Branched Chain Volatile Fatty Acids Profile of Rumen Fluids Supplemented by Different Meal Protein Sources and Protein-Energy Synchronization Index

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Abstract. The aim of this study is to examine the interaction between the meal protein source with the protein-energy synchronization index (PES) in the dairy ration on the profile of branched chain volatile fatty acids (BCVFA). The study was carried out in vitro, using factorial completely randomized design (CRD-Factorial). The first factor was 2 types of meal protein source (soybean meal and coconut meal) and the second factor was 3 levels of PES index (0.5, 0.6, and 0.7), there were 6 treatment combinations, each treatment was repeated 4 times. The results of the study showed that the interaction between the meal protein source and the PES index was not significantly affected (P> 0.05) on the levels of iso butyrate, isovalerate and valerate. The study concluded that the low PES index ration (0.5) produced a decent BCVFA profile using coconut or soybean meal.

Keywords: branched chain volatile fatty acids, meal protein source, synchronization protein-energy index

1. Introduction

The strategy for preparing rations on ruminants is not only aimed at the availability of the amount of nutrients in each gift, but also based on improving the performance of rumen microorganisms. It is the main actors in the digestive process of feed in ruminants. Microorganisms grow and develop by utilizing feed substrates and producing the main energy source for ruminants, namely volatile fatty acids (VFA). Improving the performance of rumen microorganisms will be in line with the increasing of rumen fermentation products which will ultimately have an impact on increasing the productivity.

The growth of microorganisms is influenced by energy, ammonia, and cofactors. Energy and ammonia are the main factors that are mutually limiting. It means the unequal availability of energy and ammonia would keep them less useful in the process of microbial protein synthesis. Both of them...
have to be available simultaneously (synchronous). The synchronization availability of energy and ammonia is influenced by the rate of degradation of protein and carbohydrate feed. The simultaneous provision of ammonia and energy can be achieved by composing of rations based on the protein-energy synchronization index. The index is expressed on a scale of 0-1. In other word, the ration with an index close to one (1) illustrates the availability of ammonia and energy more harmonious [1].

The growth of rumen microorganisms can not be extracted from the influence of the cofactor. The cofactor of branched chain volatile fatty acids (BCVFA) is used by most rumen microorganisms. Cellulolytic microorganisms primarily utilize BCVFA as the main source of carbon chains for growth. BCVFA is a side product of amino acid deamination in the rumen. Therefore, an increase in BCVFA levels in the rumen can be done by supplementing high protein sources in the ration [2]. Soybean and coconut meal are potential sources of protein. Each has a high protein content, sequentially 49.09% and 22.86%. In addition, they degradation level of protein and organic material ingredients are also good. This can be seen from the synchronization index, respectively sequentially 0.77 and 0.74 [3].

The studies attempted by Syamsi et al.[4] and Waldi et al.[3] has proved that the ration based on protein-energy synchronization index (PES) using different potential protein sources could increase the microbial protein synthesis (MPS). The medium level of PES rations with legume protein sources would improve VFA products [5]. The increase of MPS and VFA production affected the BCVFA profile in the rumen. The activity of rumen microorganisms will produce and utilize BCVFA in the different metabolic processes. Thus, the study of interaction effect between the meal protein source and the PES index on the BCVFA profile of rumen fluid is important to do.

2. Methodology

Experiment Design

This is an experimental research using in vitro techniques [6]. This research used rumen fluid from local Friesian Holstein (PFH) bull taken immediately after slaughtered. The measured variables were BCVFA profiles such as iso butyrate, iso valerate, and valerate. This research used completely randomized design (CRD) experimental design with factorial pattern: the first factor is 2 types of meal (soybean meal and coconut meal), and the second factor was 3 levels of the PES index (0.5, 0.6, and 0.7). It made 6 combinations of treatment, each treatment was repeated 4 times. These treatment combinations arrangement were based on Waldi et al.[3] and illustrated in Table 1.
Table 1. The arrangement of rations treatment

| Feedstuff             | R1  | R2  | R3  | R4  | R5  | R6  |
|-----------------------|-----|-----|-----|-----|-----|-----|
| King grass            | 60  | 60  | 58  | 60  | 60  | 59  |
| Soybean meal          | 0   | 0   | 0   | 10  | 10  | 10  |
| Coconut meal          | 10  | 10  | 10  | 0   | 0   | 0   |
| Dried cassava         | 3   | 5   | 21  | 3   | 5   | 20  |
| Rice bran             | 10  | 4   | 2   | 10  | 4   | 2   |
| Pollard               | 6   | 10  | 2   | 6   | 10  | 2   |
| Soybean curd dregs    | 10  | 10  | 6   | 10  | 10  | 6   |
| Mineral               | 1   | 1   | 1   | 1   | 1   | 1   |
| **Total**             | 100 | 100 | 100 | 100 | 100 | 100 |

**Synchronization index**

| Nutrient               | 0.5 | 0.6 | 0.7 | 0.5 | 0.6 | 0.7 |
|------------------------|-----|-----|-----|-----|-----|-----|
| Dry matter (%)         | 93.74 | 93.49 | 92.80 | 93.74 | 93.49 | 92.91 |
| Ash (% DM)             | 9.01  | 8.19  | 7.13  | 8.97  | 8.34  | 7.21 |
| Crude protein (% DM)   | 11.91 | 12.09 | 10.2  | 14.5  | 14.67 | 12.87 |
| Crude fat (% DM)       | 5.82  | 5.82  | 5.40  | 4.54  | 4.54  | 4.12 |
| Crude fiber (% DM)     | 23.78 | 23.16 | 21.70 | 23.20 | 22.60 | 21.41 |
| Nitrogen free extract (% DM) | 48.14 | 49.23 | 53.80 | 47.30 | 48.42 | 52.52 |
| TDN %                  | 59.99 | 60.21 | 61.60 | 60.40 | 60.65 | 61.75 |

* The arrangement of rations and level of nutrient based on Waldi et al.(2017) [3]

**Measure Protein-Energy Synchronization Index [PES] of Feed Ingredients**

The PES index of feed ingredients was measured based on Syamsi et al.[4] by measuring in vitro protein degradation and organic material (OM) of feed ingredients. The time intervals for concentrates degradation were 2, 4, 6, 8, 12, 24 and 48 hour, while for forages were 2, 4, 6, 8, 12, 24, 48, and 72 hour. The protein and OM degradation rates at each time interval then analyzed by regression to obtain the degradation rate on g N and Kg OM per hour. Then, it was used to calculate the PES index of each feed ingredient which used as the basis for the preparation of rations treatment on next step, with the following equation.

\[
\text{Synchronization Index} = \frac{1}{20} \sum_{n=1}^{20} \left( \frac{N_{\text{OM per hour}}}{20-N_{\text{OM per hour}}} \right)^2
\]

Note: 
- n: observation time,
- \( N_{\text{OM per hour}} \): rate of protein degradation compared to the rate of degradation of organic matter every hour [7].

**Variable Analysis**

The levels of isobutyrate, isovalerate, and valerate of rumen fluid were measured using gas chromatography with standard solution = acetate 52.54% molar, propionate 13.42% molar, isobutyrate 5.40% molar, n-butyrate 10.89% molar, isovalerate 4.23% molar, n-valerate 4.61% molar. Calculations can be done with the following formula.

\[
\text{VFA [% molar]} = \frac{\text{area of VFA Sample} \times \text{VFA standard}}{\text{area of VFA standard}}
\]
The measurement procedure for iso butyrate, isovalerate, and valerate of rumen fluid begins with taking the rumen fluid supernatant from the in vitro digestion using a 4 ml pipette then centrifuged at 10,000 rpm for 15 minutes. Two milliliters of supernatant were added into a small 5 ml plastic tube. Add 30 mg 5-sulphosalicylic acid then the solution is shaken, centrifuged at 3000 rpm for 10 minutes at 4°C then filtered with milipore until clear liquid is obtained. One μl of clear liquid was injected into gas chromatography, which was previously injected with a standard VFA solution. The concentration in the sample is calculated by using the following formula.

\[ \text{VFA [Individually]} = \frac{\text{The high sample solution}}{\text{The high standard solution}} \times \text{standard concentration} \]

3. Results and Discussion

Branched chain volatile fatty acids (BCVFA) are a by-product of amino acid deamination in the rumen. These products are iso butyrate, isovalerate, valerate, and 2-methylbutyric acid [8]. The main raw material producing the products is feed protein. Proteins will be hydrolyzed by proteolytic microorganisms resulting oligopeptides and amino acids. Both then deaminated to be α-keto, VFA, CO₂, and NH₃. The α-keto acid then undergone further hydrolysis to be BCVFA [9]. The data of BCVFA on rumen fluid which received ration treatment based on protein-energy synchronization index (PES) with different meal protein sources is presented in Table 2.

| Variables | R1 (coconut meal + Index 0.5) | R2 (coconut meal + Index 0.6) | R3 (coconut meal + Index 0.7) | R4 (soybean meal + Index 0.5) | R5 (soybean meal + Index 0.6) | R6 (soybean meal + Index 0.7) |
|-----------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Isobutyrate (mM) | 10.38±2.11 | 9.89±2.73 | 10.24±0.36 | 6.57±1.55 | 7.36±0.53 | 6.78±0.45 |
| Isovalerate (mM) | 0.26±0.24 | 0.28±0.19 | 0.57±0.47 | 0.46±0.22 | 0.56±0.41 | 0.63±0.10 |
| Valerate (mM) | 1.11±0.26 | 1.05±0.08 | 1.23±0.30 | 0.73±0.44 | 1.03±0.24 | 0.98±0.26 |

The results of the variance analysis showed that the interaction between the meal protein source with the PES index was not significantly affected (P> 0.05) to BCVFA profile. Likewise, the types of protein source meal and the PES index, each was not significantly affected (P> 0.05) to BCVFA profile. The research of Syamsi et al.[10] found a similar results that the ration based on PES index with supplementation of legume protein sources did not affect to the production of isovalerate and valerate, but had positive effect to the production of iso butyrate. This may caused by the level of protein sources was not different from each treatment, so the protein content of each treatment was also not different.

Protein levels that not different between treatments may cause similarities in BCVFA production. This is supported by the opinion of Ropotă et al.[11] which explains that BCVFA production is largely determined by the availability and rate of degradation of feed proteins in the rumen. Considering at the protein availability of each treatment [Table 1], the range of protein ration treatment is between 10.2-14.67%. The reseracher of Syamsi et al.[10] stated that equal protein levels on each treatment ration will produce several different BCVFA products. Based on the rate of protein degradation compared to OM,
soybean or coconut meal has the same PES index, which is at the medium level (0.77 and 0.74 respectively) [3].

The average BCVFA production as follows: iso butyrate 6.57-10.38 mM, lower than Syamsi et al. [10] which is 9.96 - 12.62 mM; iso valerate 0.26-0.63 mM lower than Zhang et al.,[12] (1.86 - 2.58 mM); and valerate 0.73 - 1.23 mM lower than Suwandyastuti and Rimbawanto [13] (1.26-1.51 mM). The BCVFA is a source of carbon body for MPS and the excess of carbon body would be used for VFA production [14]. Based on that, the results of this study are in line with Waldi et al. [3] who elucidate that the MPS increases by increasing protein-energy synchronization index in soybean or coconut meal. Causing the lower levels of BCVFA of this study than others. In addition, Syamsi et al. [5] also proved that rations with the SPE index and supplemented with high protein feed sources were able to produce higher VFA than ordinary level.

4. Conclusion
The study concluded that the low PES index ration (0.5) produced a decent BCVFA profile using coconut or soybean meal.

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