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Maggot oil as a feed supplement for reducing methanogenesis of rumen microbial culture in vitro

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Abstract. *Hermetia illucens* larvae or known as maggot contains considerable amount of oil in its body. This experiment aimed to evaluate the use of maggot oil as a feed supplement for reducing methanogenesis of rumen microbial culture in vitro. The oil was supplemented into a feed substrate that consisted of forage: concentrate mixture (3:2 w/w). Supplementation of the oil was performed at different levels, i.e., 0, 1, 2, 3, 4 and 5% from substrate dry matter. All incubation bottles were tightly closed and incubated for 24 h in a water bath that maintained at 39°C. Results revealed that maggot oil at 4 and 5% supplementation level was able to reduce methane emission by 20.7 and 26.9% ($P < 0.05$) in comparison to control, respectively. However, its supplementation at 3% or lower did not alter the methane emission. The organic matter digestibility parameter was linearly reduced with increasing level of maggot oil supplementation ($P < 0.05$), but volatile fatty acid concentration was not affected. In contrast to our expectation, maggot oil at 3 to 5% increased log protozoa population and ammonia concentration than those of control ($P < 0.05$). In conclusion, maggot oil may serve as a promising supplement for mitigating ruminal methanogenesis and the effect is dose-dependent.

1. Introduction

Larvae of black soldier fly (*Hermetia illucens*) or known as maggot has been recently addressed as a promising protein source for human and animal. Like other insect species, maggot has various advantages as compared to plant and animal protein sources such as low water requirement, efficiently convert feed into its body mass, and low greenhouse gas emission [1]. Previous research on maggot as a feed ingredient had focused on exploring its chemical composition and the effect on animal production performance. Our previous study revealed that protein content of maggot is above 40% on dry matter basis and comparable to soybean [2]. Despite the potency of maggot, it has been reported that the insect contains some components that may negatively affect nutrient digestion in the digestive tract of animal when present in excessive amounts, and among them are chitin and oil. Chitin content in maggot was reported to be higher than 8% [3] and this component was responsible for the lower nutrient digestibility in livestock [4].

With regard to oil, maggot contains approximately 30% oil of its body [2]. The oil is rich in energy and C12:0 fatty acid (lauric acid), making it promising to be used as a multi-functional feed supplement. More than 40% of fatty acids in maggot oil is lauric acid [5]. Generally, oil or more specifically oil rich in medium-chain fatty acids such as C12:0 or C14:0 has methane-mitigating property in the rumen of ruminant livestock [6]. The influence of maggot oil on methanogenesis rumen microbial culture, however, has not experimentally been tested. This experiment therefore aimed to evaluate the use of



maggot oil as a feed supplement on methanogenesis and fermentation profile of rumen microbial culture *in vitro*.

2. Materials and methods

Maggot was cleaned from impurities and then dried in an oven at 60°C for 24 h. The dried maggot was subsequently ground to a powder form. Maggot was chemically extracted to obtain the oil, carried out using hexane solvent by the Soxhlet method by following the protocol of Jayanegara et al [7]. Extraction was performed at the boiling point for 6 h, and the sample was evaporated with a rotary evaporator to remove the residual hexane from the oil.

Maggot oil was added into a basal substrate that comprised of napier grass and concentrate mixture (3:2 w/w). An amount of 0.75 g basal substrate was added with either 0, 1, 2, 3, 4 or 5% maggot oil, and then added with 25 ml rumen fluid and 50 ml McDougall buffer. These were mixed in a serum bottle glass (125 ml capacity). This *in vitro* procedure was performed according to Theodorou et al [8]. Rumen fluid was taken from two fistulated Friesian Holstein cows, and filtered through four layers of gauze prior to use. The serum bottle was covered with a rubber cap and then sealed using a crimper. All incubation bottles were placed into a water bath that maintained at 39°C for 24 h. Arrangement of experimental treatments into incubation units followed a randomized complete block design in which different incubation runs served as the blocks. Incubation was performed in three incubation runs, and each run was conducted in duplicate.

After the incubation was completed, gas production was recorded by using a syringe. Measurement of methane emission was conducted by injecting the gas produced into a gas chromatograph. The incubation fluid was determined for pH, volatile fatty acid (VFA) concentration, ammonia concentration and protozoa population. The residue was further digested with pepsin-HCl solution for another 24 h to generate dry matter digestibility (DMD) and organic matter digestibility (OMD) values.

Data were analyzed by using analysis of variance. When a variable showed significantly different at $P < 0.05$, the statistical analysis was continued with the post-hoc test namely Duncan's multiple range test for comparison among different treatment means.

3. Results and discussion

Addition of maggot oil at various levels did not alter total gas production (Table 1). The gas production for all treatments ranged from 70.2 to 74.1 ml. Maggot oil at 4 and 5% supplementation level was able to reduce methane emission by 20.7 and 26.9% ($P < 0.05$) in comparison to control, respectively. However, its supplementation at 3% or lower did not alter the methane emission. Gas production due to the activity of rumen microbes is mainly influenced by level and type of carbohydrate present in the substrate rather than other components. Lipid or fat or oil has little influence on ruminal gas production since it is minimally utilized by the microbes [9]. Triglyceride, a main form of lipid, is separated into glycerol and various fatty acids in the rumen by lipolytic microbes. Further, unsaturated fatty acids are hydrogenated to generate other fatty acids with higher saturation degree. These fatty acids are not, however, degraded into smaller molecules and thus generate only little amount of gas during the process.

To our knowledge, based on literature search from Scopus database, there is no previous report that evaluate the use of maggot oil on rumen methanogenesis, and therefore this is the first study to do so. There have been a number of papers reported the ability of C12:0 fatty acid, the main fatty acid present in maggot oil, for reducing enteric methane formation. The C12:0 was found to be toxic for methanogens as shown by the reduced population and its associated methane emission [6]. In addition, C12:0 contributes further to lowering methane by partially eliminating ciliate protozoa and depressed fiber fermentation of cellulolytic microbes [10]. At present, mitigating enteric methane emission from livestock has been generating a lot of attention in order to decelerate the accumulation of the greenhouse gas in atmosphere. Therefore, various nutritional attempts have been performed for achieving such lower methane emission [11,12]. It is expected that, in turn, all these efforts would contribute to reduce the problem of global warming.

Table 1. *In vitro* gas production and methane emission of maggot oil supplementation on feed substrate (forage: concentrate mixture 3:2 w/w).

Maggot oil (%)	Gas 24 h (ml)	Methane (ml/l)
0	70.7	3.23 ^c
1	70.2	3.05 ^{bc}
2	74.1	3.26 ^c
3	72.5	3.22 ^c
4	73.5	2.80 ^{ab}
5	74.0	2.64 ^a
SEM	1.41	0.351
P-value	0.580	<0.001

Maggot oil supplementation did not influence pH and total VFA concentration in the *in vitro* rumen microbial culture (Table 2). The DMD and OMD parameters were linearly reduced with increasing level of maggot oil supplementation ($P < 0.05$, Table 3). In contrast to our expectation, maggot oil at 3 to 5% increased log protozoa population and ammonia concentration than those of control ($P < 0.05$).

Table 2. *In vitro* ruminal pH, volatile fatty acid (VFA) and ammonia concentration of maggot oil supplementation on feed substrate (forage: concentrate mixture 3:2 w/w).

Maggot oil (%)	pH	VFA (mmol/l)	Ammonia (mmol/l)
0	6.68	109.5	11.1 ^a
1	6.63	109.8	11.2 ^a
2	6.66	115.7	14.0 ^b
3	6.63	105.8	13.0 ^b
4	6.68	101.8	14.6 ^b
5	6.65	110.2	14.5 ^b
SEM	0.010	4.83	0.380
P-value	0.123	0.818	<0.001

Table 3. *In vitro* gas production and methane emission of maggot oil supplementation on feed substrate (forage: concentrate mixture 3:2 w/w).

Maggot oil (%)	DMD (%)	OMD (%)	Protozoa (log/ml)
0	54.6 ^d	54.3 ^d	4.74 ^a
1	51.7 ^c	51.8 ^{cd}	4.70 ^a
2	49.7 ^{bc}	49.1 ^{bc}	4.74 ^a
3	48.2 ^{ab}	47.3 ^{ab}	5.00 ^b
4	47.3 ^{ab}	46.3 ^{ab}	5.02 ^b
5	46.3 ^a	45.2 ^a	5.13 ^b
SEM	1.04	1.08	0.046
P-value	<0.001	<0.001	<0.001

Lower nutrient digestion due to maggot oil supplementation is in accordance with some other previous studies. Lipid, particularly at higher level, is regarded to cause a negative effect on carbohydrate digestion and fermentation in the rumen [13]. Lipid is negatively affecting the growth of cellulolytic bacteria in the rumen that contribute to cell wall degradation [14]. In insects including maggot, another component that causes negative effect on nutrient digestion is chitin [3, 4] in which the effect is closely similar to plant tannin that able to bind protein [15].

4. Conclusion

Maggot oil may serve as a promising supplement for mitigating ruminal methanogenesis under *in vitro* rumen environment and the effect is dose-dependent. Further *in situ* and *in vivo* studies are required in order to comprehensively elucidate the influence of maggot oil on digestion, enteric methane emission, metabolism and performance of ruminant livestock.

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