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In vitro study: Soybean meal substitution with maggot on sheep digestibility and rumen fermentation

Studi in vitro: Substitusi bungkil kedelai dengan maggot terhadap kecernaan dan fermentasi rumen domba

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ABSTRACT

The in vitro study aimed to evaluate the utilization of maggot flour from selected substrates as a substitute for soybean meal in sheep rations on digestibility and rumen fermentation in vitro. Maggot were reared using three different substrates i.e., sheep feces, household waste and bread waste. The BSF maggot product with feces substrate showed the highest protein was used in this study. The treatments in this study were T1=100% soybean meal, T2=50% soybean meal + 50% maggot flour, T3=100% maggot flour. In vitro rumen fermentation and digestibility were conducted and the parameters were pH, NH_3, volatile fatty acid (VFA), dry matter digestibility (DMD) and organic matter digestibility (OMD). Maggot rearing data were presented descriptively while the in vitro data were analyzed using analysis of variance. The result showed that the treatments had no significant effect (P>0.05) on pH, VFA, DMD and OMD, but replacement of soybean meal (100% maggot flour) affected NH_3 production. The utilization of maggot as a substitute for soybean meal in sheep ration did not have a negative impact on in vitro fermentability and rumen digestibility.

Kata kunci:
Black soldier fly
Fermentasi rumen
In vitro
Kecernaan

ABSTRAK

Penelitian in vitro ini bertujuan mengevaluasi penggunaan tepung maggot dari substrat terpilih sebagai substitusi bungkil kedelai pada ransum domba terhadap kecernaan dan fermentasi rumen secara in vitro. Budidaya maggot menggunakan media feses domba, limbah rumah tangga dan limbah roti. Maggot hasil budidaya yang menggunakan media feses domba digunakan pada tahapan selanjutnya karena maggot tersebut memiliki kandungan protein tertinggi dibandingkan maggot dari substrat lainnya. Perlakuan pada penelitian ini adalah T1=bungkil kedelai 100%, T2=bungkil kedelai 50% + tepung maggot 50%, T3=tepung maggot 100%. Pengujian fermentasi rumen dan kecernaan secara in vitro dilakukan pada semua perlakuan dan parameter yang diukur adalah pH, NH_3, volatile fatty acid (VFA), kecernaan bahan kering (KcBK) dan kecernaan bahan organik (KcBO). Data pemeliharaan maggot disajikan secara deskriptif sedangkan data in vitro dianalisis menggunakan analisis sidik ragam. Hasil riset menunjukkan bahwa perlakuan tidak berpengaruh nyata (P>0,05) terhadap pH, VFA, KcBK dan KcBO, tetapi substitusi maggot 100% mempengaruhi produksi NH_3. Penggunaan maggot sebagai substitusi bungkil kedelai pada pakan domba tidak berdampak negatif terhadap fermentabilitas dan kecernaan rumen secara in vitro.

INTRODUCTION

The use of soybean meal (SBM) as a source of protein in livestock rations has limitations because soybean meal is an imported product. Therefore, an alternative of SBM is urgently needed and an alternative protein source feed ingredient that can be used from insects, such as

the black soldier fly (BSF). BSF has a life phase in the form of a larvae which is commonly referred to as a maggot. Maggot can be an alternative feed ingredient because of its protein content because its value can resemble the protein content of SBM. Based on Fitriana et al. (2022), the crude protein content of maggot was 30%-50% while the ether extract was 8%-46%.

On the other hand, maggot has an ability to degrade organic waste into its body. Maggot as bioconversion agent can degrade 56%-70% of organic waste (Suciati & Faruq, 2017). The quality of nutrient media as food as well as growing media/substrate is very important for its effect on body mass and/or individual size and egg production in BSF rearing (Gobbi, Martínez-Sánchez, & Rojo, 2013). Various types of organic waste tend to have different characteristics in terms of nutrients, especially macronutrients in the form of carbohydrates and proteins.

The organic waste that can be used as a substrate for maggot were sheep feces, household waste and bread waste. Household waste has the potential to be used as a substrate because it is 4-6 times more effective as a maggot growing medium than vegetable or fruit waste (Salman, Nofiyanti, & Nurfadhilah, 2019). Bread waste which can be used as a substrate because it has a nutrient content of crude protein (13.56%), ether extract (8.55%), crude fiber (5.86%), nitrogen free extract (NFE) (46.83%), ash (2.5%) ash and total digestible nutrient (TDN) (68.74%) (Yamashita, Rachmat, Tarmidi, Ayuningsih, & Hernaman, 2020). There is also sheep feces which can be used as a substrate, which contains 75.35% organic matter, 17.84% crude protein, 0.92% ether extract, and 2.85% crude fiber. The nutrient content of sheep feces before being composted based on fresh ingredients was C (46.51%), N (1.41%), C/N (32.98), P (0.54%) and K (0.75%) (Cholis, Setyowati, Ita, & Nursita, 2016).

Balhis, Indriyanti, Widiyaningrum, & Setiati (2022) had performed bioconversion study of maggot bioconversion on bread waste. It showed that the chemical composition of maggot from this substrate promising higher crude protein content. Sheep feces as maggot substrates had been conducted, but the utilization was mixed with other animal feces so the bioconversion and maggot chemical composition from this substrate have not been known. Various studies were evaluated the bioconversion of the household waste by using maggot and it showed the household waste were decrease and the maggot could be used as feedstuffs (Amrul et al., 2022; Dafri, Nahrowi, & Jayanegara, 2022). The utilization of these substrates had been performed, yet the in vitro digestibility and rumen fermentation of maggot from these substrates were rarely performed. The three substrates were evaluated based on the maggot chemical compositions in this research. Moreover, the highest crude protein of maggot from these substrates was used as the soybean meal substitution. The purpose of this study was to evaluate the utilization of maggot flour from selected substrates as a substitute for soybean meal in local breed sheep rations on digestibility and rumen fermentation in vitro.

MATERIALS DAN METHODS

Maggot Rearing

Maggot rearing was used three different substrates i.e., sheep feces, household waste and bread waste and lasted for 21 d. Sheep feces were from sheep rearing in sheepfold located in Faculty of Animal Science, IPB University whereas the household waste was gained from nearby restaurants and the majority of household waste was rice. The bread waste was obtained from local bread factory in Bogor and the expired breads were used as maggot substrate. All substrates were given to maggot in fresh condition.

For the first seven days, maggot was fed with fine rice bran and given with the main substrates until 21 d. The BSF egg was weighed 0.5 g and substrates were water-moisturized in rice bran substrate. An amount of 150 g was moved in each substrate after 7 d rearing, feces substrate was water-moisturized periodically so it had the similar moisture content as other substrates, and the humidity was maintained. Maggot aged 19-20 d was harvested by filtering and washing it so the maggot was separated from the substrate. Maggot was frozen and analyzed for the nutrient composition and the substrates (Table 1). The analysis was based on AOAC (2005).

Ration Preparation

Maggot from feces substrate was used as a substitution of soybean meal because its higher crude protein content. The ration was formulated in iso protein and iso energy based on Ariyanto et al. (2020). The ration treatments were T1 = 100%

Table 1. Chemical composition of substrates in dry matter-based (100%)

Chemical composition	Substrates		
	Sheep Feces	Household Waste	Bread Waste
Dry matter[a] (%)	93.75	90.61	94.22
C-organic[a] (%)	43.76	55.34	57.47
C/N ratio[a]	22.00	31.00	38.00
Protein[b] (%)	13.00	11.25	10.08

[a]Analysis results from Indonesian Soil Research Institute (2021), [b]Analysis results from Saraswanti Indo Genetech Laboratory (2021).

soybean meal (as control), T2 = 50% soybean meal + 50% maggot flour, T3 = 100% maggot flour. The ratio of forage (napier grass) and concentrate in formulated feed was 40:60. The composition of formulated feed was presented in Table 2.

Table 2. Ration composition and the nutrient composition of each treatment

Feedstuffs (%)	Treatment		
	T1	T2	T3
Napier grass	40.00	40.00	40.00
Cassava meal	12.60	12.60	10.80
Pollard	10.20	10.20	10.20
Copra meal	11.10	11.10	12.60
Corn	13.80	13.80	14.10
Molasses	3.00	3.00	3.00
Soybean meal	9.00	4.50	0.00
Maggot flour	0.00	4.50	9.00
Premix	0.30	0.30	0.30
Total	100.00	100.00	100.00
Dry matter[a]	89.29	89.14	89.37
Ash[a]	8.58	9.06	9.24
Crude protein[a]	14.82	14.52	14.95
Ether extract[a]	2.50	2.60	2.92
Crude fiber[a]	16.81	17.01	17.23
Nitrogen free extract	57.29	56.81	55.66
Total digestible nutrient[b]	67.95	67.29	66.88

[a]Analysis results from PAU laboratory (2022), [b]TDN was calculated based on Wardeh (1981) TDN of concentrate = 2.6407 + (0.6964 x CP) + (1.2159 x EE) – (0.1034 x CF) + (0.9194 x NFE), TDN of forage = 1.6899 + (1.3844 x CP) – (0.8279 x EE) + (0.3673 x CF) + (0.7526 x NFE); T1 = 100% soybean meal, T2 = 50% soybean meal + 50% maggot flour, T3 = 100% maggot flour.

Rumen Fluid Collection

A thirteen-months male sheep (local breed) was used to collect the rumen fluid. The collection was performed by using stomach tube. The water flask was filled with warm water (±39 ℃) and rumen fluid was taken from sheep using stomach tube which was inserted into sheep mouth. Rumen fluid that had been filtered with gauze was put into water flask which had been emptied so the temperature remained constant. The water flask was closed tightly and the rumen fluid was analyzed by in vitro analysis.

In Vitro Rumen Fermentation and Digestibility

In vitro rumen fermentation and digestibility was based on Tilley & Terry (1963). An amount of 0.5 g feed sample was put into fermenter tube and added 40 mL McDougall buffer solution (pH 6.9) and 10 mL rumen fluid. The mixture was flowed with CO_2 for 15 s and closed tightly with rubber. The tube was put into shaker water bath for 4 h and 48 h at 39 ℃ to create similar condition as rumen. The mixture sample was dripped with 2 drops of saturated $HgCl_2$ solution to kill rumen microbes, so the fermentation was stopped. The sample was centrifuged in 3500 rpm for 15 mins and the supernatant (after 4 h incubation) was used to measure pH, NH_3 production and total VFA.

Digestibility analysis was started with rumen fluid and feed sample incubation in 100 mL fermenter tube at shaker water bath for 48 h. After incubation, the mixture was dripped with 2 drops of $HgCl_2$ solution and moved to centrifuge tube. The sample was centrifugated in 3000 rpm for 15 mins. The pellet was moved to fermenter tube and the tube was added pepsin solution and 50 mL HCl 0.2%. The tube was incubated at shaker water bath (39℃) for 48 h and the residue after incubation was filtered with filter paper (Whatman 41). The residue was stored in crucible and dried in the oven (105℃) for 24 h. The crucible was weighed and the dry matter digestibility was calculated. Meanwhile, the organic matter digestibility was analyzed by putting the crucible into furnace (600 ℃) for 4 h. The crucible was weighed and the organic matter digestibility was calculated.

$$DMD\ (\%) = \frac{sample\ DM\ (g) - (residue\ DM\ (g) - blank\ DM\ (g))}{sample\ DM\ (g)} \times 100\%$$

$$OMD\ (\%) = \frac{sample\ OM\ (g) - (residue\ OM\ (g) - blank\ OM\ (g))}{sample\ OM\ (g)} \times 100\%$$

Total VFA Analysis

Total VFA was analyzed by steam distillation technique General Laboratory Procedures (1966). An amount of 5 mL supernatant from in vitro fermentation was put into distillation tube, which was heated using steam, mixed with 1 mL of 15% H_2SO_4 and closed tightly. The distillation solution was collected into Erlenmeyer flask containing 5 mL of 0.5 N NaOH until the volume reached 250 mL. 2 drops of phenolphthalein (PP) were added into flask and titrated with 0.5 N HCl until the color changed from pink into light pink.

$$\text{Total VFA concentration (mM)} = \frac{(a - b) \times N\ HCl \times (\frac{1000}{5})}{\text{sample weight} \times \%DM\ \text{sample}}$$

NH_3 Analysis

Concentration of NH_3 was analyzed by using Conway (1957) method. An amount of 1 mL supernatant from in vitro fermentation was mixed with 1 mL saturated Na_2CO_3. Petroleum gel was smeared on the edge of Conway glass and the supernatant was dripped on the right end of Conway glass while Na_2CO_3 solution was dripped on the other side. Boric acid was dripped on the middle of the Conway glass and the Conway glass was closed in anaerobic condition for 1 d. Color of boric acid was changing from red into blue and the solution was titrated with H_2SO_4 until the color changed into pink (as indicator).

$$NH_3 = \frac{\text{Volume } H_2SO_4 \text{ x N } H_2SO_4 \text{ x } 1000}{\text{sample weight x \%DM sample}}$$

Data Analysis

Substrates analysis was presented as descriptive data. The data from pH, NH_3, total VFA, DMD and OMD were analyzed using analysis of variance and followed by Duncan test if there were significant differences between the treatments (Steel & Torrie, 1993).

RESULTS AND DISCUSSIONS

Maggot Rearing

Maggot was rearing in three different substrates i.e., sheep feces, household waste and bread waste. The nutrient composition of each maggot flour from each substrate was shown in Table 3.

Table 3. Chemical composition of maggot flour based on substrates in 100% dry matter-based

Chemical composition (%)	Substrates		
	Sheep Feces	Household Waste	Bread Waste
Dry matter[a]	91.70	38.87	95.95
Ash[a]	11.12	4.37	4.04
Crude protein[a]	42.91	28.61	20.56
Ether extract[a]	8.83	47.47	50.08
Crude fiber[a]	12.86	4.81	3.53
Nitrogen free extract	24.28	14.74	21.27
Ca[a]	0.67	0.12	0.08
P[a]	1.46	0.63	0.44
Carbohydrate[a]	21.32	19.63	25.29

[a]Analysis results from Saraswanti Indo Genetech Laboratory (2021); Ca = Calcium, P = Phosphorus

The crude protein in feces substrate produced the highest content among household waste media and bread waste which were 42.91%, 28.61% and 20.56% respectively. The protein contained in the maggot was sourced from the protein found in the growing media because the maggot utilized the protein in the media to form its body proteins. Katayane et al. (2014) stated that if the quantity and quality of the medium were high, it would have a positive effect on the quantity and quality of protein of maggot. The higher protein content of the media, the higher the protein content and vice versa (Suciati & Faruq, 2017).

The C/N ratio in the feces media was 22 and it resulted in a higher crude protein content compared to other substrates. The C/N ratio of the substrate would affect degradation, in which this process required organic carbon (C) to fulfill energy and growth, and nitrogen (N) to fulfill protein as a building block for metabolic cells. According to Suntoro (2002), the value for the C/N ratio which could be immediately decomposed was less than 20. Ismayana et al. (2012) stated that nearly 50% of larvae bodies were composed of protein and converted N into protein and broke down C compound as an energy source. The level of a C/N ratio would determine the nutritional quality of BSF larvae. Nugrahani et al. (2018) stated that if the C/N ratio was too high, organisms would lack N for protein synthesis so that the decomposition process ran slowly and if the C/N ratio was too low, there would be excess

N. Excess nitrogen could not be assimilated by microbes so N would be denitrified and produced ammonia.

Based on the results on maggot rearing, maggot from sheep feces was used as the soybean meal substitution. The highest crude protein content of maggot among the maggot from all substrates made this maggot was enlisted in sheep ration formula. Also, the crude protein on this maggot is similar to crude protein content of soybean meal.

Rumen Fermentability

The results of the fermentability analysis of soybean meal substitution with maggot flour using in vitro were presented in Table 4.

Table 4. In vitro rumen fermentability

Parameter	Treatment		
	T1	T2	T3
pH	5.87±0.04	5.87±0.03	5.86±0.02
NH_3 (mM)	5.33±0.21^b	5.05±0.18^b	6.08±0.58^a
VFA (mM)	109.03±4.36	107.47±4.65	106.21±3.14

Different superscripts on the same line show significant differences ($p<0.05$); T1 = 100% soybean meal, T2 = 50% soybean meal + 50% maggot flour, T3 = 100% maggot flour

pH

The pH of rumen fluid is one of the factors that determined the process of fermentation and degradation of feed in the rumen. The rumen pH was one of the main factors in determining an appropriate environment for rumen microbial life (Suharti, Aliyah, & Suryahadi, 2019). The results showed that there was no significantly different effect ($P>0.05$) in maggot substitution and soybean meal. The results were quite normal, where Bayne & Edmondson (2021) stated that pH conditions in the rumen for ideal fermentation required a pH in the range of 5.5 – 7. Rumen pH in the normal range indicated a good process of feed degradation so that rumen microbes could perform optimally to help ruminants digest high-fiber feed ingredients and converted feed nutrients fermentatively into other compounds, including NH3 and total VFA (Fassah, Nurhazizah, Astuti, & Khotijah, 2022).

The rations in all treatments had pH values that tended towards 5. It could be seen that the percentage of concentrate in the ration was higher than the percentage of forage in the ration so that the feed ration had a higher soluble content. Grünberg & Constable (2009) mentioned that animal that fed containing more structural fiber or carbohydrates, the pH tends towards 7. However, if the feed contained more starch or soluble carbohydrates, the pH tends towards 5. Providing more concentrate containing easily soluble carbohydrates compared to forages could increase rumen microbial activity. Thus, the production of lactic acid would increase and caused the pH to decrease. Rumen pH could be influenced by several factors, including the type of feed, feeding time, rumination time, feed flow rate, VFA levels in the rumen, and effectiveness of VFA absorption (Budiasa, Suryani, & Suarna, 2018).

NH_3 concentration

Ammonia (NH_3) is an indicator to determine feed fermentability which is related to feed protein digestibility, activity, and rumen microbial population. Ammonia itself is the product of the degradation of protein and non-protein nitrogen in the rumen. NH_3 is an important component for optimizing the synthesis of amino acids and microbial proteins in the rumen. The results showed that 100% maggot substitution in ration produced a significant difference ($p<0.05$) rather than other treatments. The NH_3 concentration of each treatment in T1, T2 and T3 were 5.33 mM, 5.05 mM, and 6.08 mM, respectively. The low concentration of NH_3 in control and 50% maggot substitution showed that rations containing soybean meal increased resistance to protein degradation in the rumen when compared to rations supplemented with maggot flour. Soybean meal showed lower protein solubility and easily escaped protein degradation so that ammonia production in the rumen was low. Harahap et al. (2017) stated that the high protein content of the feed and the protein was easily degraded would result in an increase the concentration of NH_3 in the rumen.

The NH_3 concentration in each treatment showed quite optimal condition for the growth and development of rumen microbes and activities. These results were in accordance with McDonald et al. (2010) which stated that the optimum concentration of NH_3 to support microbial growth in the rumen as a source of N for cell synthesis processes was 4.9 – 17.6 mM. Factors that affect NH_3 production in the rumen were the duration of feed in the rumen, carbohydrates in rations,

and the pH of the rumen itself. Rumen bacteria are highly dependent on NH_3 concentrations, if the ammonia concentration in the rumen is low then the activity of bacteria in the rumen will be hampered and consequently the value of feed degradation will decrease (Rosmalia, Permana, & Despal, 2022).

Total VFA

VFA is the end product of carbohydrate fermentation found in the rumen and the main source of energy from the rumen (Muslimah, Istiwati, Budiman, Ayuningsih, & Hernaman, 2020). VFA productions in this study were not significantly different (P>0.05). This was because each treatment contained similar NFE. VFA increased as the content of NFE increased and the crude fiber content in the ration decreased. NFE was a compound that is easily soluble compared to crude fiber, so it was more easily degraded. According to Muslimah et al. (2020) stated that the high content of NFE in rations was easily digested by rumen microbes and converted into VFA compared to rations that have a high crude fiber content.

The results in this study were in the range of Rahayu et al. (2018) study who stated that normal rumen microbial VFA concentrations produced in the range 80-160 mM. Moreover, Jayanegara et al. (2017) performed a study of BSF larvae as single feed and the VFA concentration was 103 mM. The value of total VFA concentration was influenced by several factors such as the fermentability level of ration, the amount of soluble carbohydrate content, rumen pH, the ration digestibility, the amount of feed given, and the types of rumen bacteria (Wijayanti, Wahyono, & Surono, 2012). Total VFA was the result of bacterial activity during fermentation in the rumen so more bacteria were produced which would produce higher VFA. This was in line with this study because the highest VFA concentration values were found in high TDN.

Rumen Digestibility

Data on the average in vitro dry matter digestibility and organic matter digestibility were presented in Table 5.

DMD describes compounds in the form of proteins, carbohydrates and fats, which can be digested by animal while OMD describes the digestibility of organic matter in food ingredients other than minerals (Suharti et al., 2019). There were not significantly different (P>0.05) in each treatment. The digestibility was in accordance with the study of Lestari et al. (2015) who stated that the normal ration digestibility value for ruminants was above 60%. DMD had the same pattern as VFA concentrations, when high VFA would have an impact on high DMD as well. Saripudin et al. (2019) stated that the level of DMD correlated positively with VFA concentrations.

Table 5. In vitro dry matter digestibility and organic matter digestibility

Parameter	Treatment		
	T1	T2	T3
DMD (%)	69.01±1.77	68.87±2.32	68.31±1.86
OMD (%)	67.10±0.95	66.44±1.36	65.89±1.12

DMD = dry matter digestibility, OMD = organic matter digestibility; T1 = 100% soybean meal, T2 = 50% soybean meal + 50% maggot flour, T3 = 100% maggot flour

The OMD was affected by the level of ash in the ration. High ash content could cause low OMD (Nugroho, Muhtarudin, Erwanto, & Fathul, 2020). The ash in each treatment was within normal limits, namely in the range of 8% - 9%, where the utilization of ash in sheep rations was 15% (maximum) (Wulandari, Fathul, & Liman, 2015). It could be seen that the increase in DMD affected the value of OMD. (Harahap et al., 2017) stated that the OMD increased in line with the increase of DMD, this was because some of the dry matter was part of the organic matter. Both DMD & OMD were affected by the chemical composition of sheep ration. All treatments had similar chemical composition because the ration was formulated in iso energy and iso protein thus it made the DMD & OMD were not significantly different (P>0.05).

CONCLUSION

Maggot from sheep feces substrate had higher crude protein content than the other substrates. The utilization of maggot unaffected the pH, VFA, DMD and OMD and maggot could replace 100% soybean meal as protein source in sheep ration although it could increase NH_3 production in rumen fermentation.

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