The evaluation by passes energy based on in vitro gas production digestibility and palatability

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Abstract. The purpose of this study was to evaluate the influence of combined treatments of the fish oil protected with different NaOH concentration and drying method as bypass energy based in vitro total gas production and palatability. Lemuru fish oil, starch and NaOH were used for capsulation and optimizing saponification, along with CaCl₂ and required reagents were used for in vitro gas test. The indicators for protected fish oil palatability test were 18 female lamb, aged 7-8 months, with average weight 15.37 ± 1.09 kg. A completely randomized design was deployed for in vitro gas production with three replications in which different NaOH concentrations and drying methods were applied. Then, to see the results of the animal preference level, it was by reducing the feeding time and feeding residue in 24 hours. All data was analysed using analysis of variance (ANOVA) with Duncan test. As a results, fish oil protected with different NaOH concentration and drying method have no significant effect on total gas production. Furthemore, the results on the palatability analysis showed that there was no significant different in all treatments.

1. Introduction
Energy is an important nutrient for ruminants. In ruminant animals, especially those with high production, the rumen requires the availability of more energy or degraded energy. The energy density of the daily ration can be increased by adding cereal grains; however, the risk of subclinical rumen acidosis may limit the inclusion rates [1]. An alternative strategy to reduce this risk can be done by using rumen bypass fat in the ration [2]. Fat is one of the source of energy. Fatty acids will produce higher energy compared to other nutrients such as carbohydrates or protein when metabolized in the body. Fat is an energy source with a calori value of about 2.25 times higher than carbohydrates. The recommended fat content in ruminant animal feed should not exceed 5% since high fat content will affect the microbial activity of rumen, which is by reducing the microbial population that digests fibre in the rumen [3]. Nevertheless, the use of fats, especially fats containing high-unsaturated fatty acids in ruminants, is constrained by the bio hydrogenation mechanism by microbes in the rumen. One of the efforts to overcome this problem is by manipulating the feed. This method aims to protect fat from bio hydrogenation by microbes in the rumen. The sources of unsaturated fatty acids for feed supplementation in ruminants can be obtained from animal oils. One of the animal oils from fish
processing waste is Lemuru fish oil. It has a high potential for use as an energy source since it has a high-energy content of 8400 kcal/ kg [4].

Several technologies that can be used to protect fat have been carried out physically and chemically with the aim of reducing the negative effects of fat on carbohydrate digestibility and rumen bacterial populations and suppressing the hydrogenation process of fat in the rumen. Saponification process technology is one of the most advanced technologies compared to formaldehyde technology which is widely applied commercially [5]. To protect fat from being degraded in the rumen can be done by means of protection. This method can be done by binding carboxyl groups with minerals, such as Ca and Mg. It is known as calcium soap or magnesium soap formation. Polyunsaturated fatty acids such as linoleic or linoleic can be protected in this way so that the animal without any previous degradation processes can readily utilize them. Protection method to shield fat from being degraded in the rumen, can be conducted by binding carboxyl group with minerals, such as Ca and Mg. This method is known as formation of calcium soap or magnesium soap. Polyunsaturated fatty acids such as linoleate or linolenic can be protected using this method in order that animals can immediately use it without the previous degradation process [3]. In making calcium soap (Ca-SOap) by using a chemical process, it is by reacting the fat material with NaOH solution which is known as the saponification process. After that, the solution is reacted again with CaCl solution to obtain calcium soap which is insoluble in water. Drying method is intended to remove a certain amount of water from the dried material by evaporation.

Based on the above explanation, the aim of this study was to evaluate the effect of bypasses fat adding as an energy source based on in vitro gas production and palatability. Studies to evaluate the availability of organic matter can be carried out using in vitro gas production techniques [6].

2. Materials and methods
2.1. Preparing sample (protected lemuru fish oil)
The material used as protected fatty acid in this study was lemuru fish oil. While the capsules were starch, caustic soda (technical NaOH) as materials for optimizing the saponification, the technical CaCl2, and the chemicals for in vitro gas analysis. The optimized saponification aids were buckets, scales, digital scales, sieves, knives, sunbeds, ovens, and in vitro gas test instrument. Lemuru fish oil was saponified using NaOH (caustic soda) and 10% of starch solution, with a ratio of 1: 2: 1 [7]. The concentration of NaOH can be divided into 10%, 20% and 30%. The treatment at this stage was carried out using a completely randomized factorial design. Factor A is the concentration of NaOH, which consists of A1: 10% of NaOH, A2: 20% of NaOH and A3: 30% of NaOH concentration. Factor B is the drying method, which categorized into two method, B1: sun drying and B2: oven drying.

2.2 In vitro gas production
For gas production analysis, 0.2 g sample was used [8]. Rumen fluid was collected from a ruminally-fistulated cow were fed 70% elephant grass, 30% concentrate and drinking water was provided ad libitum. Rumen fluid was collected before the morning feeding and immediately transported to the laboratory for use. The medium was prepared by mixing 474 mL distilled H2O, 0.12 mL micro-mineral solution, 237 ml macro-mineral solution and 1.22 ml resazurin solution (0.1%). The buffer solution contained 35 g NaHCO3 in 1 L of distilled water. The macro-mineral solution contained 5.7 g Na2HPO4.12H2O, 6.2 g KH2PO4 and 0.6 g MgSO4.7H2O in 1 L of distilled water. These were prepared freshly before use. The micro mineral solution contained 13.2 g CaCl2.2H2O, 10.0 g MnCl2.4H2O, 1 g COCl2.6H2O and 0.8 g FeCl3.6H2O in 1 L of distilled water. Micro-mineral and resazurin solutions were prepared prior to the experimentation and stored in the dark at 48 °C until required. The syringe was warmed to 39°C before being filled with a 30 ml rumen fluid buffer mixture. After the fluid was inserted into each syringe, this activity is followed by incubation in a water heater at 39°C. Gas production (GP) readings were recorded at 0.2,4,6,8,12,24, and 48 hours after incubation. The syringe was shaken gently at each hour of the incubation reading.
2.3. Evaluating palatability
The palatability test in this study was carried out based on a modified method [9] for 6 days. Total Mixed Ration and bypass fat was given twice a day, at 08.00 am and 04.00 pm. The feeding was based on 3% body weight. The bypass fat supplementation was given by 5% of the total concentrate. The results of the animal preference levels can be seen by reducing the feeding time and the feeding residue. The feeding residue was observed in 24 hours.

The 18 female lambs, aged 7-8 months, with average body weight 15.37 ± 1.09 kg were divided into 6 groups (Groups A, B, C, D, E, and F) with completely randomized design. Each group consists of 3 female lambs which were fed with Total Mixed Ration (TMR) with fish oil protected supplementation, with the following design:

A = TMR + protected fish oil with 10% of NaOH, sun drying method
B = TMR + protected fish oil with 20% of NaOH, sun drying method
C = TMR + protected fish oil with 30% of NaOH, sun drying method
D = TMR + protected fish oil with 10% of NaOH, oven drying method
E = TMR + protected fish oil with 20% of NaOH, oven drying method
F = TMR + protected fish oil with 20% of NaOH, oven drying method

2.4. Data analysis
The data were analysed by using an Analysis of Variance (ANOVA) and the significant difference of the treatments was tested by using Duncan test with a statistical software of SPSS 16.

3. Results and discussion
The chemical composition of the protected fish oil was analysed in the laboratory to determine dry matter (DM) and organic matter (OM). The analysis results are shown in Table 1.

|     | DM (%) | OM (%) |
|-----|--------|--------|
| A1B1| 84.69  | 54.58  |
| A1B2| 96.89  | 64.37  |
| A2B1| 85.37  | 41.39  |
| A2B2| 97.41  | 51.46  |
| A3B1| 78.91  | 29.58  |
| A3B2| 97.10  | 37.73  |

DM = dry matter, OM = organic matter,
A1B1 = fish oil with 10% of NaOH and sun drying; A1B2 = fish oil with 10% of NaOH and oven drying; A2B1 = fish oil with 20% of NaOH and sun drying; A2B2 = fish oil with 20% of NaOH and oven drying; A3B1 = fish oil with 30% of NaOH and sun drying; A3B2 = fish oil with 30% of NaOH and oven drying.

3.1. In vitro gas production
The curve on gas production for each treatment are shown in Figure 1. A concatenation of fermentation processes is expressed in gas production profiles. Alternatively, it is possible to determine the kinetics of feed degradation from the output of fermentative gas, which calculates the quantity of gas emitted directly as the result of fermentation and indirectly from the buffered fluid of the rumen. These processes either start at their highest rate during the incubation and subsequently decline, or the rate increases to a maximum and decreases thereafter. This allows processes that vary in their maximum fermentation rate to be separated during the time at which this maximum rate is reached.
Figure 1. Gas production kinetics.

Note: A1B1 = Treatment combination of fish oil with 10% NaOH and sun drying
A1B2 = Treatment combination of fish oil with 10% NaOH and oven drying
A2B1 = Treatment combination of fish oil with 20% NaOH and sun drying
A2B2 = Treatment combination of fish oil with 20% NaOH and oven drying
A3B1 = Treatment combination of fish oil with 30% NaOH and sun drying
A3B2 = Treatment combination of fish oil with 30% NaOH and oven drying

The highest increase in the gas production occurs during the 24-hour incubation period, then slows down with the incubation period. The in vitro gas production rate in all treatments decreased with the increasing of incubation time [10], [11]. The gas production within 24 hours of incubation produces gas more than 75% of the maximum gas production in most treatments. This indicated that the in vitro gas production rate decreases with the increasing incubation time since the amount of the fermented substrate also decreasing [10], [12]. Generally, the potential of gas production for non forage high fibre tropical feed is quite high, because of the high carbohydrate fraction (particularly NDF). Thus, it was found that gas production is the result of the fermentation of carbohydrates into acetate, propionate and butyrate [13], [14]. Meanwhile, the fermentation of protected fish oil does not produce much gas.

The method of preparation and drying of feed samples could affect the volume of gas production. The use of fresh samples produces higher volumes of gas production than freeze-dried or oven samples. Thus, for the best simulation of fresh in vivo feed conditions, in vitro feed evaluation should be carried out using fresh samples [14].
Table 2. The gas production from the insoluble fraction (mL), and potential gas production (mL) of fish oil protected.

| Parameter | Drying method | NaOH Concentration | Average |
|-----------|---------------|---------------------|---------|
|           |               | NaOH 10%            | NaOH 20% | NaOH 30% |         |
| b         | Sun drying    | 21.42±0.86          | 21.69±1.86 | 20.94±1.13 | 21.35±1.21b |
|           | Oven          | 19.16±0.75          | 18.76±0.30 | 17.62±0.33 | 18.51±0.81a |
|           | Average       | 20.29±1.43a         | 20.23±2.00a | 19.28±1.96a |
| a+b       | Sun drying    | 21.83±0.75          | 20.55±0.99 | 21.23±1.55 | 21.20±1.14b |
|           | Oven          | 17.92±1.17          | 17.59±0.66 | 17.54±0.43 | 17.68±0.73a |
|           | Average       | 19.87±2.32a         | 19.07±1.79a | 19.38±2.26a |
| c         | Sun drying    | 0.08±0.003          | 0.08±0.006 | 0.07±0.005 | 0.08±0.007a |
|           | Oven          | 0.08±0.004          | 0.07±0.012 | 0.06±0.000 | 0.07±0.010a |
|           | Average       | 0.08±0.003a         | 0.08±0.011a | 0.07±0.006b |

Means within rows and column with unlike superscript differ significantly (P<0.05).

b = the gas production from the insoluble fraction (ml)
c = the gas production rate constant for the insoluble fraction b (h)
a+b = potential gas production (ml)

The protected fish oil is a form of protected fat and is an effective source of fat in ruminant feed ingredients since the rumen fermentation system remains normal. Thus, it is expected that it will not have a negative impact on the rumen microbes. Gas production from slowly fermentable fraction (b) and the potential gas production (a+b) were not significant with the protected fish oil from all levels of NaOH concentrations. Kustantinah et al., [15] defined the observation of the maximum gas production (a + b) of the degradable fraction showing that the sample preparation at high temperature gave lower yields compared to the sample preparation at a low temperature. The gas production rate constant for the insoluble fraction b (c) was significantly (p<0.05) higher than the other NaOH concentrations. The gas production from fractions a, b, and a+b in the sun drying method was significantly higher than the oven drying method.

3.2. Palatability
The palatability test results in this study showed that there was no significant difference (P> 0.05) in all treatments (Table 3), due to the total mixed rations (TMR) feeding. [16], [17] studied the total mixed rations (TMR) to increase the ruminant nutrient intake and utilization. Feed with low palatability can be mixed in the TMR to increase the intake [17].

Table 3. Intake of the protected fish oil in the palatability test.

| No | Group | Consumption (g DM/ 24 h) |
|----|-------|--------------------------|
| 1  | A     | 561.19±2.62<sup>ns</sup> |
| 2  | B     | 560.02±1.03<sup>ns</sup> |
| 3  | C     | 559.92±2.14<sup>ns</sup> |
| 4  | D     | 555.43±5.68<sup>ns</sup> |
| 5  | E     | 559.44±2.60<sup>ns</sup> |
| 6  | F     | 561.19±5.77<sup>ns</sup> |

Ns = non significant
A = TMR + protected fish oil with 10 % of NaOH, sun drying method
B = TMR + protected fish oil with 20 % of NaOH, sun drying method
C = TMR + protected fish oil with 30 % of NaOH, sun drying method
D = TMR + protected fish oil with 10 % of NaOH, oven drying method
E = TMR + protected fish oil with 20 % of NaOH, oven drying method
F = TMR + protected fish oil with 20 % of NaOH, oven drying method
Several methods have been used to evaluate palatability. Some of these methods differ based on the intake or behavioural parameters recorded and whether only one or more feeds were offered. However, neither of these methods can avoid the effects of previous feed characteristics studies. The differences in voluntary intake cannot be attributed solely in using palatability as they are due to the sensory responses and post-meal digestive, metabolic, and hormonal disturbances. The recording intake during the first minutes after feed exposure limits the confusion of palatability by posting the positive factors [18].

Thus, the palatability test must be carried out in the selected situation. However, the result of the choice of test was influenced by the duration of the test and the amount of feed given to the animal. If an unlimited number is presented within a short test duration, the animal may show an exclusive preference for one feed [19]. Therefore, the test procedure in the chosen situation must be carefully determined. For example, to test the delicacy of the concentrate in goats, Morand-Fehr et al., [20, 21] established the following procedure: i) the feed is tested in pairs; ii) in different combinations (six for the four feeds to be tested) are specified in the Latin square design; iii) each test lasts for 30 seconds and is repeated four times with 200 g of each feed given to the animals. The differences in voluntary intake cannot be attributed solely to the palatability since they are due to the sensory responses and post-meal digestive, metabolic, and hormonal disturbances.

4. Conclusion
Fish oil that was protected with NaOH concentration treatment did not have a significant effect on the gas production. From the results of in vitro gas production, it can be concluded that the protected fish oil does not affect the rumen fermentation. The palatability of the results of this study indicated that there was no significant difference in all treatments due to the total mixed ration (TMR) feeding. Diets with low palatability can be mixed in the TMR to increase the intake.

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