Estimation rumen degradable protein of local feeds in dairy cattle using in sacco method

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Abstract. There is a lack of nutrition information on local feeds protein in Indonesia, especially to determine protein fraction of dairy feed. The objective of this study was to determine rumen degradable protein (RDP) of local feeds in dairy cattle using in sacco method. The local feeds are copra meal, palm kernel meal, coffee husk, tofu waste, soy-sauce waste, brewer waste, and habbatussauda waste. Two ruminal fistulated male Frisian Holstein were used to determine rumen degradable protein using in sacco method. The parameters observed include the estimated kinetic parameters, effective degradability (ED), and RDP. The parameters were evaluated with analysis of variance using SAS University software. The result showed that tofu waste and habbatussauda waste had high potential rapid degradation of CP following by brewer waste, palm kernel waste, soy-sauce waste, copra meal, and coffee husk. Habbatussauda waste had higher RDP followed by brewer waste, tofu waste, copra meal, palm kernel meal, soy-sauce waste, and coffee husk. There was a positive correlation between RDP and crude protein content, and a negative correlation between RDP and crude fiber. It is concluded that local feeds have various characteristics of quality feed protein, which is shown by rumen degradable protein and rumen undegradable protein.

1. Introduction

Protein is one of the most expensive nutrient components. The amount of protein needs to be considered according to livestock requirements so that protein can be used optimally and efficiently. The requirement of protein for ruminant is unique because of the rumen. Feed protein in the rumen will be degraded into ammonia and it will be used to form microbial protein synthesis. The other protein fraction that is not degraded will bypass from rumen (rumen undegradable protein). Rumen degradable protein (RDP) is required for ruminal fermentation to provide peptides, free amino acids, and ammonia for the synthesis of microbial protein. However, rumen undegradable protein (RUP) provides a direct source of amino acid that is digested in the intestine of the animal.

Few methods are developed to estimate RDP and RUP of dairy cattle feed, such as in sacco method, in vitro enzymatic method, in vitro chemical method, and in vitro multi-chemical method [1]. In sacco or in situ method is commonly used because it can be simulated the ruminal environment [2]. The method consists of incubations of feed samples in nylon bags in the rumen for various times. Variation among feed samples, animals, and laboratories can be observed with this method [3].

Dairy cattle in Indonesia is commonly fed concentrate of local feed as a protein source. There is a lack of nutrition information on local feeds protein in dairy cattle, especially to determine protein fraction of feed. The objective of this study was to determined rumen degradable protein (RDP) of local feeds in dairy cattle using in sacco method.
2. Methods

2.1. Location and samples preparation
This study was conducted from September to October 2020 at the Laboratory of Dairy Nutrition, Faculty of Animal Science, IPB University, Indonesia.

Local feed proteins used in this study were copra meal, palm kernel meal, coffee husk, tofu waste, soya-sauce waste, brewer waste, and habbatussauda waste. All local feeds were dried at 60 °C and ground through a 2-mm screen prior to analysis. Nutrient content of local feeds (dry matter, ash, crude protein, crude fat, crude fiber, and nitrogen free extract) were analyzed using NIRs near-infrared spectroscopy [4].

2.2. In sacco procedures
In sacco method was used to determine rumen degradable protein (RDP) and rumen undegradable protein (RUP) [5]. This study was conducted using two ruminal fistulated male Frisian Holstein with average body weight 519±80.61 kg. Dairy cattle fed twice daily at 7.00 a.m and 3.00 p.m. Diets contained 60% Napier grass and 40% concentrate mix on a DM basis. Nylon bags (ANKOM, 5 x 10 cm with ±50 micron porosity) containing 5 g of each local feed were placed in triplicate in the rumen for 0, 3, 6, 9, 12, 15, 24, and 48 h. The nylon bags were placed into the rumen before morning feeding. For 0 h incubation, the nylon bags were only rinsed under tap water. After incubation, the nylon bags were removed from the rumen and washed in the tap water until clear. Then, the nylon bags were dried at 60 °C for 2 days and weighed. The residual samples at each incubation time were taken for crude protein analysis (CP) with the Kjehdal method [6].

Ruminal degradabilities of CP or the kinetic parameters were estimated by fitting the data to the exponential equation [7] (CP versus time) (Eq. 1):

\[ y = a + b \left( 1 - e^{-ct} \right) \]  

(1)

where y is CP disappearance in the rumen (%) at time t, a is the soluble fraction (%), b is the insoluble fraction but a potentially degradable fraction (%), c is the degradation rate constant of the b fraction (%/h), t is the time of incubation degradation time.

The effective degradation of CP was calculated using the formula [8] (Eq.2) by assuming a rumen outflow rate of 6%/h.

\[ ED = a + b \frac{c}{(k+c)} \]  

(2)

where (a), (b), and (c) are the same as in Eq.1 and k is the rumen outflow rate. The RDP and RUP values (% CP) were calculated [1] with the following equation (Eq. 3 and 4):

\[ RDP = a + b \left[ \frac{c}{c+k} \right] \]  

(3)

\[ RUP = 100 - RDP \]  

(4)

where (a), (b), and (c) are the same as in Eq.1, and (k) is the same as in Eq.2. The RDP value was correlated among each nutrient content of local feeds.

2.3. Statistical analysis
One way analysis of variance (ANOVA) was performed using SAS University to evaluate significant differences in the means of all parameters among samples. The animals were treated as blocks. The model was described by [9] and if it was significantly different (P<0.05) further tested by Duncan test.

3. Results and discussion

3.1. Nutrient content and protein degradation
The nutrient content of local feeds is present in Table 1. The average dry matter is 93.68%±3.79. The crude protein content of local feeds ranges from 10.08% - 27.64%. Habbatussauda waste and brewer waste had the highest crude protein, and coffee husk had the lowest.
Table 1. The nutrient content of local feeds

| Feed                  | Moisture | DM (%) | Ash (%) | Crude Protein (%) | Crude Lipid (%) | Crude Fiber (%) | NFE (%) |
|-----------------------|----------|--------|---------|-------------------|-----------------|-----------------|---------|
| Copra meal            | 6.22     | 93.78  | 11.11   | 18.91             | 7.36            | 11.09           | 45.31   |
| Palm kernel meal      | 1.78     | 98.22  | 5.14    | 12.12             | 10.49           | 16.35           | 54.12   |
| Coffee husk           | 13.4     | 86.6   | 5.97    | 10.08             | 0.16            | 25.08           | 45.31   |
| Tofu waste            | 5.49     | 94.51  | 6.84    | 18.35             | 9.72            | 14.00           | 45.60   |
| Soy-sauce waste       | 4.33     | 95.67  | 23.39   | 21.16             | 17.87           | 21.95           | 11.30   |
| Brewer waste          | 4.19     | 95.81  | 12.30   | 27.43             | 12.07           | 18.92           | 25.09   |
| Habbatussauda waste   | 8.85     | 91.15  | 8.10    | 27.64             | 6.79            | 3.8             | 53.07   |

DM: dry matter; NFE: nitrogen-free extract

The data in Table 2 showed CP degradation of local feed. Ruminal degradabilities of CP local feeds protein were significantly different affected by feed, incubation time, and feed x incubation time (P<0.01). Crude protein degradation increased following by incubation time. Degradabilities of CP local feeds are various. Figure 1. showed trends of CP degradation of local feeds protein. The graph shows habbatussauda waste had rapidly degraded, in contrast, coffee husk had slowly degraded. Habbatussauda waste has a lower level of crude fiber, also higher content of nitrogen-free extract. Nitrogen-free extract represents non-nitrogen substances and the soluble carbohydrate included sugars, starches, fructans, galactans, pectins, beta-glucans, and organic acid. Pectin is a rapidly degraded complex carbohydrate and is almost completely degraded in the rumen [10]. Meanwhile, coffee husk has higher crude fiber that is dominated with lignin so difficult to be degraded in the rumen [11]. Lignin has the cross-linking with the other cell wall polysaccharides that can limit CP degradation [12].

Table 2. Degradability of crude protein (CP loss) local feeds

| Incubation time (h) | Copra meal | Palm kernel meal | Coffee husk | Tofu waste | Soy-sauce waste | Brewer waste | Habbatussauda waste | Feed | Time | Feed x time | P-value |
|---------------------|------------|------------------|-------------|------------|----------------|--------------|---------------------|------|-----|-------------|---------|
| 0                   | 25.04      | 23.01            | 0.00        | 27.45      | 10.73          | 38.43        | 86.30               | <0.0001 | <0.0001 | <0.0001 |         |
| 3                   | 40.23      | 41.00            | 15.09       | 37.66      | 24.28          | 62.94        | 95.35               |       |     |             |         |
| 6                   | 44.96      | 45.20            | 15.66       | 45.31      | 37.68          | 71.54        | 96.39               |       |     |             |         |
| 9                   | 62.08      | 46.76            | 20.05       | 51.04      | 49.42          | 77.48        | 97.43               |       |     |             |         |
| 12                  | 59.83      | 64.23            | 24.84       | 53.96      | 66.46          | 79.70        | 97.88               |       |     |             |         |
| 15                  | 60.17      | 66.36            | -6.58       | 61.96      | 65.04          | 82.32        | 98.31               |       |     |             |         |
| 24                  | 70.76      | 65.78            | 19.64       | 80.74      | 80.02          | 86.23        | 98.60               |       |     |             |         |
| 48                  | 78.35      | 85.61            | 15.56       | 94.12      | 80.49          | 91.17        | 98.72               |       |     |             |         |
Figure 1. Crude protein degradation of local feeds protein

The result of estimation kinetic parameters and effective degradation (ED) are presented in Table 4. The data showed that soluble fraction (a), potentially degradable fraction (b), potential degradation (a+b) are significantly different affected by feed (P<0.001), meanwhile the rate of degradation (c) had no significant (P>0.05). The potential degradation is “a+b”, which is a summary of soluble material and the potential for material to be degraded. The potential degradation (a+b) CP was higher for tofu waste and habbatussauda, intermediate for brewer waste, palm kernel meal, soy-sauce waste, copra meal, and lower for coffee husk. The calculated ED of local feeds protein shows various ED values with a range from 14.38%–96.70%. Habbatussauda was the greatest ED value among other samples local feed protein (P<0.01).

Table 3. Estimated of kinetic parameters and effective degradation of CP

| Parameters | Copra meal | Palm kernel meal | Coffee husk | Tofu waste | Soy-sauce waste | Brewer waste | Habbatussauda waste |
|------------|------------|-----------------|-------------|------------|----------------|--------------|---------------------|
| a (%)      | 26.23c     | 26.00c          | 0.00d       | 27.53c     | 7.79d          | 39.92b       | 86.40a             |
| b (%)      | 51.26c     | 60.44b          | 15.04e      | 79.69a     | 76.53a         | 47.75c       | 11.82e             |
| c (%/h)    | 0.09       | 0.06            | 3.66        | 0.04       | 0.10           | 0.18         | 0.41               |
| a+b (%)    | 77.49b     | 86.44b          | 15.04c      | 107.22a    | 84.32b         | 87.67b       | 98.22a             |
| ED (%)     | 57.22c     | 57.04c          | 14.38d      | 59.19c     | 54.82c         | 76.62b       | 96.70a             |

a: soluble fraction; b: potentially degradable fraction; a+b: summary of a and b values; c: rate of degradation; ED: effective degradation.

3.2. Rumen degradable protein

Data in Table 4 shows the estimated RDP and RUP for local feeds protein. RDP and RUP values are significantly different affected by feed (P<0.01). Habbatussauda waste is a local feed protein that had the highest RDP value (96.70%), following by brewer waste, tofu waste, copra meal, palm kernel meal, soy-sauce waste, and coffee husk. RUP value is calculated as 100 minus RDP. In contrast, coffee husk had the highest RUP value (85.62%), following by soy-sauce waste, palm kernel meal, copra meal, tofu waste, brewer waste, and habbatussauda waste.
Table 4. Estimated of rumen degradable protein and rumen undegradable protein

| Parameters       | Copra meal | Palm kernel meal | Coffee husk | Tofu waste | Soy-sauce waste | Brewer waste | Habbatussauda waste |
|------------------|------------|------------------|-------------|------------|----------------|--------------|---------------------|
| CP (%)           | 18.91      | 12.12            | 10.08       | 18.35      | 21.16          | 27.43        | 27.64               |
| 100% CP          |            |                  |             |            |                |              |                     |
| RDP (%)          | 57.22^a    | 57.04^c          | 14.38^d     | 59.19^e    | 54.82^c        | 76.62^b      | 96.70^a             |
| RUP (%)          | 42.78^b    | 42.96^b          | 85.62^a     | 40.81^b    | 45.18^b        | 24.38^c      | 3.30^d              |
| % of CP          | 10.82      | 6.91             | 1.45        | 13.59      | 11.60          | 20.74        | 26.73               |

CP: crude protein; RDP: rumen degradable protein; RUP: rumen undegradable protein.

The calculated RDP and RUP with the percentage of CP show habbatussauda waste had higher RDP value compare to other local feed. Soy-sauce waste had a higher RUP value (9.56% of CP), followed by coffee husk, copra meal, brewer waste, palm kernel meal, and habbatussauda waste. This result differs from 100%CP because the calculation depends on the CP content of local feeds protein. There was a positive correlation between RDP and crude protein content (r=0.85604). It shows that the high protein content of the feed will increase RDP value. In contrast, there was a negative correlation between RDP and crude fiber (r=-0.78548). High crude fiber content of feed will decrease RDP value and increase RUP value. It can be seen, coffee husk and soy-sauce waste had a high of crude fiber (25.08% and 21.95%), so the RDP values (100%CP) are lower than other local feeds.

4. Conclusion
It is concluded that local feeds have various characteristics of quality feed protein, which showed by rumen degradable protein and rumen undegradable protein.

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