The Estimation of Metabolizable Energy Using an Analysis of Ruminal Fermented Gas Production in Protected Lemuru Fish Oil

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Abstract. This study has purpose to estimate the metabolic energy of protected lemuru fish oil using the rumen fermentation gas production analysis. This study applied a combination of treatments, which is differences in NaOH concentrations and drying methods on the total gas production in vitro. The gas production was determined at the incubation of 0, 2, 4, 8, 12, 24, 48, and 72 hours. This study applied a Completely Randomized Design on 3x2 factorial. The parameters observed included the total gas production, the data were tested using an analysis of variance and continued with the Duncan test. The results of this study indicated that at the incubation time of 48 hours and 72 hours, there was no difference in the interaction between the NaOH concentration and the drying method on the total gas production, and there was a significant difference between the NaOH concentration and the drying method in terms of metabolizable energy (ME). The results of the highest ME value in the protected lemuru fish oil were 10% NaOH concentration and the sun drying method was 4.56 MJ/ Kg BK, it can be concluded that the ME estimation is influenced by the yield of incubation gas production for 24 hours.

1. Introduction
Energy is an important nutrient for ruminants. Fat is a source of energy. Fatty acids will produce higher energy than other nutrients such as carbohydrates or protein when metabolized in the body. Ruminants, especially high production ones, either require more available energy or are degraded in the rumen. It is often said that the quality of the feed is very dependent on its energy content. In particular, measuring the energy value of feed is a key aspect of the animal nutrition. The available feed energy content must be determined before formulating feed [1]. In vitro gas production techniques can help to better measure the nutrient utilization and its accuracy in describing digestibility in animals has been validated in various experiments. In vitro gas production techniques have been widely used to assess the nutritional value of ruminant feed ingredients based on the pattern of gas accumulation when incubated with the rumen fluid under anaerobic conditions. This technique was originally proposed by [2] to assess the digestibility and metabolizable energy (ME) content of feed normally fed to ruminants. The empirical equation developed by [3] using gas production and chemical composition of feed was used to predict the ME and the net energy required for lactation (NEI). So far, this technique has been widely used to evaluate the energy value of several feed classes.
[4], especially hay [5], agro-industrial by-products [6], compound animal feed [7] and various tropical feeds [8].

The recommended fat content in ruminant feed is not more than 5% since the high fat content will affect the rumen microbial activity, which is by reducing the microbial population that digests fiber [9]. However, the use of fats, especially those containing high unsaturated fatty acids in ruminants, is constrained by the biohydrogenation 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 the biohydrogenation by microbes in the rumen. The sources of unsaturated fatty acids for feed supplementation in ruminants can be obtained from the animal oils. Lemuru fish oil is one of the animal oils from fish processing waste and it has a high potential to be used as an energy source due to its high energy content of 8400 kcal/ kg [10].

Various technologies for fat protection have been implemented both 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 that has been applied commercially [11]. Another treatment in this study is the drying method. Drying is intended to remove a certain amount of water from the dry matter through evaporation.

This study is intended to estimate the metabolizable energy of protected lemuru fish oil with different levels of NaOH concentrations and the drying method using the rumen fermentation gas production analysis method.

2. Materials and Methods

2.1. Preparing sample (protected lemuru fish oil.

The fatty acid soap ingredients in this study were lemuru fish oil and the capsules were starch, caustic soda (technical NaOH) for the optimization of saponification, the technical CaCl2, and the chemicals for in the vitro gas test analysis. The optimized saponification instruments were buckets, scales, digital scales, sieves, knives, sunbeds, ovens, and in vitro gas test analyzers. Lemuru oil was saponified with NaOH (caustic soda) and 10% starch solution, using a ratio of 1: 2: 1 [12]. The concentration of NaOH various to 10%, 20% and 30%. The treatment at this stage was done using a completely randomized factorial design. Factor A is the concentration of NaOH, which consists of: A1: 10% NaOH, A2: 20% NaOH and A3: 30% NaOH concentration. Factor B is the drying method, such as B1: sun drying and B2: oven drying. The lemuru fish oil soap after soaking in the technical CaCl2 was then dried in 2 ways, they are drying and oven at 70° C for 24 hours [13] with modification.

2.2. Gas test

The rumen fluid was collected from two cows fitted with ruminants. Donor cows were fed with 70% of elephant grass and 30% of concentrate according to their needs and drinking water is provided ad libitum. The rumen fluid was collected before breakfast or morning feed and immediately transported to the laboratory for use in the experiments. The medium was prepared by mixing 474 ml of distilled water, 0.12 ml of micro-mineral solution, 237 ml of buffer solution, 237 ml of macro-mineral solution and 1.22 ml (0.1%) resazurin solution. The buffer solution contains 35 g NaHCO3 in 1 litre of distilled water. The macro-mineral solution contains 5.7 g Na2HPO4.12H2O, 6.2 g KH2PO4 and 0.6 g MgSO4.7H2O in 1 litre of distilled water. These solutions were prepared immediately before being used for the experiments. The micro-mineral solution contains 13.2 g CaCl2.2H2O, 10.0 g MnCl2.4H2O, 1 g COCl2.6H2O, and 0.8 g FeCl3.6H2O in 1 litre of distilled water. Micro-mineral solutions and resazurin were prepared prior to use for the experiment and stored in the dark at 48°C until needed. Three replicates of about 200 mg of dry substrate were weighed into a syringe and this material was incubated with 30 ml of the rumen fluid suspension mixture. The samples were incubated for 24 hours, in a thermostatically controlled water bath at 39 °C. The gas measurements were made at 0, 2, 4, 8, 12, 24, 48 and 72 hours after incubation.
2.3. Metabolizable energy
The metabolizable energy (ME) was calculated using the value of in vitro gas production (GP) for 24 hours using the formula as follow [2]:

\[(MJ/kg \text{ DM}) = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP} + 0.0029 \text{ CF}^2\]

Where:
GP: 24 h net gas production (ml/200 mg).
CP: crude protein (%).
CF: crude fat (%).

2.4. Data analysis
The data in this study were analyzed using an Analysis of Variance (ANOVA) and for the significant differences between treatments, it would be further tested by Duncan's test using the statistical software SPSS 16.

3. Result and Discussions

3.1. Gas production

| Incubation Time (H) | Drying method | NaOH Concentration | Average |
|---------------------|---------------|--------------------|---------|
|                     |               | 10% NaOH | 20% NaOH | 30% NaOH |
| 2                   | Sun drying    | 3.67±0.55 | 2.37±0.58 | 2.97±0.55 | 3.00±0.74b |
|                     | Oven          | 1.57±0.40 | 1.30±0.35 | 2.13±0.25 | 1.67±0.47a |
|                     | Average       | 2.62±1.22b | 1.83±0.72a | 2.55±0.59b | 2.17±1.16b |
| 4                   | Sun drying    | 6.33±0.75 | 4.67±0.29 | 5.3±0.6  | 21.74±1.16b |
|                     | Oven          | 3.9±0.62  | 3.4±0.26  | 3.87±0.06 | 18.68±0.90a |
|                     | Average       | 5.12±1.47b | 4.03±0.74a | 4.58±1.11a | 4.03±1.55b |
| 8                   | Sun drying    | 10.50±0.50 | 9.67±0.58 | 9.37±0.57 | 9.84±0.70b |
|                     | Oven          | 7.97±0.65 | 6.97±0.45 | 6.8±0.00  | 7.24±0.67a |
|                     | Average       | 9.23±1.48b | 8.32±1.55a | 8.08±1.45a | 8.22±1.55a |
| 12                  | Sun drying    | 13.93±0.73 | 13.13±0.63 | 12.37±0.57 | 13.14±0.88b |
|                     | Oven          | 10.57±0.60 | 9.53±0.71  | 9.43±0.25  | 9.84±0.73a |
|                     | Average       | 12.25±1.94b | 11.33±2.06a | 10.90±1.65a | 10.90±1.65a |
| 24                  | Sun drying    | 18.60±1.13 | 17.07±1.11 | 17.07±0.81 | 17.58±1.17b |
|                     | Oven          | 14.90±0.96 | 13.90±0.00 | 13.67±0.38 | 14.15±0.77a |
|                     | Average       | 16.75±2.23b | 15.48±1.87a | 15.37±1.94a | 15.37±1.94a |
| 48                  | Sun drying    | 21.37±0.68 | 20.43±1.10 | 20.63±1.30 | 20.81±1.01b |
|                     | Oven          | 17.57±1.07 | 16.93±0.15 | 16.60±0.35 | 17.03±0.71a |
|                     | Average       | 19.47±2.23a | 18.68±2.04a | 18.62±2.37a | 18.62±2.37a |
| 72                  | Sun drying    | 22.47±1.17 | 22.07±0.58 | 22.03±1.36 | 22.19±0.97b |
|                     | Oven          | 18.50±1.18 | 18.10±0.30 | 17.67±0.46 | 18.09±0.74a |
|                     | Average       | 20.48±2.41a | 20.08±2.21a | 19.85±2.56a | 19.85±2.56a |

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

The total gas production from the study for 72 hours of incubation time ranged from 17.67 ml/ 200 mg (30% NaOH, oven) to 22.47 ml/ 200 mg (10% NaOH, sun drying). The highest gas production occurred at the 24-hour incubation time produced in the protected lemuru oil with 10% NaOH, 18.60 ml/ 200 mg sun-drying, while the lowest gas production was protected lemuru oil with 30% NaOH.
content, oven drying was 13.67 ml/ 200 mg. Sample preparation using low temperature drying gives a tendency to produce gas from the total degraded fraction higher than using high temperature [14].

In the analysis of variance after 48 and 72 hours of incubation, it can be seen that there is no significant difference (p> 0.05) in the difference level of NaOH concentration and the drying method with gas production. The gas production begins to slow down within 48 hours of incubation. The gas production within 24 hours of incubation produces gas more than 75% of the maximum gas production in most treatments. These results indicated that the in vitro gas production rate decreases with the increasing incubation time, since the amount of substrate that can be fermented is also decreasing. [15, 16].

3.2. Metabolizable energy

| Parameter       | Drying method | NaOH Concentration | Average |
|-----------------|---------------|--------------------|---------|
|                 |               | NaOH 10%           | NaOH 20%| NaOH 30% | Average |
| ME              | Sun drying    | 4.79±0.16          | 4.59±0.15| 4.56±0.11| 4.66±0.16b |
|                 | Oven          | 4.33±0.14          | 4.17±0.01| 4.11±0.06| 4.20±0.12a |
|                 | Average       | 4.56±0.28b         | 4.38±0.25a| 4.35±0.27a | 4.35±0.27a |
| GP(24h)         | Sun drying    | 18.60±1.13         | 17.07±1.11| 17.07±0.81| 17.58±1.17b |
|                 | Oven          | 14.90±0.96         | 13.90±0.00| 13.67±0.38| 14.15±0.77a |
|                 | Average       | 16.75±2.23b        | 15.48±1.87a| 15.37±1.94a | 15.37±1.94a |

Means within rows with unlike superscript differ significantly P<0.05
ME = Metabolizable energy (MJ/kg DM)
GP(24h) = Gas production in 24 hour incubation time

The metabolizable energy is presented in Table 2. As can be seen in Table 2. There is a significant difference between the NaOH concentration and the drying method in terms of metabolite energy. The results of the value, the highest Metabolizable Energy (ME) in the protected lemuru fish oil with a concentration of 10% NaOH and a sun-drying method of 4.56 MJ/ Kg BK and the lowest metabolic energy value in the protected lemuru fish oil with 30% NaOH concentration and drying method using the oven is 4.35 MJ/ Kg BK. Metabolizable energy in this study was lower than those reported by [17] (4.6 to 6.1 MJ/day). The high ME value was influenced by the yield of 24-hour incubation gas and PK content. This result is supported by [4] which states that the in vitro gas production measured after 24 hours incubation is highly correlated with the energy value.

4. Conclusion

In this study, it can be conclude that there is no interaction between different NAOH concentration and drying method to 48h and 72h ruminal fermented gas production, and there were significant difference among NaOH concentration and drying method in terms of metabolizable energy (ME). The highest ME value is influenced by the result of 24-hour incubation gas production. So it can be conclude that in vitro gas production measured after 24-hour incubation is highly correlated with energy values.

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