



Nutritional Evaluation of Total Mixed Ration Silages Containing Maggot (*Hermetia illucens*) as Ruminant Feeds

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Abstract | The purpose of this study was to evaluate the nutritive value of total mixed ration (TMR) silage containing intact and defatted black soldier fly larvae (BSFL) as ruminant feeds. The BSFL was included as an ingredient in TMR according to the following treatments: ensiled TMR (R1), ensiled 80% TMR + 20% intact BSFL (R2), ensiled 80% TMR + 20% chemically defatted BSFL (R3), and ensiled 80% TMR + 20% mechanically defatted BSFL (R4). Each treatment was performed in five replicates. Ensiling was performed in lab scale silo and stored for 30 d. All dietary treatments were subjected to chemical composition determination and *in vitro* incubation with buffered-rumen fluid. Results showed that R2, R3, and R4 had higher crude protein (CP) contents than R1 both before and after ensiling. The ether extract (EE) contents in R3 and R4 were lower compared to R2. Ruminal ammonia concentration of R2 was the lowest compared to all treatments, while treatment R3 had the highest ammonia concentration ($p < 0.05$). *In vitro* organic matter digestibility (IVOMD) parameter showed that R1, R3, and R4 treatments were higher than R2 ($p < 0.05$). Total gas production in R1 and R2 was lower than treatments in R3 and R4, and the total gas production of R3 was the highest compared to all treatments ($p < 0.05$). Methane production was not altered due to dietary treatments. In conclusion, defatted BSFL can be included in TMR silage without causing any adverse effect on the nutritional value of the silage.

Keywords | Total mixed ration, Silage, Black soldier fly, Rumen, *In vitro* digestibility

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INTRODUCTION

Feeding of high-quality feedstuffs is a determining factor for the success of livestock production. In many countries, the source of protein for animal feed is primarily based on plant protein. However, protein is considered as the most expensive component in feed in comparison to other nutrients. Thus, economically, the fulfilment of protein sources burdens production costs. The increasing prices of protein sources and the threat of food security have made livestock businesses seek alternative feeds. Insects just recently have been proposed as future food and feed resources for human and animals, respectively. The use of insects may simultaneously alleviate the problem of organic waste disposal since they easily grow on various organic

substrates and convert the substrates into their body mass. Insects are typically characterized by their high protein contents with relatively balance amino acid composition (Jayanegara et al. 2017a). Maggot or black soldier fly larvae (*Hermetia illucens*) is considered as a potent animal feed because it has a fast production system, grow rapidly, and high protein content, i.e., approximately 40% (Liland et al., 2017). However, BSFL contains high fat of 29.65% (Fahmi et al. 2007). The use of insects as a feed ingredient is apparently a potential solution to solve, at least partially, the problem of feed shortage especially as a source of protein. The present study aimed to evaluate the nutritive value of total mixed ration silage containing intact and defatted black soldier fly larvae as ruminant feeds.

ETHICAL APPROVAL

This study used the rumen content from two non-lactating Holstein Friesian cows located at field experimental station, Faculty of Animal Science, IPB University, Indonesia. Animals were cared for according to IPB University standard for animal welfare and approval of this study was obtained from the same faculty.

SAMPLE PREPARATION AND ANALYSIS

Sample of BSFL aged 15 d was obtained from PT Biocycle, Bogor. Fat from BSFL was removed mechanically and chemically by using an expeller and a hexane solution, respectively. BSFL was dried in an oven at 50°C for 24 h. The BSFL was included as an ingredient in total mixed ration (TMR) according to the following treatments: ensiled TMR (R1), ensiled 80% TMR + 20% intact BSFL (R2), ensiled 80% TMR + 20% chemically defatted BSFL (R3), and ensiled 80% TMR + 20% mechanically defatted BSFL (R4). Each treatment was performed in five replicates. Ensiling procedure was performed according to Kondo et al. (2014). All ingredients in TMR were mixed homogeneously and put into a lab scale silo (1000 ml capacity). The silo was tightly closed to ensure anaerobic condition. All silos were stored in a place without any direct exposure to sunlight for 30 d, and then silage was harvested for further experimental procedures.

Silage samples were dried at 50°C for 24 h in an oven. The samples were milled to pass a 1 mm screen. These ground samples were determined for their chemical composition and subsequent *in vitro* incubation with buffered-rumen fluid. Samples were subjected to determination of nutrient composition that included dry matter (DM), organic matter (OM), ash, crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract (NFE) by following the procedure of AOAC (2005). Samples were also determined for their neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Van Soest et al., 1991). Hemicellulose and cellulose were obtained by difference from NDF – ADF and ADF – lignin, respectively. Insoluble CP in neutral detergent (NDICP) and acid detergent solutions (ADICP) were analyzed by following the procedure of Licitra et al. (1996). Determination of the chemical composition of each ration was carried out in duplicate.

IN VITRO PROCEDURE

The experimental TMR silage samples were incubated with rumen fluid and bicarbonate buffer mixture (Theodorou et al., 1994), performed in five replicates. Rumen fluid was filtered before use to remove large forage particles. Approximately 750 mg sample was added into a serum bottle (150 ml capacity) and added with 15 ml rumen fluid and 60 ml

bicarbonate buffer. All serum bottles were gassed with CO₂ for 30 s and then closed with a butyl rubber plug and an aluminum crimp seal to begin the incubation. Incubation was carried out in a water bath at 39°C for 48 h. Gas was released and recorded regularly at a determined interval using a syringe. Shaking was performed manually at each time the gas production was taken. Gas samples were then injected into a gas chromatograph to measure the methane concentration. Separation between supernatant and residue was performed by using a centrifuge after 48 h of incubation. Supernatant was taken to analyze pH, ammonia, total bacteria population and total volatile fatty acid (VFA) (Jayanegara et al., 2016). The residue was added with 75 ml of pepsin-HCl mixture and put into the water bath for another 48 h. The remaining material was dried in an oven at 60°C for 24 h and subsequently put into a furnace at 550°C to determine *in vitro* dry matter digestibility (DMD) and *in vitro* organic matter digestibility (OMD), respectively (Tilley & Terry 1963).

STATISTICAL ANALYSIS

Data were analysed by using analysis of variance with four treatments and five replicates. When the ANOVA results for a certain parameter show a significant difference at $p < 0.05$, Duncan test was employed for comparison among different treatments. Statistical analysis was performed by using SPSS statistics software version 23.

RESULTS AND DISCUSSIONS

The chemical composition of the total mix ration (TMR) based on the dietary treatments is shown in Table 1. The R2, R3, and R4 had higher CP contents than R1 both before and after ensiling. An increase in CP content also followed the increase in the NFE content in R2, R3, and R4 compared to R1. It is because of the addition of BSFL to TMR, thus increasing the value of CP ration. BSFL is a potential source of protein feed ingredients from insects. Based on its nutritional content, insects generally have high levels of crude protein and balance amino acid composition (Sanchez-Muros et al., 2014). It has been reported that the value for BSFL crude protein (aged 15 days) is in the range of 40-44% DM (Sprangers et al., 2017; Jayanegara et al., 2017a). However, extreme variability in EE content (15.0-34.8% DM) and ash (14.6%-28.4% DM) was observed in BSFL, which primarily due to the different diets (Makkar et al., 2014). With regard to individual fatty acid compositions, BSFL was reported to contain a high percentage of saturated fatty acids particularly lauric and palmitic acids, i.e., above 40 and 8%, respectively. BSFL also contained C18:2 n-6 of 4.5-11.6%, which was relatively high among other polyunsaturated fatty acids (Sprangers et al., 2017).

The EE content in R2 was higher compared to R3. How

Table 1: Chemical composition of total mixed ration (TMR) silage containing black soldier fly larvae (BSFL) before and after 30 days of fermentation.

Chemical composition**	Treatments*			
	R1	R2	R3	R4
Before ensiling				
Dry matter, %	87.02	87.03	87.06	87.03
Ash, %DM	8.00	7.10	8.09	8.05
Crude protein, %DM	14.06	14.15	15.87	15.14
Crude fiber, %DM	21.33	23.20	24.78	25.00
Ether extract, %DM	2.94	2.72	2.62	3.38
Nitrogen free extract, %DM	35.46	41.73	35.70	38.82
After ensiling				
Dry matter, %	82.75	91.03	91.16	90.93
Ash, %DM	9.01	8.60	8.60	8.69
Crude protein, %DM	12.08	13.98	15.26	14.38
Crude fiber, %DM	16.20	15.93	13.34	19.63
Ether extract, %DM	2.10	2.84	2.52	2.65
Nitrogen free extract, %DM	43.36	49.60	51.31	45.79
NDICP, %DM	9.51	9.95	10.76	10.89
ADICP, %DM	8.25	8.65	8.43	8.61
Neutral detergent fiber, %DM	59.39	55.99	58.00	58.77
Acid detergent fiber, %DM	45.52	37.60	40.14	41.18
Hemicellulose, %DM	13.87	18.39	17.86	17.59
Cellulose, %DM	7.04	5.16	5.06	5.34
Lignin, %DM	38.35	31.81	34.20	34.64
Silica, %DM	0.13	0.63	0.88	1.20
pH	5.08	5.06	5.14	5.14

*R1: TMR without BSFL; R2: 80% TMR + 20% BSFL; R3: 80% TMR + 20% chemically defatted BSFL; R4: 80% TMR + 20% mechanically defatted BSFL; **DM, dry matter; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein.

ever, the EE content in the chemical composition of R4 was higher than that of R2. The CP content in R3 and R4 were also higher than R2 which was influenced by the defatted method in BSFL both before and after silage. It happened because of differences in the defatted method on BSFL raw materials. The soxhlet extraction method is a chemical extraction method commonly used to remove lipids using petroleum ether. This method effectively removes the ingredients so that their EE content becomes <1% DM from various oilseed (Bouallegue et al., 2016; Castejón et al., 2018; Jayanegara et al., 2018a). Mechanical extraction is a technique to extract lipid (Savoire et al., 2013) but it produces lower lipid compared to chemical extraction methods using organic solvent. It has been reported that the extraction of lipid oil from flaxseed using mechanical oil expeller is lower, which is 22.6% compared to organic solvent extraction methods, which is 36.3% (Ali & Watson, 2014).

BSFL has a high fiber content as shown by the CF, NDF, and ADF parameters that increased in R2, R3, and R4 compared to R1 both before and after ensiling. It is due to the exoskeleton content of insects that contribute to the high fiber content. The main component present in the exoskeleton of insects is chitin. Chitin is a polymer of N-acetylglucosamine (Chaudhari et al., 2011). In the proximate analysis, chitin is recovered as crude fiber according to Finke (2012). Another study reported that BSFL contained chitin 8.7% DM (Diener et al., 2009) and 9.6% DM (Kroeckel et al., 2012). The NDICP and ADICP contents in R2, R3, and R4 were higher compared to R1 after the 30 days ensiled, because chitin contains nitrogen groups in the molecule. However, the increasing age of BSFL will be followed by the development of exoskeletons, which increases the proportion of chitin per unit of DM (Zhu et al., 2016). The generally high pH values observed in R2, R3, and R4 containing BSFL after silage appear to be related to high CP content, which has been known to have an enhanced

Table 2: *In vitro* rumen fermentation and digestibility of total mixed ration (TMR) silage containing black soldier fly larvae (BSFL).

Treatments*	Parameters**				
	pH	VFA (mmol/l)	NH ₃ (mmol/l)	IVDMD (%)	IVOMD (%)
R1	7.28 ± 0.14	135 ^c ± 4.90	7.47 ^c ± 0.53	79.20 ^c ± 1.14	77.20 ^b ± 1.01
R2	7.24 ± 0.08	112 ^a ± 1.90	5.99 ^{ab} ± 0.52	52.50 ^a ± 0.65	50.70 ^a ± 0.78
R3	7.04 ± 0.05	138 ^c ± 3.17	10.40 ^d ± 0.78	79.20 ^c ± 0.64	77.80 ^b ± 0.94
R4	7.16 ± 0.11	125 ^b ± 2.49	5.92 ^a ± 0.49	67.40 ^b ± 0.65	77.20 ^b ± 1.00

^{abc}: means in the same column with varying superscript differ significantly (P<0.05); *R1: ensiled TMR; R2: ensiled 80% TMR + 20% BSFL; R3: ensiled 80% TMR + 20% chemically defatted BSFL; R4: ensiled 80% TMR + 20% mechanically defatted BSFL; **IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility.

buffering capacity (Chaikong et al., 2017; Jayanegara et al., 2019). However, the content of % DM TMR in each treatment after silage did not decrease compared to before silage which means the quality of the ration was maintained. The characteristics of *in vitro* rumen fermentation and digestibility of TMR silage containing BSFL based on the treatment are shown in Table 2. The pH values at each treatment were not significantly different. The total volatile fatty acid (VFA) value of R2 was the lowest compared to all treatments, while R3 was higher than that of R4 (p<0.05). Likewise, the ammonia concentration at R2 was the lowest compared to all treatments, while treatment R3 had the highest ammonia (NH₃) concentration (p<0.05). The IVDMD parameter showed that the lowest IVDMD value was R2 compared to all treatments, while the R1 and R3 treatments were higher than R4 (p<0.05). The IVO-MD parameter showed that R1, R3, and R4 treatments were higher than R2 (p<0.05). The pH value of each treatment is in the range of 7.04–7.28, which is included in the category of high pH values. It is consistent with the statement of Nagaraja & Lechtenberg (2007) which divides the rumen pH range into four categories, i.e., including acidic (pH < 5.6), optimal (5.6 to 6.0), suboptimal (6.0 to 6.4), and high (above 6.4). Rumen pH value is very influential in fermentation activity and feed degradation. The pH value of 5.5 within 24 hours has the potential for cattle to experience SARA (Subacute Ruminal Acidosis) (Zosel et al., 2010) and interfere with the stability of the rumen microbes.

Concomitantly, the high NDF and ADF contents in BSFL led to lower total VFA, IVDMD and IVOMD. Such high chitin content as fiber in BSFL affects in lower IVDMD and IVOMD (Jayanegara et al., 2017b). Fermentation of rumen microbes, especially carbohydrates, produces VFA which contribute in providing energy for the host animals after being absorbed (Nozière et al., 2011). The addition of BSFL in TMR increases the CP content so that the ammonia concentration becomes higher in rumen fluid. Ammonia is obtained from the degradation of proteins that enter the rumen by proteolytic microbes through proteolysis and deamination (Owens et al., 2014), and it is a precursor for

microbial protein synthesis (Pengpeng & Tan, 2013). Protein degradation that generates ammonia in the rumen is influenced by a number of factors such as protein fraction, protein degradation rate, the efficiency of converting ammonia to protein microbes, and ammonia absorption rate in the rumen (Bach et al., 2005). The low concentration of ammonia, IVDMD, and IVOMD in R2 can be due to the high lipid and fiber content in BSFL. BSFL contains high proportion of medium-chain fatty acids particularly lauric acid (C12:0), amounting to 21.4–49.3% (Tran et al., 2015). Many variations of NH₃-N values are affected by lipids in the feed rations that have been reported, but the results were inconsistent, either decrease (Wanapat et al., 2011), lack of effect (Shingfield et al., 2010; Toral et al., 2018) or increase (Homem Junior et al., 2010). Previous studies reported that lauric and myristic contained in palm oil could be toxic to bacteria and archaea (Soliva et al., 2011). It occurs through the mechanism of MCFAs dissociation in bacterial cells as antimicrobials (Goel et al., 2009). Another study has reported that MCFAs have a strong antiprotozoal effect both in pure form and in oil extraction products (Newbold et al., 2015), so it has an impact on the ability to degrade proteins and is directly proportional to the low IVDMD and IVOMD.

Total gas production, methane concentration, and total bacterial population of TMR silage containing BSFL are shown in Table 3. Total gas production in R1 and R2 is lower than treatments in R3 and R4, but the total gas production of R3 was the biggest compared to all treatments (p<0.05). Methane production in each treatment was not significantly different. Total *in vitro* rumen bacteria on R4 treatment was greater than other treatments, while R1 was the lowest (p<0.05). The pattern of low gas production in R2 seems to follow the pattern of low total VFA as well because both parameters are the final product of microbial fermentation in the rumen. *In vitro* gas production is positively correlated with total VFA concentration (Getachew et al., 2004). The addition of defatted BSFL to TMR gained a larger bacterial population compared to intact BSFL, and this was true when comparing between R2 and R4. It is because BSFL contains fat MCFAs, which

Table 3: *In vitro* total gas production, methane concentration, and total bacteria population of total mixed ration (TMR) silage containing black soldier fly larvae (BSFL).

Treatments*	Total gas production (ml)	Methane (% total gas)	Total bacteria (10 ⁹ /ml)
R1	133 ^a ± 3.00	16.06 ± 1.05	2.53 ^a ± 0.12
R2	130 ^a ± 4.32	16.19 ± 1.20	3.63 ^b ± 0.16
R3	139 ^b ± 4.38	16.35 ± 0.99	3.60 ^b ± 0.86
R4	135 ^{ab} ± 3.65	16.39 ± 1.09	5.11 ^c ± 0.32

^{abc}: means in the same column with varying superscript differ significantly ($P < 0.05$); *R1: ensiled TMR; R2: ensiled 80% TMR + 20% BSFL; R3: ensiled 80% TMR + 20% chemically defatted BSFL; R4: ensiled 80% TMR + 20% mechanically defatted BSFL.

were reported to be toxic to bacteria and archaea (Soliva et al., 2011).

Methane originated from enteric fermentation of livestock is a major greenhouse gas that significantly contribute to global warming (Jayanegara et al., 2018b). Methane production can decrease in feed ingredients that contain lipids. It has been reported that fat supplementation can reduce methane emissions by several mechanisms. Medium-chain saturated fatty acids, i.e., between C10-C14, have been shown to reduce methane emissions. One of the MCFAs is C12:0 or lauric acid. Oil supplementation containing C12:0 fatty acids in ruminant animal diets proved to be very useful in competing with rumen methanogens (Dohme et al., 2001). According to Guyader et al. (2014), there is a linear relationship between protozoan concentrations and methane emissions. Some studies suggest that lauric acid supplementation can defoliate protozoa, impacting the reduction in CH₄ production (Newbold et al., 2015). In the present study, however, methane emission was similar between TMR silage containing intact and defatted BSF. Apparently, although fat is removed in the defatted BSF, the process indirectly increases the proportion of chitin/chitosan that may possess a methane-mitigating effect as well, depending on its de-acetylation degree (Jayanegara et al., 2020).

CONCLUSION

Defatted BSFL can be included in TMR silage without causing any adverse effect on the nutritional value of the silage. However, inclusion of intact BSFL in TMR silage may reduce its nutritional value. Removal of fat from BSFL is therefore important for utilizing the insect as a feed ingredient of ruminant livestock.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

RM conducted the experiment, performed data analysis and wrote the article draft; RPH checked data analysis and revised the article draft; EBL and MR supervised the experiment; AJ designed the experiment, checked data analysis and revised the article draft.

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