Fermentability and Digestibility Responses of Prill Fat Supplementation in Dairy Ration

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Abstract. Prill fat is a non-hydrogenated vegetable oil and contains more than 85% palmitic acid with high melting point. Due to this reason it does not melt at rumen temperature and bypass rumen degradation. This research was to measure the characteristic of fat high in palmitic fatty acid by its utilization in ruminant. This research held in the Laboratory of Dairy Science, IPB University. This research used Randomized Block Design with 4 treatment and 4 replication: P1 (control without treatment), P2 (control + 2% of high palmitic acid fat 78%), P3 (control + 2% of high palmitic acid fat 86%), P4 (control + 2% of high palmitic acid fat 96%). The variables observed included rumen fermentation characteristics (pH, volatile fatty acid/VFA, and ammonia (NH₃) concentration), dry matter and organic matter digestibility. Data obtained were analyzed using Analysis of Variance (ANOVA) and the significant different among treatments were further tested using Duncan. The result showed prill fat supplementation was significantly affected the total VFA concentration (P<0.01) and dry matter digestibility (P<0.05). Supplementation of prill fat had no effect on pH, NH₃ concentration, and organic matter digestibility. It was concluded that prill fat supplementation of 96% palmitic fatty acid can be used to augment the VFA production and ruminal digestibility at the level of 2%.

1. Introduction

Ruminants are animals that have privileges on their digestive apparatus because they have a rumen fermentation which aids to digest feed with high and low quality of crude fiber becomes a source of energy to produce milk, meat, wool, and others [1]. Dairy cow is one of ruminants which produce milk. Forage is the main energy source of fiber for ruminant feed. The most widely forage used in dairy cattle is Pennisetum purpureum which contain crude protein (CP) and digestible energy expressed as total digestible nutrient (TDN) only 8.69% and 52.4% [2].

Improvements in the availability of nutrients and metabolic processes in digestive tract of animals need to be done. Supplementation is a strategic action to improve the digestibility of feed and microbial metabolism. An increasing of the energy requirement in ruminants can be done by enhancing the density of energy through the use of lipid [3].

Fats and oils are lipid compounds that are widely available in nature. Fat is needed as an energy source for ruminants. The difference between them lies in the consistency or physical properties at
room temperature, whereas fats are solid while oil is a liquid. The differences of fat and oil are the melting point due to the number of double bonds, the carbon chain length, and the cis or trans form contained in unsaturated fatty acids [4]. There are several functions of fat, such as an energy source, part of the cell membrane, mediators of biological activity, insulator of the body temperature, protective organs of the body, and as a solvent of vitamin A, D, E, and K [5].

Fat as a feed supplement for dairy cows can be given to the cattle which cultivated in hot regions, whereas dairy cattle in hot areas prone to heat stress. Heat stress that occurs in dairy cattle would cause the animal’s metabolic impaired. The supplementation of fat required to protect the rumen from biohydrogenation. Prill fat is a by-product of cooking oil process, which prepared by liquefying mixture of fatty acid by spraying it under pressure into a cooled atmosphere. Prill fat remain inert in the rumen and against degradation of the ruminal bacteria. Prill fat is a non-hydrogenated vegetable oil which contain more than 85% palmitic fatty acid with high melting point. Due to this reason, it does not melt at rumen temperature and bypass rumen degradation which does not interfere the activity of rumen microbes so that these fatty acids are absorbed directly in the small intestine and its digested in small intestine by lipase enzyme [6]. In addition to protect the rumen fermentability, fat supplementation in dairy rations that cared in hot areas has a beneficial to be used as a post rumen energy source, overcome the low quality of forage, and increase milk production. In the other side, the addition of fat often causes termination in the rumen fermentation due to the nature of fat that surrounds the protozoa, so that the protozoa immobilization will be agitated, but fat is needed as an energy source in a certain amount.

The number of fat supplementation products circulating in the community are still very limited and there are companies that have developed this product but they had no scientific testing related to the characteristics of prill fat as a feed supplement for ruminants. Following the above-mentioned background, this research needs to be implemented to assess the prill fat supplementation effectiveness in dairy ration fermentability and nutrient digestibility.

2. Materials and methods

2.1. Treatment ration

The ration is made for dairy cattle. Ration used are elephant grass, concentrate, and soybean curd with ratio 72.19%:23.93%:3.88% DM. The ratio of feed is based on the provision which made at the KANIA dairy farm, Cijeruk, Bogor. The composition and nutrient content of feed ingredients is shown in Table 1.

| Feed Ingredients | Dry Matter | Ash | Crude Protein | Ether Extract | Crude Fiber | Nitrogen Free Extract | TDNd |
|------------------|------------|-----|---------------|---------------|-------------|-----------------------|------|
| Elephant grassa | 24.67      | 13.43 | 7.24          | 1.43          | 35.11       | 42.79                 | 55.629 |
| Soybean curdb   | 13.25      | 12.19 | 16.98         | 2.75          | 25.51       | 42.58                 | 50.773 |
| Concentratec    | 81.77      | 9.10  | 14.15         | 8.05          | 15.15       | 53.01                 | 76.11 |

*a Result of Dairy Laboratory, using NIRS (2019)
*b Result of Feed Technology Laboratory (2019)
*c Result of Laboratory of analysis and certification Quality Testing Center Feed, Bekasi
*dTDN = total digestible nutrient; calculation results Wardeh [7], forage = 1.6899 + 1.3844(CP) + 0.7526(NFE) – 0.8279(EE) + 0.3673(CF), concentrates as an energy source = 2.6467 + 0.6964(CP) + 0.9194(NFE) + 1.2159(EE) – 0.1043(CF), source as a protein = -37.3039 + 1.3048(CP) + 1.3630(NFE) + 2.1302(EE) + 0.3618(CF)
The nutrient requirement is based on NRC [8], where the needs of cattle with an average body weight of 417 kg and the production of 12 liters need of 12.20% crude protein and 61.30% TDN. Based on the calculations, crude protein and TDN are not fulfilled if only using the feed that provide from local farm, supplementing with prill fat is expected to improve the energy. Prill fat was added to the dairy cattle’s rations to maintain the production of heat derived from the ration. Prill fat addition needs to fulfil the energy of the dairy cow which has a low energy in their rations. Ration calculation results are presented in Table 2.

2.2 Research procedure
This study was conducted in February 2019 to April 2019 at the Laboratory of Dairy Nutrition, Faculty of Animal Science, IPB University. Research variables observed consisted of fermentation characteristics (pH, ammonia concentration (NH₃), and total VFA production) and nutrient digestibility (dry matter and organic matter).

The research aims to evaluate the effects of prill fat supplementation against fermentability and feed digestibility. Feed used in this form of forage and concentrate. Analysis was conducted on the concentration of ammonia, total VFA production, dry matter digestibility and organic matter digestibility. Materials used in this study was prill fat, rumen fluid, the ration of dairy cows consists of forage such as grass, concentrate, and soybean curd, NaOH, HCl, buffer solutions McDougall, CO₂, HgCl₂ solution, solution of Na₂CO₃, 0.2% pepsin solution, 0.005 N H₂SO₄ solution, and distilled water. The feed composition and nutrient content of the research ration can be seen in Table 1 and Table 2. Equipment used in in vitro studies include tube fermenter, autoclaves, centrifuges, shaker water bath, Conway, tube centrifuge, erlenmeyer flasks, pH meter, burettes, pipettes, oven 105°C, a set of steam distillation, porcelain, pump vacuum, digital scales, and the filter paper.

| Table 2. Composition and nutrient content of ration |
|----------------------------------|------------------|
| Item                            | Percentage (%) DM |
| **Composition**                 |                  |
| Elephant grass                  | 72.19            |
| Soybean curd                    | 3.88             |
| Concentrate                     | 23.93            |
| **Nutrient Content**            |                  |
| TDN                             | 60.34            |
| Crude protein                   | 9.27             |
| Ether extract                   | 3.07             |
| Crude fiber                     | 29.96            |
| Calcium                         | 0.614            |
| Phosphorus                      | 0.441            |

In vitro analysis in this study was carried out following Tilley and Terry [9] method. Sample of the ration (0.5 gr) and McDougall solution (40 mL) for each treatment was poured into fermenter tubes. Then, the rumen fluid from different cattle (10 mL) added to the tube followed by the addition of CO₂ (anaerobic condition). Fermenter tubes were incubated in the shaker water bath for 48 h at 39°C. The pH, ammonia (NH₃), and total VFA were carried out in 4 h after the incubation. Nutrient digestibility (DMD and OMD) measurements were conducted by incubating the fermenter tubes filled with the sample of ration at 39°C for 48 h in the shaker water bath.
2.3 Collection and measurement samples
Characteristics of rumen fermentation observed in this study were the concentration of ammonia (NH$_3$) which analyzed through Conway micro diffusion method [10], pH (analyzed by pH meter), and steam distillation method used to analyzed the total VFA concentration. Nutrient digestibility (dry matter digestibility and organic matter digestibility were measured after 48 h of incubation. The 2 drops of HgCl$_2$ was added to the substrate after 48 h incubation period (to stop microbes activity), then it was centrifuged at 3000 rpm in 15 minutes to separate the supernatant and solid component. Supernatants were eliminating and 50 mL of pepsin solution were added to the solid component in each tube. Tubes were incubated in the shaker water bath at 39°C for 48 h. Then, supernatants were eliminated and the solids were washed with hot water and filtered using vacuum pump and Whatman filter paper. Substrate placed in the porcelain cup and dried at the oven (105°C in 24 h) for dry matter measurements. Then, substrates were incinerated in the furnace at 6 h (600°C) for the organic matter measurements.

2.4 Experimental design
The experimental diets of in vitro using Randomized Block Design with 4 treatments and 4 replications, as follows:

P1 = ration of control in the form of elephant grass 72.19% + concentrate 23.93% + 3.88% soybean curd
P2 = P1 + 2% PF A (prill fat with 78% palmitic acid content)
P3 = P1 + 2% PF B (prill fat with 86% palmitic acid content)
P4 = P1 + 2% PF C (prill fat with 96% palmitic acid content)

2.5 Data analysis
The data were analyzed using ANOVA and the significant different among treatments were further tested by Duncan multiple range test using the SAS University edition. Differences were considered significant at P<0.01 and P<0.05.

3. Results and discussion

3.1 Fermentation characteristics
The data showed that treatments of prill fat addition have relatively similar pH values. The pH values among treatments were around 6.75-6.86, which was a normal pH level to support the fermentation in the rumen [11]. The normal pH level reflected that the addition of prill fat had no negative effects on rumen ecosystem. The concentration of pH had an important role in microbial growth and rumen stability maintenance [12]. The prill fat supplementation in the ration resulting in the normal pH level which contributed by fat prilling technique that protect the olein byproduct (prill fat) in term of microbes degradation. Table 3 showed the effect of prill fat supplementation on ruminal pH.

Ammonia (NH$_3$) is a product of protein degradation and it is important for the formation of microbial protein synthesis. Ammonia concentrations indicate a change in feed protein in the rumen and microbial protein synthesis. Growth and synthesis of microbial proteins is influenced by the concentration of ammonia because 60%-80% of N bacteria come from N-ammonia [13].

The average ammonia concentration of the rumen fluid with prill fat supplementation in this experiment ranged from 9.44-9.90 mM (Table 3). According to Sutardi [14], this value is in the normal range of ammonia concentration in rumen fluid needed for rumen microbial growth which is 4-12 mM. McDonald et al. [15] stated that NH$_3$ concentration in the rumen was around 5-17.65 mM. Ammonia will be used by microbes to synthesize de novo their amino acids essential for the tissues of the mammals [16], then microbial protein will be used by ruminants to fulfil protein needs. Table 3 showed the result of the concentration of NH$_3$. 

Table 3: Concentration of NH$_3$
Concentration of ammonia is influenced by rumen fluid groups (P<0.01), because it was taken from different individual cows and at different times. This condition can be predicted that cows consume different feed so that the population and microbial activity in rumen fluid are also different. Bacterial activity that affects the concentration of ammonia is proteolytic bacteria. Feed protein that enters the ruminants will be partially broken down by rumen microbial proteolytic enzymes and converted to ammonia [17]. Results of analysis of variance showed that the addition of prill fat in the ration did not affect the NH₃ concentration. This condition was expected due to the absence of protein content contained in prill fat, so it did not affect the availability of ammonia in the rumen. Study of Montgomery et al. [18] reported that lipid supplementation did not affect N metabolism in the rumen.

Volatile Fatty Acid (VFA) consists of acetate, propionate, butyrate, valerate, and formate which are carbohydrate fermented products in ruminants [19]. The availability of carbohydrates in sufficient quantities is needed by microbes. Carbohydrates will be fermented to produce VFA which is used as a source of energy in ruminants [20]. VFA act to role as an energy source for livestock and a carbon source for microbial protein synthesis [21]. Data on total VFA concentration are showed in Table 3.

The average of total VFA concentration with prill fat supplementation ranged from 100.19-136.70 mM. This value is still in the range of normal VFA concentrations that found in ruminants. According to McDonald et al. [15], VFA concentrations that support microbial growth ranged from 80-160 mM. The amount of VFA formed is strongly influenced by the digestibility and quality of fermented rations [21]. The results showed that supplementation of prill fat with 96% palmitic fatty acid content in the ration was good for feed fermentation activities. Grouping based on rumen fluid did not have a significant effect on total VFA concentration, but the effect of prill fat supplementation had a very significant effect on total VFA concentration (P<0.01). Duncan’s analysis showed that the P4 treatment produced the highest level of total VFA concentration compared to the other treatments. Nathani et al. [22] stated that the VFA are extremely important as an energy supply for ruminants. The addition of oil will increase the total VFA due to the increased process of feed degradation by rumen microorganisms.

Ruminants that consume feed with fat supplementation tend to have a high ability to absorb FFA (Free Fatty Acid) which results in FFA synthesis de novo a low of acetate in adipocytes [23]. Stearic fatty acid (C18:0) and palmitic fatty acid (C16:0) are produced in large quantities in the rumen when the FFA content in the rumen increases [24]. The factor that influenced the concentration of total VFA in this study is the value of free fatty acids (high value in PF C). FFA is a source of energy that will be directly absorbed in the body of ruminants and this condition was expected to affect the concentration of VFA in the rumen.

### Table 3. Effect of prill fat supplementation on in vitro fermentation products

| Variables       | Treatments                        | P-value |
|-----------------|-----------------------------------|---------|
|                 | P1                                 | P2      | P3      | P4      |
| pH              | 6.75 ± 0.09                        | 6.71 ± 0.05 | 6.83 ± 0.10 | 6.86 ± 0.05 | 0.081 |
| NH₃ (mM)        | 9.69 ± 1.63                        | 9.54 ± 1.91 | 9.44 ± 1.82 | 9.90 ± 1.99 | 0.769 |
| Total VFA (mM) | 112.74 ± 9.46<sup>a</sup>          | 108.64 ± 6.85<sup>a</sup> | 100.19 ± 6.73<sup>a</sup> | 136.70 ± 17.87<sup>b</sup> | 0.003 |

P1 = Control rations (72.19% elephant grass+23.93% concentrate+3.88% soybean curd); P2 = P1 + 2% prill fat A, P3 = P1 + 2% prill fat B, P4 = P1 + 2% prill fat C; NH₃, ammonia; VFA, volatile fatty acid; Different superscripts on the same row show significant differences (P<0.01).
3.2 Nutrient digestibility

Digestion is a sign of the availability of various kinds of nutrients from a particular feed ingredient for animals. Determining digestibility is useful for obtaining the value of feed ingredients because only the digestible food can be utilized by the body [25]. Data on nutrient digestibility showed in the Table 4.

| Variables (%) | Treatments | P-value |
|---------------|------------|---------|
|               | P1         | P2      | P3       | P4       |
| DMD           | 48.89 ± 3.32<sup>a</sup> | 46.40 ± 2.26<sup>ab</sup> | 42.56 ± 4.35<sup>b</sup> | 50.21 ± 2.52<sup>a</sup> | 0.046 |
| OMD           | 47.60 ± 4.18 | 44.88 ± 1.98 | 41.01 ± 4.67 | 49.19 ± 3.05 | 0.063 |

Based on the results of variance analysis, it was found that the treatment of prill fat addition has a significant effect (P<0.05) on DMD and tend to affect OMD (0.05<P<0.1). This is presumably because the use of prill fat with 96% palmitic acid content at 2% level in the ration can increase the activity of rumen bacteria to degrade feed. This can be seen in the highest value of total VFA production in P4 treatment. However, the treatment had no effect on OMD. In general, in vitro DMD is higher than OMD in vitro. This condition is due to the organic matter component consisting of crude fiber, protein, fat carbohydrates, and NFE so that the reduction in DMD is followed by a decrease in OMD [26]. According to Setiyaningsih et al. [27], DMD is higher than OMD because of the low ash degradation in the DM component and the ability of microbes to degrade the dry matter component is higher than the organic matter.

Dry matter digestibility (DMD) in treatment 4 (P4) has a relatively higher digestibility value than the other treatments. This can be caused by iodine value (IV) on PF C with 96% palmitic acid content which has the lowest value. According to Firkins and Eastridge [28], iodine value can affect the digestibility of the ration, where the lower IV value will affect the higher digestibility. However, these results are very varied and there are many factors that cause a low ration digestibility. This is indicated in P3 which has a lower digestibility than P4. These results found that at the level of 2% prill fat supplementation with 86% palmitic acid content was not optimal in increasing feed degradation or there was a toxicity in the content of certain substances that cause disruption of microbial activity in the rumen.

The digestibility value in this study is relatively low. According to Sutardi [14], the normal standard digestibility value is ranged 50%-60%. This condition was because the rations that used are low-quality with high crude fiber and high proportion of elephant grass (72%) in the ration. In addition, elephant grass that used is in the old phase with a crude fiber more than 35%. The results are also suspected that prill fat can interfere the rumen microbial activity because of the fat’s nature that can envelop rumen microbes, so that it can reduce the digestibility. Factors that influence the digestibility of dry matter in this experiment were rumen fluid quality, lignin content of feed ingredients, rumen pH, temperature of shaker water bath, physical condition of feed ingredients, and nutrient content of feed [29].

4. Conclusion

Prill fat supplementation in the ration had no negative effects on the pH and ammonia concentration of ruminal activity among treatments. The addition of prill fat contains 96% palmitic fatty acid can be used to augment the total VFA production and ruminal digestibility at the level of 2% compare to the other treatments.
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