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THE INHIBITION OF METHANE RELEASE FROM THE CELLULOLITIC FERMENTATION AS AN EFFECT OF LEMURU FISH OIL ADDITION

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ABSTRACT

Ruminants, especially cattle fed by high level of forages, has become a special interest for nutritionist, because of its considerable contribution to the methane release to atmosphere. Ruminant nutrition research has been focused on finding the method to reduce methane production, because of the methane effect on global warming and its effect on the decreasing of energy efficiency of the diets. The compounds with the ability to capture electron such as poly unsaturated acids which were found in the fish oil was studied in this experiment. Two head of Ongole cattle were used in this experiment, as the donor animal to get the rumen fluid for fermentation. A small pieces filter paper was used as a source of cellulose for the substrate in the fermentation. Lemuru fish oil as poly unsaturated fatty acid in the level of 2.5, 5.0 and 7.5 % were used in this experiment to reduce methane produced by cellulolytic fermentation. The result showed that the addition of Lemuru fish oil in the fermentation medium could reduce methane production significantly. Fish oil in the level of 2.5, 5.0 and 7.5 % reduced methane production 1%, 17% and 31%. The addition of fish oil did not affect on cellulolytic enzyme activity. The CMC-ase activities in the medium solution with 2.5, 5.0 and 7.5% addition of Lemuru fish oil, were 5.00, 4.08 and 4.01 U/g protein. The enzyme activities were not significantly different compare with the enzyme activity in the control medium solution (4.35 U/g protein). The average microbial protein content were 0.56, 0.43 and 0.46 mg/ml for the medium added by 2.5, 5.0 and 7.5% Lemuru fish oil respectively. Those values were not significantly different compare with the microbial protein in the control medium (0.62 mg/ml). In the closed fermentation experiment, with the same condition gave the result that acetic acid as well as the propionate acid were increased as an effect of the compounds, while the butyric acid remain constant. The total volatile fatty acid contents increased significantly from 22.08 μmol in the control to 34.42, 50.31 and 36.78 μmol as an effect of 2.5, 5.0 and 7.5% Lemuru fish oil addition respectively. It could be concluded that Lemuru fish oil could be used as a compound to reduce methane production in celulolityc fermentation by rumen microbes, without any negative effects on the fermentation products as well as the CMC ase activity.

Key Words: Methane Inhibition, Lemuru Fish Oil, Cellulase

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INTRODUCTION

About 70% of methane production arises from anthropogenic sources, and two third of those sources was considered come from agricultural sources included flooded rice paddies, enteric fermentation and animal waste (Moss *et al.*, 2000). Stabillising global methane concentration at current levels would require reductions in methane emission or increased sink for methane at the same level. Livestock is one of the large source of methane emission with 80-115 tons produced per year. The global cattle population is responsible for 73% methane emission of all livestock, and methane produced during rumen fermentation represents a loss of 2-15% of gross energy intake and may contribute to global warming (Johnson and Johnson, 1995). Many researches had been carried out to find ways to decrease methane production especially from ruminants. The compound with the ability to capture the electron such as unsaturated fatty acid was tested to reduce methane production (Minami et al., 1992). Another compounds were also used to reduce methane production, such as, methanol and nitrate (Neuman et al., 1999), fumaric acid (Asanuma *et al.*, 1999), acrylic acid, fumaric acid and 3-oxoglutaric acid (Lopez et al., 1999), coconut oil (Machmuller et al.,1998 and Machmuller *et al.*, 2003), 9,10- anthraquinone (Lopez et al., 1996, Fievezl et al., Kung et al., 2003) and *Cassia alata L* leaf which contains anthraquinone (Yusiati , et al., 2005). The most promising research for reducing methanogenesis are the development of new product or delivery system for anti methanogenic compounds or alternative elecfron acceptors in the rumen (Moss et al., 2000). In this present in vitro study, Lemuru fish oil which contain unsaturated fatty acids (Supadmo,1999), was tested for its ability in decreasing methane production, rumen fermentation pattern as well as the activity of carboxy methyl cellulase.

MATERIALS AND METHODS

Donor Animal. Two head of rumen fistulated Ongole cattle as donor animals for rumen fluid, were fed twice a day, at 8.00 am and 15.00 pm. with King grass and rice bran (70:30). The rumen fluid samples were collected in the morning before feeding time and it was kept at 39° C, an aerobically, mixed with buffer solution to keep its pH.

Substrate and reducing methane production agent. Filter papers in small pieces were used as substrate. Lemuru fish oil, a waste product in fish canned industry was used as a source of unsaturated fatty acids which can act as a reducing methane production agent.

Fermentation by "gas production technique" as described by Menke, K.H. and Steingass, H. (1988). The fermentation was done in the syringes as fermentor to get the gas produced measuring. Filter paper as much as 300 mg was put into the syringes and let them incubated in the oven at 39^0C, over night. Rumen fluid/buffer mixture (1 : 2) was pumped in 30 ml doses into syringes and followed by addition of Lemuru fish oil with the levels of 0, 2.5; 5.0 and 7,5 % for T0 (control), T1, T2 and T3 respectively. The experiments were done in three replicates. The syringes were put in the incubator and the temperature was kept at 39^0C for 72 hours. Gas produced were read and then taken out for methane analysis, which was done by GC. At the end of the fermentation

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process, the fermentation medium about 25 ml were taken and prepared for microbial protein and Carboxy methyl cellulase (CMC-ase) activity (Halliwell et al.,1985), while protein measurement was done by Lowry method (Plummer, 1987).

Closed Fermentation. Fermentation was done in the serum bottles, with the same substrate and medium as in the fermentation by "gas production technique". Incubation was done at 39^0C for 24 hours. At the end of the process, the medium was taken out and prepare for volatile fatty acid (VFA), including acetic acid, propionic acid and butyric acid analysis. The measurements were done by HPLC.

RESULTS AND DISCUSSION

The addition of Lemuru fish oil with the levels of 5% and 7.5% decreased significantly methane production 17 and 31% respectively (Table 1). In vivo methane production has positive correlation with in vitro methane production measured by gas production technique (Moss and Givens, 1998). The volumes of methane produced by in vitro experiment were lower than in vivo. Methane production during fermentation of various diets arranged 15 – 35 ml/ g dry matter/24 hours, or 20-50 ml/g of DM/24 h by the assumption that dry matter digestibility was 70%. In the present studies, the average of methane production was 15 ml/g digested dry matter/ 24 h. It could be understood as the fermentation did not run in optimum condition due the substrate used in this present studies was filter paper which does not contain any other nutrient except cellulose.

It was reported that supplementation diet with coconut oil in the doses of 25 g/kg DM and supplementation with 57 g/kg DM sunflower seed which contained fatty acids as much as 36 and 34 g/kg DM reduced methane production 26% and 27% respectively (Machmuller et al., 2000). Compare with those results, the decreasing of methane produced in the present finding were higher. The addition of Ketepeng Cina which were equal to 0.5 ; 1.0 and 5.0 ppm anthraquinone in the fermentation medium also gave higher effect on the reducing methane production with the value of 47% in average (Yusiati et al., 2005).

The activity of CMC ase, the concentration of enzyme dan microbial protein in the fermentation medium, were shown in Table 2. The addition of Lemuru fish oil didn't give significant effects on the activity of CMC ase. The activities of CMC ase in the present studies were in the range values of previous finding. Yusiati et al. (1996) reported that CMC ase activity of anaerobic thermophilic bacteria arranged 0.32 – 25.36 U/g protein, depended on the temperature and incubation time. It was also reported that the CMC-ase activities in the medium solution with the addition of Ketepeng Cina leaf meal equal with 0.5, 1.0 and 5.0 ppm of anthraquinone, were 3.41, 4.30 and 4.20 U/g protein, while the control was 3.77 U/g protein (Yusiati, et al., 2005).

Table 1. Total gas and methane production in cellulose fermentation with addition of Lemuru fish oil .

Gas production	Levels of fish oil added			
	0.0%	2.5%	5.0%	7.5%
Total gas (ml/g DDM/72h	427.0 [a]	481.0 [a]	388.0 [b]	360.0 [b]
Methane production (ml/DDM/72h)	67.7 [a]	67.5 [a]	56.3 [b]	46.9 [b]

[a,b.] The value in the same row with different superscript showed the different effect of treatment (P< 0.05)

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Table 2. Carboxy Methyl Cellulase activity and microbial protein in cellulose fermentation with addition of Lemuru fish oil.

Gas production	Levels of fish oil added			
	0.0%	2.5%	5.0%	7.5%
CMC ase activity (U/g protein) [ns)]	4.35	5.00	4.08	4.01
Enzyme protein (mg/ml) [ns)]	1.39	1.26	1.35	1.31
Microbial protein (mg/ml) [ns)]	0.62	0.56	0.43	0.46

[ns)] Not significant

Table 3. Volatile fatty acid produced in the cellulose fermentation with addition of Lemuru fish oil (μmol)

Volatile Fatty Acids	Levels of fish oil added			
	0.0%	2.5%	5.0%	7.5%
Acetic acid	14.31 [c]	22.05 [bc]	33.91	25.70
Propionic acid	6.16 [b]	10.59 [ab]	14.15	6.63
Butyric acid	1.60	1.78	2.25	4.46
Total Volatile Fatty Acid	22.08 [b]	34.42 [ab]	50.31 [a]	36.78 [ab]

[a,b,c.] The value in the same row with different superscript showed the different effect of treatment ($P < 0.05$)

The result of in vitro fermentation study showed that the addion of Lemuru fish oil didn't give any effect on enzyme protein concentration as well as microbial protein content (Table 2.).

Microbial protein content in the fermentation fluid was 0.48 mg/ml in averages. Those value was in the ranges of the values (0.39-0.48 mg/ml) reported by Yusiati, et al. (2005) when the fermentation medium were added by *Cassia alata.*L. as a source of anthraquinone. It is considered that Lemuru fish oil addition does not give negative effect on rumen microbe metabolism. Cheng et al. (1999) reported that canola oil, cod liver oil and coconut oil depressed methane production. Total viable, amylolitic and cellulolitic bacterial number were reduced by coconut oil but not by canola and cod liver oil. The most inhibitory to methane, was the most depressed fibre digestion.

In the closed fermentation experiment, with the same condition of in vitro rumen fermentation gave the result that the addition of Lemuru fish oil, increased significantly propionic acid and acetic acid content. Although butyric acid were not affected by the treatment, total VFA increased significantly.

The decreasing of methane production was accompanied by the increasing of VFA especially propionic acid. Fish oil addition at the level of 5% increased acetic acid, propionic acid and total VFA more than 100%. Maximum production of VFA was reached when the fermentation fluid was added by 5% fish oil. The increasing of VFA production was not found anymore at 7.5% fish oil addition. The addition of fish oil seems to change metabolic activity of rumen microbe, as described by Macmuller *et al.*, (2003), that the methane suppressing effect of coconut oil was mediated through a changed metabolic activity and / or composition of the rumen methanogenic population.

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CONCLUSION

It could be concluded that Lemuru fish oil can be used as a compound to reduce methane production without negative effect on cellulolytic fermentation by rumen microbes.

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