Catfish oil supplementation in Bali cattle diet: Effects on rumen fermentation parameters, carboxymethylcellulase and protease activity in vitro

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Abstract. This study aimed to measure the effect of Catfish oil supplementation on rumen pH, volatile fatty acid (VFA), acetate : propionate, NH3, microbial protein, carboxymethyl cellulase (CMCase), and protease activity. There are five treatments conduct on the current study i.e. T0 (control diet: Pennisetum purpureoides (60): wheat pollard (30): soybean meal (10)), T1 (T0 + 2% DM CFO), T2 (T0 + 4% DM CFO), T3 (T0 + 6% DM CFO), T4 (T0 + 8% DM CFO) and each treatment replicated 3 times. The fermentation method was in vitro gas production technique described by Menke and Steingass by incubating samples in the fermentation medium for 48 hours of the incubation period. The statistical analysis result showed that Catfish oil supplementation did not change the value of rumen pH, total VFA, partial VFA, rumen microbial protein and protease activity. Supplementing Catfish oil at the level of 2% and above decreasing the C2:C3 ratio, and when the supplementation increased to the level of 8% could decrease rumen NH3 and CMCase activity. It was concluded that the supplementation of Catfish oil in a ruminant diet could be done up to the level of 6% without giving any negative effect on rumen fermentation.

1. Introduction

Methane production in the rumen described as one of rumen hydrogen sinks [1], following the reaction of carbon dioxide reduction with the existence of hydrogen conducted by archaea methanogen [2]. This reaction causing the loss of diet gross energy up to 12% [3], it leads scientists to study the strategy of rumen enteric methane inhibition [4], although the background of methane inhibition study in the latest study was aimed to decrease greenhouse gas emission of livestock production. Methane is a greenhouse gas that has a greater impact on the environment than carbon dioxide in an equal amount [5]. These problems provide a strong background in conducting the study about rumen enteric methane production inhibition. The current study utilizes fish oil as methane inhibition supplement in ruminant diet. Fish oil is a potential rumen methane production inhibitor related to its unsaturated fatty acid content [6], besides the oil main function in a feed as an energy source [7]. Catfish oil was used in this study to eliminate conflicts of interest among feed and food because Catfish oil was a by-product of the Catfish meal industry [8].

Oil supplementation, especially PUFA-rich at a high level to ruminant diet lead to the changing of the rumen fermentation product related to its toxic effect on rumen microbes [9]. Based on the statement above, the utilization of fish oil as a rumen methane production inhibitor supplement needs to be done
carefully. Patra [10] stated that oil supplementation on a ruminant diet could not be done at the level higher than 6%, but the studies reported by [11] and [12] stated that the addition of Carotino oil and Cakalang fish oil respectively at the level of higher than 6% did not affect rumen fermentation parameters. In vitro fermentation study was a screening method that could be done before in vivo study, because the in vitro fermentation study could provide rumen fluid to measured rumen fermentation parameters such as pH, volatile fatty acid (VFA), NH₃, microbial protein, and enzyme activities. Related to the statement above, Catfish oil is a potential rumen methane production inhibitor, but the optimum supplementation level without affecting the rumen fermentation parameter was remaining unknown. This present in vitro study aimed to evaluate the effects of Catfish oil supplementation in diet on rumen fermentation parameters, carboxymethylcellulase, and protease activity in vitro.

2. Materials and methods

2.1. Materials

Materials used in this study were king grass (Pennisetum purpureoides), wheat pollard, soybean meal, Catfish oil (CFO), rumen fluid, and buffer solution. Catfish oil obtained as a by-product of Catfish meal, which contains unsaturated fatty acid >90g/100 g fatty acid. Rumen fluid was taken from rumen fistulated Bali cow 1 hour before morning feeding. One liter of buffer solution consist of 474 mL aquades, 0.12 mL micro mineral (CaCl₂·H₂O; MnCl₂·4H₂O; CoCl₂·6H₂O; FeCl₃·6H₂O), 237 mL buffer (NaHCO₃, (NH₄)HCO₃), 237 macro mineral (Na₂HPO₄; KH₂PO₄; MgSO₄·7H₂O), 1.22 mL resazurin, and 49.5 mL reducing solution (NaOH; Na₂S·7H₂O).

2.2. Research design and treatments

This research was in vitro study using a completely randomized design consists of 5 treatments, each treatment replicated 3 times. Five treatments in the current study were mention as T0 = control diet (king grass: wheat pollard: soybean meal, 60:30:10), T1 = T0 + 2% DM basis CFO (v/w), T2 = T0 + 4% DM basis CFO, T3 = T0 + 6% DM basis CFO, T4 = T0 + 8% DM basis CFO.

2.3. In vitro fermentation

In vitro gas production technique procedure described by [13] were used in this study. Diet substrate as much as 300 mg of dry matter was put into 100 mL glass syringe and incubated for one night in a waterbath (39° C). On the next day, the fermentation medium was prepared. Rumen fluid and buffer solution were mixed in the ratio of 1:2 in the glass jar continuously flushed by CO₂ to maintain the anaerobic condition. Each substrate-contained syringe filled with 30 mL of fermentation medium, then incubated at waterbath for 48 hours. After 48 hours of incubation, the fermentation medium was separated from the substrate (its mention as filtrate) for further analysis.

2.4. Sample analysis

Parameter measured in this study were pH, volatile fatty acid (VFA), NH₃, microbial protein, CMCase, and protease activity. The value of filtrate pH was measured using calibrated pH-meter. VFA concentration of filtrate was analyzed using gas chromatography following the method described by [14]. NH₃ concentration was measured by the method described by [15]. Determination of microbial protein following the Lowry protein assayment [16]. CMCase activity and protease activity were determined by the method described by [17] and [18] respectively. All of the data obtained in this study were analyzed as a completely randomized design using one-way analysis of variance followed by Duncan multiple ranges tests (DMRT) as post hoc analysis [19] using IBM SPSS Statistics 24.

3. Results and discussion

The values of rumen pH, total volatile fatty acid (VFA) concentration, partial VFA (acetate, propionate, butyrate) concentration, and NH₃ of Catfish oil supplementation in the diet with different levels were
shown in Table 1. The rumen microbial protein, carboxymethylcellulase, and protease activity was shown in Table 2.

Table 1. The effects of Catfish oil (Clarias sp.) supplementation as unsaturated fatty acid source on rumen fermentation parameter.

| Parameter | Level of Catfish Oil Supplementation (% of DM) |
|-----------|---------------------------------------------|
|           | 0%  | 2%  | 4%  | 6%  | 8%  |
| pH<sup>a</sup> | 6.87 | 6.87 | 6.88 | 6.90 | 6.91 |
| VFA (Mm)<sup>a</sup> | 87.53 | 93.99 | 89.50 | 87.92 | 86.21 |
| C2 (Mm)<sup>a</sup> | 57.99 | 61.57 | 59.05 | 57.19 | 55.90 |
| C3 (Mm)<sup>a</sup> | 20.15 | 21.99 | 21.01 | 20.65 | 20.30 |
| C4 (Mm)<sup>a</sup> | 9.38 | 10.43 | 9.44 | 10.07 | 10.02 |
| C2:C3<sup>a</sup> | 2.88<sup>a</sup> | 2.81<sup>b</sup> | 2.80<sup>b</sup> | 2.77<sup>b</sup> | 2.75<sup>b</sup> |
| NH<sub>3</sub> (mg/100 mL)<sup>a</sup> | 57.39<sup>a</sup> | 55.56<sup>ab</sup> | 53.95<sup>ab</sup> | 58.56<sup>a</sup> | 50.83<sup>b</sup> |

<sup>a,b,c,d</sup>: not significant; <sup>a,b</sup>: different superscript in the same line show significantly different (P<0.05)

Table 1 showed that Catfish oil supplementation in the diet did not affect rumen pH value, which ranges from 6.87-6.91. The value of rumen pH recorded in this study was classified as normal. Bodas et al [20] stated that the normal value of ruminal fluid pH for optimum rumen microbes ranges from 5.78 to 7.19. A study by [21] reported that supplementation of combined unsaturated fatty acid-rich sunflower oil and fish oil (20 g/kg + 10 g/kg) into the RUSITEC substrate did not affect the rumen pH values. Rumen pH value correlates with the acid compound in the rumen fluid, it is consistent with the result of VFA concentration in this study. Catfish oil supplementation into the diet up to the level of 8% did not change the value of rumen total VFA and partial VFA (acetate, propionate, butyrate) concentration, but the supplementation at the level of 2% could decrease the ratio of C2:C3. Patra and Yu [21] reported that the addition of fish oil into the diet did not give any negative effect on rumen VFA concentration. Oil supplementation on diet consist of 40% of concentrates reported by [12] did not affect rumen VFA concentration. The decreasing value of C2:C3 ratio was a result of the decreasing concentration of acetate followed by the increasing of propionate concentration, although in this study noted there was no change in concentration value of VFA. Unsaturated fatty acid-rich oil supplementation into the ruminant diet was reported by [22] could decrease the acetate; propionate ratio of rumen fluid. The inhibition in rumen methane production caused the increase of propionate synthesis related to this component role as one of the rumen hydrogen sink [23]. It's related to the decreasing of methane production by the Catfish oil supplementation recorded in this study (data not shown). The increase of glycerol content in the diet by the increasing of oil supplementation level also presumed that caused the decrease of acetate: propionate ratio, related to the glycerol role as propionate precursor [24].

As shown in Table 1, the supplementation of Catfish oil did not change the concentration of NH<sub>3</sub> concentration, but the increase of supplementation level up to the level of 8% decreased the NH<sub>3</sub> concentration. Similar results also reported by [12] that supplementation of unsaturated fatty acid source oil, Cakalang fish oil at the level of 7.5% decrease the NH<sub>3</sub> concentration. The decreasing concentration of rumen NH<sub>3</sub> as an effect of oil supplementation in the diet, related to the protozoal population in this study (data not shown) and antiprotozoal properties of unsaturated fatty acids-rich Catfish oil. Rumen protozoa playing role in utilizing protein as its diet then releasing NH<sub>3</sub> as metabolic product [25].

The result of statistical analysis showed that Catfish oil supplementation in the diet up to the level of 8% did not give any negative effect on rumen microbial protein. A similar result reported by [26] et al that adding Lemuru fish oil as a methane inhibitor had no significant effect on rumen microbial protein with the value ranges from 0.43-0.62 mg/mL. Unsaturated fatty acid, especially PUFA content of Catfish oil reported as a toxic compound to rumen microbes [23]. Based on those statement, it's considered that
Catfish oil has low toxicity effect on rumen microbes and its supplementation in diet did not give a negative effect on rumen microbial protein.

**Table 2.** The effects of Catfish oil (*Clarias* sp.) supplementation as unsaturated fatty acid source on microbial protein, Carboxymethyl cellulose activity, and protease activity.

| Parameter                  | Level of Catfish Oil Supplementation (% of DM) |
|----------------------------|-----------------------------------------------|
|                            | 0%                | 2%              | 4%              | 6%              | 8%            |
| Microbial protein (mg/mL)  | ns 0.48           | 0.47            | 0.42            | 0.43            | 0.42          |
| CMCCase activity (U/g protein) | 14.32<sup>a</sup> | 14.51<sup>a</sup> | 12.15<sup>ab</sup> | 13.09<sup>a</sup> | 8.71<sup>b</sup> |
| Protease activity (U/g protein) | 21.58            | 22.51           | 22.65           | 21.69           | 21.58         |

*ns.* : not significant;  
<sup>a,b,c,d</sup> : different superscript in the same line show significantly different (P<0.05)  
CMC : Carboxymethyl cellulose

Data in Table 2 showed that Catfish oil supplementation up to the level of 6% did not change the value of rumen CMCCase activity but at the supplementation level of 8% it decreased the rumen CMCCase activity. The CMCCase enzyme is responsible for fiber digestion in the rumen and its related to b fraction value of gas production kinetics (data not shown). Fish oil supplementation had a higher effect on decreasing CMCCase activity than canola and soybean oil supplementation related to its unsaturated fatty acid concentration [27]. Oil content in the diet capable of surrounding feed substrate [28], especially fiber, furthermore the enzyme unable to create enzyme-substrate complex [29]. A study by Tiven also reported that the increasing level of crude palm oil decrease CMCCase activity of rumen fluid [30]. Protease activity did not affect by the supplementation of catfish oil. It's related to the rumen microbial protein (Table 2) associated with its capability on protein degradation [31] by secreting protease.

**4. Conclusions**

Supplementation of Catfish oil up to the level of 6% of DM (v/w) could be used as a methane inhibition supplement without having any negative effect on rumen fluid pH, total volatile fatty acid, partial volatile fatty acid, NH3, CMC, and protease activity of Bali cattle rumen fluid in vitro.

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