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Effect of Cinnamaldehyde From Cinnamomum (*Cinnamomum burmanni* Ness ex Bi.) as an Encapsulation Agent Of Lemuru Fish Oil on *In Vitro* Gas and Methane Production

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ABSTRACT

Polyunsaturated fatty acids (PUFA) supplementation in animal feed is expected to increase unsaturated fatty acids content in livestock products. Lemuru fish oil supplementation as a source of PUFA can function as a hydrogen sink to reduce methane production. The contribution of methane gas emissions in livestock reaches 15-17% of the world's methane gas emissions. Encapsulation of PUFA using natural ingredients of cinamaldehyde is expected to be a solution to increasing PUFA in livestock product and improving environmentally friendly animal husbandry. This study aimed to determine the effect of using cinnamaldehyde as an encapsulation agent for lemuru fish oil as a source of PUFA on *in vitro* gas, methane and CO_2 productions. Treatments consisted of different levels of cinnamaldehyde, namely 0, 250, 500, 750 and 1000 (mg/kg feed DM), with the 5% lemuru oil as PUFA source. The experimental design used a one-way ANOVA in completely randomized design pattern consisting of five treatments and three replicates. Each replicate was duplicate, and then, if there were significant differences, it was continued with Duncan's New Multiple Range Test (DMRT) with SPSS version 23. This study discovered that the use of cinnamaldehyde did not affect the *in vitro* kinetics of gas and CO_2 production. The use of cinnamaldehyde level of 500 mg/kg DM feed is the optimal level that can be used as an encapsulation agent for lemuru oil without causing an increase in methane production.

Keywords: *Cinnamaldehyde, Lemuru fish oil, In vitro gas production*

Introduction

Polyunsaturated fatty acids (PUFA) supplementation in animal feed is expected to increase PUFA content in livestock products. However, the provision of fat in ruminant feed is limited to 5% (McAllister & Newbold, 2008). It is due to oil in feed can inhibit the work of cellulolytic bacteria to degrade fiber into energy sources for host livestock (Szczechowiak *et al*., 2016). Adding high oil to feed can also increase the content of saturated fatty acids in livestock products (Bauman *et al*., 2003). It requires a mechanism to defend the PUFA from biohydrogenation.

Previous research reported that encapsulation of crude palm oil showed good results (Tiven *et al*., 2013) It did not adversely affect rumen bacteria and could reduce the hydrogenation process in the rumen. Cinnamaldehyde is an aldehyde compound contained in cinnamon that can be used as an alternative to formaldehyde (Yusiati *et al*., 2014). Research by Chaves *et al* (2009) discovered that adding cinnamaldehyde up to 400 mg/kg DM can be used as an aldehyde source. Hadianto *et al.* (2020) asserted that adding 4.6% cinnamaldehyde is the optimum level as a protein protection agent.

on the other hand, providing unprotected PUFA provides benefits. Research by Yusiati *et al*. (2006) showed that adding lemuru fish oil at the 5% level reduced methane production by 17% and adding at the 7% level reduced methane by 31%. Lemuru fish oil is one of the sources of long-chain fatty acids used as a methane-reducing agent, Lemuru fish oil has a high content of unsaturated fatty acids ranging from 15-20%, especially the content of *Eicosapentaenoic acid* (EPA) and *Docosahexaenoic acid* (DHA), (Khoddami *et al*, 2009).

As we know that livestock farming produces greenhouse gases (GHG) like CH_4, N_2O and CO_2 (Lascano and Cardenas, 2020). The

contribution of up to 15-17% of methane gas emissions impacts global warming (DPCC, 1992; Jhonson & Johnson, 1995). Methanogenic bacteria will use CO_2, H_2 and formate fermentation products to produce CH_4 (Attwood and Mc Sweeney, 2008). The formation of methane will result in energy waste of 2-12% of the gross energy consumed by animals (Jhonson & Johnson, 1995). On this basis, many studies have been conducted to reduce these effects. It will decrease the loss of net feed energy and promote environmentally friendly livestock husbandry (Zhou *et al.*, 2021).

Based on these considerations, the provision of fatty acid sources in ruminant feed should be considered. Encapsulation is done to increase the content of PUFA that escape biohydrogenation in the rumen, but considering the function of fatty acids as methane reducers. It is expected that cinnamaldehyde from cinnamon also has the ability as an encapsulation agent for lemuru fish oil to increase the PUFAs escaping from biohydrogenation without increasing methane production.

Materials and Methods

Preparation and Treatment

We followed Tiven *et al*.'s method (2021) for encapsulating lemuru oil with 5% lemuru oil as a source of fatty acids mixed with 10% soybean meal as a source of protein in the basal feed. Cinnamon as a source of cinnamaldehyde was added at five different levels: 0, 250, 500, 750, and 1000 (mg/kg feed DM) or equivalent to the addition of cinnamon flour of 14; 29; 43 and 58 g/kg feed DM. The protection was added to the basal feed, with the feed analysis results shown in Table 1.

Table 1. Composition and nutrient content of basal ration

Feed Materials	%
Pennisetum purpureum grass	60
Bran pollard	30
Soybean meal	10
Total	100

Nutrient contents	%DM
Crude protein	22.00
Crude fat	2.23
Crude fiber	23.52
Organic mater	87.45
Nitrogen Free Extract (NFE)	39.70
Total Digestible Nutrient (TDN)	64.60

In vitro fermentation

Rumen fluid as a source of rumen microbes was obtained from Balinese cattle undergoing an adaptation period for one week, Rumen fluid was taken in the morning before feeding. The collected rumen fluid was placed in an airtight place with a temperature of 39°C. The in vitro method was carried out according to the method of Menke and Steingass (1988). Rumen fluid was mixed with buffer at a 1:4 ratio. Each treatment was done in duplicate with three replicates. Fermentation was occured in an incubator at 39°C for 48 h. Gas production was recorded at 0, 2, 4, 6, 12, 36 and 48 h, and the measurement results were analyzed using the Fit Curve program to determine feed degradation and entered in the equation $Y = ax + b$ (Chen, 1994). Following fermentation, gas samples were collected using a syringe and put into an airtight countainer to analyze methane and carbon dioxide concentrations. The fermentation filtrate was filtered using silica disk and analyzed for dry matter and remaining organic matter.

Analysis of methane and carbon dioxide production

Methane and carbon dioxide gas production was measured by multiplying the methane and carbon dioxide contents in the gas by the total gas production volume. Methane and carbon dioxide levels were analyzed using gas chromatography (GC) with the Shimadzu brand (GC-8A model, Kyoto, Japan, 2010) equipped with a flame ionization detector (FID) and a recorder with the C-R61 brand and a Porapak-Q column. Following the method by applied by Bhatta *et al.* (2012), the material prepared is an activated carbon column with a length of 1m and a diameter of 0.5m. The temperature at the injector was 60°C, 100°C at the column and 110°C at the detector. Helium gas is set at a 3 ml/min speed as a carrier gas. The sample was injected into the GC, equipped with a thermal conductivity detector and a parapak-Q steinless steel column package with the previously described settings.

Data analysis

All data obtained were statistically tested using analysis a one way of variance (ANOVA) in completely randomized design (CRD) with significance ($p<0.05$). If there was a significant difference, it was followed with Duncan's New Multiple Range Test (DMRT) to determine the difference in the average value of each treatment (Astuti, 1981).

Results and Discussion

In Vitro Gas Production

The results of the study on the effect of using cinnamaldehyde *(Cinnamomum burmanni* Ness ex Bi.) as an encapsulating agent for lemuru fish oil on total gas production, fraction a, fraction

b, and fraction (a+b) are presented in Table 2. Based on the data obtained, it indicates that there is no effect of using cinnamaldehyde as a lemuru oil fatty acid encapsulation agent.

Table 2. *in vitro* gas production kinetics resulted from the use of cinnamaldehyde as an encapsulating agent for lemuru oil

Variable	Level of cinnamaldehyde (mg/kg DM)				
	0	250	500	750	1000
Total gas production (mg / 300 mg DM) [ns]	63.80 ± 3.77	66.00 ± 5.03	65.26 ± 4.50	66. 46 ± 4.89	64.39 ± 5.05
a (ml / 300 mg DM) [ns]	4.37 ± 0.82	4.97 ± 0.89	5.22 ± 0.79	4.71 ± 0.81	5.70 ± 0.87
b (ml / 300 mg DM) [ns]	63.66 ± 6.25	62.27 ± 8.67	62.54 ± 7.60	64.28 ± 4.34	63.47 ± 8.11
c (ml / hour) [ns]	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.00
a + b (ml / 300 mg DM) [ns]	68.03 ± 6.14	67.26 ± 9.55	67.76 ±7.51	68.99 ± 5.15	69.17 ± 7.24

[ns] no significant difference

Statistical results indicate that adding cinnamaldehyde cinnamon as an encapsulating agent of unsaturated fatty acids does not significantly affect the total gas production and, gas production of fractions a, b, c, and (a + b). This finding is in line with research conducted by Dewi *et al* (2020) that confirmed that there is no significant effect ($p<0.05$) Supplementation of protected lemuru oil using NaOH on total gas production, fractions a, b, and (a + b). The results of research conducted by Adeyemi *et al*, (2015) on the addition of carotino oil in rumen fermentation showed no change in the resulting gas production. Gas production describes the degradation of feed in the rumen. Chumpawadee *et al.* (2005) explained that the gas production illustrates the fermentation of the feed given, especially carbohydrates into acetate and butyrate.

Methane and Carbon Dioxide Production

The results of the study on the effect of using cinnamaldehyde *(Cinnamomum burmanni* Ness ex Bi.) as an encapsulating agent for lemuru fish oil to methane and carbon dioxide mitigation are presented in Table 3. Based on the data obtained, there is no effect on digested DM, digested OM, CO_2 gas production, CO_2 gas production / digested DM and CO_2 gas production/ digested OM but there is an increase of CH_4 gas production, CH_4 production/ digested DM, CH_4 production/ digested OM using cinnamaldehyde as a lemuru oil fatty acid encapsulation agent.

Table 3. CO_2 and CH_4 production resulted from the use of cinnamaldehyde as an encapsulating agent for lemuru oil

Variable	Level of cinnamaldehyde (mg/kg DM)				
	0	250	500	750	1000
digested DM (mg / 300 mg DM) [ns]	125.78 ± 1.62	115.64 ± 6.83	121.97 ± 5.46	112.24 ± 9.38	119.93 ± 6.94
digested OM (mg / 300 mg DM) [ns]	110.39 ± 2.45	101.34 ± 6.34	113.08 ±13.50	99.25 ±10.36	103.05 ± 5.68
CH_4 (ml/300 mgDM)[a.b]	6.46 [a] ± 0.29	6.26 [a] ± 0.63	6.87 [ab] ± 0.38	7.33 [b] ± 0.21	7.27 [b] ± 0.42
CH_4 / digested DM (ml/g) [a.b]	0.05 [a] ± 0.01	0.05 [a] ± 0.01	0.06 [a] ± 0.01	0.07 [b] ± 0.00	0.06 [a] ± 0.01
CH_4 / digested OM (ml/g) [a.b]	0.063 [ab] ± 0.01	0.063 [ab] ± 0.01	0.056 [a] ± 0.01	0.083 [c] ± 0.01	0.070 [b] ±0.00
CO_2 (ml/300 mgDM) [ns]	32.24 ± 1.45	33.35 ± 3.56	34.78 ± 3.99	37.13 ± 3.85	37.41 ± 3.79
CO_2 / digested DM (ml/g) [ns]	0.26 ± 0.01	0.30 ± 0.04	0.29 ± 0.05	0.32 ± 0.04	0.32 ± 0.01
CO_2 / digested OM (ml/g) [ns]	0.29 ± 0.01	0.33 ± 0.05	0.31 ± 0.04	0.34 ± 0.03	0.36 ± 0.02

[ns] no significant difference

[a.b.] superscripts on the same line indicate significant differences ($p<0.05$)

Based on statistical tests that have been carried out, the use of cinnamaldehyde as a capsulation agent for lemuru fish oil has no effect on CO_2 gas production, CO_2 gas production/ digested DM and CO_2 gas production/ digested OM. However, there are significant differences ($p<0.05$) in the production of CH_4, CH_4/ digested DM and CH_4/ digested OM compared to the control.

Methanogens will application the resulting CO_2 to produce methane. Haque (2018) explained that H_2 and CO_2 produced by dynamic processes in the rumen are the main substrates for methanogenic bacteria in producing methane. The highest methane production in adding cinnamaldehyde at 750 mg/DM and 1000 mg/DM was increased 13.47% and 10.82% compared to the control. Methane production without treatment is a description of methane production with the addition of the largest fatty acid source of 5%, resulting in low methane production. Research by Yusiati *et al* (2006) discovered that adding lemuru fish oil at 5% significantly reduced methane levels by 17%. Fievez *et al*. (2003), who stated that adding lemuru fish oil in in vitro experiments will reduce methane production. It occurs because protozoa will produce hydrogen which will enter the pathway of methane formation. Morgavi *et al.* (2011) stated that protozoa are microorganisms in the rumen ecosystem that produce hydrogen and will be utilized by methanogens into methane.

The encapsulation of lemuru oil using cinnamaldehyde show an increase in methane production. this is thought to be a decrease in the biohydrogenation process which causes hydrogen ions to be utilized by methanogens and increases methan. Tiven *et al*. (2013) stated that symbiosis between ciliated protozoa and methanogens prevents hydrogen accumulation through interspecies hydrogen transfer mechanisms. The addition of cinnamaldehyde may diminish the effectiveness of lemuru oil in reducing methane gas production. Darabighane *et al*. (2021) stated that the use of fish oil in ruminant feed could reduce methane production through various mechanisms by reducing the digestibility of feed ingredients, influencing the protozoa population through C12:0 and C14:0 and competition for hydrogen use for biohydrogenation in the rumen. Fievez *et al*. (2003) confirmed that methane production depends on the level of PUFA added to the feed. In this study a decrease in the level of cinnamaldehyde used can reduce the negative effects of PUFA so that there is an increase in methane production at the highest level of cinnamaldehyde administration.

Conclusion

This study discovered that the use of cinnamaldehyde did not affect the *in vitro* kinetics of gas and CO_2 production. The use of cinnamaldehyde level of 500 mg/kg DM feed is the optimal level that can be used as an encapsulation agent for lemuru oil without causing an increase in methane production.

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