The effects of catfish oil supplementation as unsaturated fatty acid source on Bali cow gas production kinetics, dry matter digestibility, and organic matter digestibility in vitro

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Abstract. The purpose of this study was to investigate the effects of catfish oil (CFO) supplementation on gas production kinetics, dry matter (IVDMD), and organic matter digestibility in vitro (IVOMD) with Bali cow rumen fluid. The design of this study was a completely randomized design with 5 treatment consist of T0 (control diet: Pennisetum purpupoides (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 3 replication. Gas production technique described by Menke and Steingass was used in this study with 48 hours of incubation time. Gas production kinetics was analyzed by Fit Curve application. The result showed that CFO supplementation did not change the value of gas production, a fraction value, b fraction value, gas production rate (c value), IVDMD, and IVOMD, but the increasing supplementation at the level of 8% decreased (P<0.05) gas production, b value, IVDMD, and IVOMD. It could be concluded that catfish oil supplementation as unsaturated fatty acid source at the level of 6% DM in the Bali cow diet did not give a negative effect on rumen substrates degradation.

1. Introduction

Environmentally friendly is one factor that must be considered in sustainable agriculture, therefore, as much as possible every agricultural production suppressed pollutant emissions from the production process [1]. The livestock industry is one of the agricultural sectors that emitted greenhouse gases such as methane and carbon dioxide from enteric fermentation of the ruminant digestive tract [2]. Although the greenhouse gas produced by large ruminant production was only 16% of total emission from human activities [3], but it is been accused of being the leading cause of global warming. However, methane mitigation of large ruminant enteric fermentation needs to be done, because rumen's methane production not only had a negative impact on the environment but also had a negative impact on the efficiency of livestock production, especially in the utilization of gross energy of diet [4].

Unsaturated fatty acids-rich oil was reported [5] could be used as a rumen modifier supplement that results in reducing of rumen methane production. Considering the agriculture sustainability this study would utilize catfish oil, which is a catfish meal by-product [6], as an unsaturated fatty acids source oil to mitigate rumen methane production. Determining the level of unsaturated fatty acids-rich oil supplementation should be done carefully because the high level of oil supplementation in the ruminant diet could interfere feed digestion in rumen, although Adeyemi et al [7] noted that the usage
of Carotino oil up to the level of 8% did not interfere rumen fermentation, which indicated by gas production. Gas production technique was a method that can provide information about the digestion kinetics of feed in the rumen in vitro [8]. Related in the description above, catfish oil was a potential by-product to mitigate rumen methane production, without giving any negative effect on rumen digestion. The current study aimed to determine the effect of catfish oil supplementation on rumen gas production, gas production kinetics, dry matter, and organic matter digestibility.

2. Materials and methods

Materials used in this research were rumen fluid from ruminal fistulated Bali cow fed Pennisetum purpupoides and wheat pollard in ratio of 60:40 DM based, in vitro gas production buffer solution, catfish oil (CFO) which contain 93.66% unsaturated fatty acid of total fatty acid content, and fermentation substrates consist of P. purpupoides, wheat pollard, and soybean meal which was ground to pass 1 mm screen and mixed homogeneously. The substrate used in this study proximately analyzed using the method of AOAC [9]. There were 5 treatments conduct in the current study, i.e. T0 (control diet, P. purpupoides, wheat pollard, and soybean meal (60:30:10)), T1 (control diet + 2% DM basis CFO (v/w)), T2 (control diet + 4% DM basis CFO (v/w)), T3 (control diet + 6% DM basis CFO (v/w)), T4 (control diet + 8% DM basis CFO (v/w)). Each treatment were replicated 3 times.

In vitro gas production test in this study following the method of Menke and Steingass [10]. Rumen fluid was collected 1 hour before morning feeding and then mixed with buffer solution (1:2). The buffered rumen fluid as much as 30ml was put into the 100 mL syringe already contains 300 mg of substrate, which was previously incubated in a water bath at 38–39° C. The syringe was incubated for 48 hours in a water bath under an anaerobic condition with a temperature of 38–39° C. Volume of gas produced during incubation was observed and recorded at 0, 2, 4, 6, 12, 24, 36, and 48 hours. After the incubation was done, filtrate and substrate inside the syringe were separated using glass wool filled Gooch crucible. The residual substrate acquired from the previous step was collected for further analysis.

The gas production recorded during the incubation was used to measure total gas production and gas production kinetics. These parameters measured by Fit Curve program mention by Chen [11] to find the a fraction value which means intercept value of gas production or representation of gas from highly soluble fraction, b fraction value which represent gas production from the insoluble fraction such as fiber, and c value which was gas production rate or constant. The filtration residual contained in the Gooch crucible was used to determine the undegraded dry and organic matter following the method of AOAC [9]. These data then used to calculate the digestibility of dry matter and organic matter. Every data obtained in the current study were analyzed using the procedure of one-way analysis of variance (ANOVA) IBM SPSS Statistics 24 as a completely randomized design, followed by Duncan’s Multiple Range Test as a post hoc if the treatment showed a significant effect [12].

3. Results and discussion

Gas production of each treatments was shown as gas production curves (mL/300 mg DM) in Figure 1 and values of gas production kinetics, dry matter, and organic matter digestibility were shown in Table 1.

Catfish oil supplementation at the level 2–6% of DM did not interfere in vitro total gas production, but increasing of supplementation at the level of 8% of DM in the diet decrease in vitro total gas production. A study of Gawad et al showed that linseed oil which rich in unsaturated fatty acid supplementation at the level of 6% did not interfere with gas production at 24 hours of incubation [13], Adeyemi et al [7] also reported that Carotino oil supplementation at the level of 2%-6% of DM did not have a negative effect on gas production. Another study by Gomaa et al [14] noted that sunflower oil supplementation as an unsaturated fatty acid source in the level of 5% decreased total gas production, and [15] reported that the usage of lemuru fish oil at the level of 5% and 7.5% decreased total gas production per digested dry matter compared with the control.
Figure 1. The effects of catfish oil (Clarias sp.) supplementation in different levels as unsaturated fatty acid source on gas production at various time of incubation

Table 1. The effects of catfish oil (Clarias sp.) supplementation as unsaturated fatty acid source on total gas production, gas production kinetics, in vitro organic matter digestibility (%), and in vitro organic matter digestibility (%)

| Parameter                  | Level of Catfish Oil Supplementation (% of DM) | 0%    | 2%    | 4%    | 6%    | 8%  |
|----------------------------|-----------------------------------------------|-------|-------|-------|-------|-----|
| Total Gas production       |                                               | 76.92 | 75.55 | 74.46 | 72.90 | 68.13 |
| (mL/300 mg DM)             |                                               |       |       |       |       |     |
| a (mL/300 mg DM)           |                                               | 1.09  | 1.03  | 1.03  | 0.97  | 1.01 |
| b (mL/300 mg DM)           |                                               | 81.90 | 81.10 | 80.49 | 80.35 | 76.39 |
| c (%)                      |                                               | 0.05  | 0.05  | 0.05  | 0.05  | 0.06 |
| IVDMD (%)                  |                                               | 58.27 | 58.15 | 57.18 | 56.81 | 53.91 |
| IVOMD (%)                  |                                               | 55.72 | 55.47 | 55.10 | 55.10 | 49.49 |

m: not significant; a,b,c,d: different superscript in the same line show significantly different (P<0.05)
a: intercept value of gas production or gas from highly soluble fraction, b: gas production from the insoluble fraction, c: constant or gas production rate, IVDMD: In Vitro Dry Matter Digestibility, IVOMD: In Vitro Organic Matter Digestibility

Table 1 showed that the catfish oil supplementation effect on gas production had similar results to IVDMD and IVOMD, so it could be said that the decreasing volume of total gas production at catfish oil supplementation level of 8% was caused by the decreasing digestibility of dry matter and organic matter. Lourenco et al [16] stated that gas was one of many rumen fermentation products, therefore the decreasing of substrate digestibility in the rumen would affect the gas were produced. This condition occurred because supplementation at the level of 8% of DM has a negative effect because the treatment had the highest UFA concentration among other treatments. Torok et al [17] noted that unsaturated fatty acid could interfere rumen microbes activity in degrading substrate. It could be said that catfish oil supplementation up to level 6% of DM did not give a negative effect on gas production.

The result in Table 1 showed that catfish oil supplementation at the level up to 8% didn't decrease the value of gas production intercept that represent gas production of highly soluble fraction (a) and rate of gas production (c), but decreased the value of gas production of insoluble fraction (b). Similar result noted by Kongmun et al [18] and Kang et al [19] that coconut oil supplementation as much as 16 mg and krabok seed oil at a level of 2.5% into the diet did not give the significant effect to a fraction value and decreased b fraction value, but both studies showed that there was an increasing c value as an effect of supplementation. Unsaturated fatty acid-rich oil supplementation in the ruminant
diet didn’t affect a fraction and c value [20]. The soluble fraction or a fraction digestibility of the diet didn’t affect by the existence of oil in the diet [21]. The decrease in b fraction value at 8% of DM catfish oil supplementation was thought to occur because of oil supplementation at high levels affected the digestibility of the fiber. This is supported by the evidence that catfish oil supplementation at the level of 8% of DM decreased the CMC-ase activity compared with control (data not published yet). Oil in the diet had the capability to surrounding the fiber substrate of the diet, which blocking enzyme to attached the substrates [22]. These conditions caused a decrease in digested fiber and a decrease in the value of fractions b. It could be said that catfish oil supplementation up to level 6% of DM did not give a negative effect on gas production kinetics.

In vitro dry matter and organic matter digestibility were recorded in this study did not affected by CFO supplementation at the level of 2–6%. The increasing of supplementation up to level of 8% significantly (P<0.05) decrease IVDMD and IVOMD. This result was in line with the study of [23] noted that the increasing concentration of fat in the diet, especially in the shape of oil could linearly decrease the dry matter and fiber digestibility. The high concentration of unsaturated fatty acids suspected to be interfering rumen digestion. Wu et al [24] reported that oleic acid could decrease dry matter digestibility. The decrease of dry matter and organic matter digestibility due to catfish oil supplementation at 8% of DM considered as high level. Although Carotino oil with high concentration of unsaturated fatty acid was reported did not have a significant effect on dry matter and organic matter digestibility at 8% supplementation [7], and the research of Patra [23] noted that the addition of rich unsaturated fatty acids fat or oil in the ruminant diet could be applied maximum at 6%. Catfish oil supplementation in the level of 8% of DM also decreased the CMC-ase activity and protozoa population (data not published yet), which caused a decrease in the digestibility of organic matter, especially fiber. The decreasing of protozoa (data not published yet) population could decrease the organic matter digestibility because protozoa had a role in dietary fiber digestibility up to 30% [25]. It could be said that catfish oil supplementation up to level 6% of DM did not give a negative effect on IVDMD and IVOMD.

4. Conclusion
Supplementation of catfish oil as unsaturated fatty acid source up to the level of 6% of DM could potentially be used as rumen methane mitigation compound without gave negative effect on rumen gas production, gas production kinetics, dry matter, and organic matter digestibility in vitro.

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