crude fiber.

The value of extract material without maggot's frass nitrogen showed significant differences (P<0.05) from the treatment of different types of feed. Significantly different NFE values were due to significantly different protein values. Barroso et al. (2014) stated that the content of extract ingredients without nitrogen (NFE) of feed ingredients is very dependent on other components such as crude protein, crude fiber, crude fat, ash. Nitrogen free extract is a soluble carbohydrate including monosaccharides, disaccharides and polysaccharides which are easily soluble in acid and alkaline solutions and have high digestibility (Chiang and Lai, 2018).

The results of the analysis of variance showed that the treatment of different types of feed affected the metabolic energy content (P<0.05). The T3 treatment had the highest energy content with 2617.45 Kcal/kg. This can be caused because fruit and vegetable waste has a fairly high energy content and there is starch that is not digested by maggot so that it is excreted with feces and mixed into frass. Barzegar et al. (2019) stated that high metabolic energy is due to the high starch content in feed ingredients. The value of the metabolic energy content is related to the nutritional content of the material (Banavar et al. 2022).

Maggot's frass pH values of the three treatments ranged from 6.75 to 6.98. Song et al. (2021) stated that frass has a pH value of around 6-7. Frass pH will affect the content of microorganisms and the activity of microorganisms contained in frass. According to Jiang et al. (2019) the pH of a material also affects the activity of microorganisms.

In the observation of heavy metals, no lead (Pb) contamination was found in all frass samples. Heavy metal contamination cannot be degraded naturally and can accumulate in the bodies of animals and humans, affecting the work of enzymes, causing allergies and being mutagenic, teratogenic or carcinogenic (Geng et al., 2022). The limit for heavy metal contamination in feed is a maximum of 10 ppm. According to Elechi et al. (2021) the threshold for heavy metals content in feed is 10 mg/kg.

There was worm contamination in the T2 treatment with a total of 16.00 eggs per gram and T3 32.00 eggs per gram (EPG). The content of worm eggs in maggot's frass is still relatively safe. Utama et al. (2021) stated that the worm egg content in the mild class ranges from 0-500 EPG. The types of worm eggs detected were Nematoda (*Trichostrongylus Sp*), Nematode *Trichostrongylus Sp* and Nematoda *Ascaridia Sp.* The content of these worm eggs is thought to come from the feed given, because most of the feed comes from vegetable waste which comes from markets (Van der Spiegel, 2013).

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Frass microbiology profile

The results of the analysis of total bacteria, total lactic acid bacteria (LAB), total fungi, *Escherecia coli* contamination, and *Salmonella sp* can be seen in Table 3. The type of feed treatment did show a significant difference (P<0.05) to the total bacteria. The highest total content of maggot frass bacteria at T2 ranged from 4.95 x 10^6 Cfu/g. The T2 treatment may contain substrates that affect the survival and activity of a microorganism. Paritosh et al. (2017) stated that the pH value, temperature and substrate content in a medium will affect the activity of microorganisms. Total bacteria include gram positive and negative bacteria that live in a material. The total content of good bacteria in feed poultry is around 3 x 10^6 CFU/g (Cegielska-Radziejewska et al., 2013).

The total LAB value in maggot's frass is around 1.30 x 10^6 - 2.52 x 10^6 Cfu/g. The insignificant LAB content was due to relatively the same pH value. Astuti et al. (2022) stated that the increase and decrease in total LAB was influenced by several factors such as nutrient availability, temperature and pH value. LAB can survive in frass made possible because of the availability of protein and carbohydrates as a source of energy for LAB to develop. Hadj Saadoun et al. (2020) stated that LAB requires easily soluble carbohydrates to be a source of energy and protein to grow. The LAB population for poultry digestion that does not have a negative effect is around 10^8 CFU (Prado-Rebolledo et al., 2016).

The results of the analysis showed that different feed treatments did not show significant differences in the total number of fungi (P>0.05). The total content of fungi in maggot's frass ranged from 0.20 - 0.70 x 10^6 Cfu/g. The appearance of mushrooms is due to the high moisture in maggot's frass. Astuti and Komalasari (2020) stated that fungi will grow more easily in environments with high moisture content or humid conditions. Fungi can appear and develop on materials that have a moisture of more than 13% and a pH of 3 - 8 (Dewi et al., 2014).

Frass samples were not found to be contaminated with E.coli bacteria. This is possible because of the antibacterial content contained in the maggot body. Kar et al. (2021) stated that the advantage of black soldier fly is that it has anti-microbial content. The anti-bacterials contained in maggot include methanol, lauric acid and saturated fatty acids. Ardiansyah et al. (2021) stated that BSF larvae have natural antibacterial and antiviral properties that can prevent the emergence of pathogenic bacteria. The content of pathogenic bacteria in maggot's frass will be harmful when given as feed (Alonso-Hernando et al., 2013).

Table 3 - Frass microbiological profile

Parameter \ Treatment (10^6 Cfu/g)	T1	T2	T3	P-Values
Total Bacteria	2.08±2.27^b	4.95±3.80^a	1.91±2.71^b	*
Total LAB	1.30±1.20	2.00±1.41	2.52±3.54	NS
Total Fungi	0.60±0.65	0.70±1.09	0.20±0.27	NS
E.Coli	0	0	0	NS
Salmonela	0	0	0	NS

Different superscripts in the same line show a significant difference (P<0.05), NS: non-significant (P>0.05); * : P<0,05.

Table 4 - Bacterial culture results from maggot's frass

Type bacteria	Bacterial morphology	Total	(%)
Gram positive	Solitary	18	25.35
	Stem	13	18.31
	Duplo	11	15.49
	Coccus	11	15.49
	Yeast	7	9.86
	Oval	7	9.86
	Row	2	2.82
	Spores	2	2.82
Total		71	100
Gram negative	Solitary	13	50
	Stem	12	46.15
	Cocobasil	1	3.85
Total		26	100

Frass samples were not found to be contaminated with *Salmonella sp. Salmonella* bacteria are included in the category of pathogenic bacteria. Tariq et al. (2022) stated that *Salmonella* is a gram negative that is harmful to livestock. The impact of *Salmonella* bacterial infection in livestock can cause *Salmonellosis*. According to Ferarri et al. (2019) *salmonella* contamination can cause *salmonellosis* with symptoms of diarrhea, in poultry the contamination can reach the eggs. One way to reduce the content of *Salmonella* in a material is by heating it to a temperature of around 90^{0}C (Santos Dalolio et al., 2017).

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Table 4 shows that the growth of bacteria, 97 bacteria where 9 types of bacterial morphology consist of 8 types of gram positive and 3 types of gram negative. 9 types of gram-positive bacteria morphology, namely solitary (25.35%), stem (18.31%), duplo (15.49%), coccus (15.49%), yeast (9.86%), oval (9 .86%), lined (2.82%), spore (2.82%) and 3 types of gram-negative morphology, namely solitary (50%), stem (46.15%) and cocobasil (3.85%).

Gram positive bacteria have a cell wall structure with a thick peptidoglycan content. The total weight of the cell wall of gram-positive bacteria is a multiplayer peptidoglycan network bound by the inner membrane (Gangwal et al., 2022). Gram positive bacteria are dominated by bacteria with rod and solitary morphology. Examples of bacteria with solitary morphology are staphylococcus and bacteria with rod morphology are lactic acid bacteria. The presence of gram-positive bacteria such as lactic acid bacteria can minimize the risk of digestive disorders caused by negative bacteria such as *Escherichia coli* (Utama, et al. 2021).

Gram-negative bacteria with rod and solitary morphology are namely *Shigella sp.* and *Ralstonia solanacearum* (Komariah et al., 2013). The content of *Shigella* sp in feed ingredients will be dangerous because they are pathogenic. *Shigella sp.* produces exotoxins that interfere with the digestive tract. The content of the *Ralstonia solanacearum* bacteria is possible because it was carried along with vegetable waste because its presence is often found in tomato plants. The presence of lactic acid bacteria can minimize the presence of gram-negative bacteria such as *Shigella* sp. Fanin et al. (2018) stated that the number of gram-negative bacteria will decrease as the number of gram-positive bacteria increases.

The results of the microbiological content of maggot's frass with different types of feed media showed no difference. The microbiological content of maggot's frass in the study showed safe results, seen from the content of total bacteria, total LAB and total fungi and there was no *E.coli* and *Salmonella* content.

CONCLUSION

Treatment of different types of feed did not affect the organoleptic quality and microbiological profile of maggot's frass but affected the chemical quality maggot's frass. Therefore, maggot feed using fruit and vegetable waste treatment is recommended because of its high crude protein (16.37%) and metabolic energy (2617.45 Kcal/kg) and also without any *E.coli* and *Salmonella* contamination.

DECLARATIONS

Corresponding author

E-mail: cahyasetyautama@gmail.com

Author's contribution

C.S. Utama and B. Sulistiyanto provided recommendations and suggestions on research topics, article preparation and finalization of scientific articles; B. Marifah and R.I. Cahya conducted research, data processing and article preparation.

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Conflict of interests

The authors declare that they have no competing interest.

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