**In vitro digestibility of ruminant diet in response to protected feed substitution**

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**Abstract.** Excessive degradation high protein feed in the rumen can reduce nutrient utilization efficiency of feed in post rumen. Protected feed is expected to increase the undegraded proteins that enter and absorbed in the intestine. *In vitro* study was conducted to determine whether the protected feed can reduce rumen degradability as well as improving post-rumen degradability of feed. The protected feed used in this study was soybean meal and crude palm oil mixture (4:1), added with 0.3% Agromix. Independent sample t-test was used to compare 2 treatments (T1/control = commercial ration; T2 = commercial ration with 4.4% protected feed substitution). Each dietary treatment was done in 6 replications. Both dietary treatments were tested for their digestibility using a 2-stage *in vitro* technique. Results showed that pH of rumen did not change. Compared to the T1, T2 showed lower (P<0.05) digestibility of dry matter (DMD), organic matter (OMD), and crude protein (CPD) in the rumen. In the post rumen, T2 showed greater (P<0.05) DMD, OMD and CPD than T1. It concluded that protected feed substitution effectively decreases digestibility in the rumen and increases post-rumen digestibility, imply that protected feed substitution can improve the nutrient utilization in the post rumen.

1. **Introduction**

Excessive degradation of feed in the rumen, especially high protein feed, can reduce the efficiency of nutrient utilization in the post-rumen digestive tract. On the other hand, high-quality protein needed to provide amino acids to support the productivity of ruminant, so the provision of protein must consider the ability of fermentation and its resistance to rumen degradation. Soybean meal is one of the foods rich in protein with high nutrient value. Soybean meal has good palatability and amino acid balance [1]. The essential amino acid content reaches 47.6% of crude protein. The content of rumen-degraded protein is 71 - 79% [2]. Decreasing feed efficiency also occurs in fat degradation, especially unsaturated fatty acids in the rumen due to the hydrolysis and hydrogenation processes. Unsaturated fatty acids also cause a negative effect on fiber degradability. Crude palm oil (CPO) is one of Indonesia's local feed ingredients containing non-saturated fatty acids and abundant production. The main component of CPO is 93% triglyceride. The other glyceride content in CPO is 4.5% diglyceride and 0.9% monoglyceride. The free fatty acids of CPO are palmitate (40-45%), and oleic (39-45%) [3].

Feed protection is one way to reduce negative effects and improve feed efficiency for ruminants. The saponification method is known to protect feed fatty acids from the degradation of rumen microbes, while formaldehyde (HCHO) is known to be able to protect feed proteins [4]. The purpose
of this study was to determine the digestibility of dairy cow ration with protected feed substitution by in vitro method.

2. Material and methods

2.1. Material
The material used in this study were soybean meal (SBM), crude palm oil (CPO), rice bran, wheat pollard, cassava pulp, copra meal, king grass, and premix mineral Agromix®. The instruments used for proximate analysis were following AOAC [5]. Other instruments used were in vitro digestibility are following Tilley and Terry [6] method, digital scales (Shanghai Yamato, Shanghai, China with precision 0.1), analytical scales (Ohaus, New Jersey, USA with precision 0.0001), oven (Memmert, Schwabach, Germany), water bath, and Willey mill (Thomas Willey Laboratory Meal, Philadelphia, USA).

2.2. Methods

2.2.1. Sample preparation and chemical analysis. The samples dried in an oven at 55 °C for one day and ground using Willey mill sieved through 1 mm screen. The sample was analysed for chemicals composition (dry matter (DM), ash, crude protein (CP), crude fat (Extract Ether, EE), and crude fibre (CF)) by AOAC [5] method.

2.2.2. Feed protected preparation and total mixed ration. The ratio between SBM and CPO is 4 : 1, while Agromix® was added as much as 0.3%. The saponification method used NaOH and CaCl$_2$ [7]. The ratio of CPO : NaOH : CaCl$_2$ was 4:1:1 [8]. A cream is a form of soap produced from the saponification process. Cream was added to soybean meal and Agromix® (produced by CV. Agromix Lestari, Yogyakarta) then mixed until homogeneous. The mixture of the three feed ingredients was protected by spraying 0.8% formaldehyde [9]. Independent sample t-test was used to compare two dietary treatments (T1/control = commercial ration, and T2 = commercial ration with 4.4% protected feed substitution). Total mixed ration ingredients and chemical composition is presented in Table 1.

2.2.3. Ruminal fluid preparation. The ruminal fluid derived from two male Bali cattle (approximately 223 to 316 kg). The cattle fed an adaptation diet of forage and concentrates (80:20) and given free access to water. The diet adaptation given twice a day at 07.00 am and 14.00 pm. A period of adaptation carried out a week before the research.

2.2.4. In vitro digestibility. A total of 0.50 g (dry weight) sample was put in 80-90 ml in vitro tubes. Ruminal fluid and artificial saliva (1:4) added, the mixture stirred, gassed with CO$_2$, and 50 ml were added to each tube. The in vitro digestibility analysis was carried out using the 2-stage in vitro method [6], was done in 6 replications for each nutrient digestibility test. The sample then analysed for in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), and in vitro crude protein digestibility (IVCPD).

2.2.5. Statistical analysis. The mean values for in vitro digestibility was analysed using T-test of SPSS Statistics 25.
Table 1. Total mixed ration ingredients and chemical composition

| Item                        | T1  | T2  |
|-----------------------------|-----|-----|
| Ingredients (% of DM)       |     |     |
| King grass                  | 60  | 60  |
| Rice bran                   | 8   | 8   |
| Copra meal                  | 8   | 8   |
| Soybean meal                | 6.40| 3.20|
| Wheat pollard               | 10  | 10  |
| Cassava pulp                | 6.40| 6.40|
| CPO                         | 0.40| 0   |
| Agromix®                    | 0.80| 0   |
| Feed protected              | 0   | 4.40|
| Nutrient composition        |     |     |
| DM (%)                      | 95.74| 94.98|
| CP (%)                      | 13.02| 13.18|
| CF (%)                      | 24.77| 24.79|
| EE (%)                      | 3.05 | 3.51 |
| Ash (%)                     | 8.03 | 8.05 |
| Total digestible nutrients (TDN) % | 63.36 | 64.22 |
| Nitrogen-free extract (NFE) % | 51.13 | 50.47 |

3. Results and discussion

3.1. In vitro digestibility

The digestibility of dry matter, organic matter, and crude protein of ruminant ration in response to protected feed substitution presented in Table 2.

Table 2. Digestibility of ruminant ration in response to protected feed substitution

| Variable                        | Treatment of ration | p-value |
|---------------------------------|---------------------|---------|
| IVDMD 1 (%)*                    | 55.10 ± 0.77        | 0.002   |
| IVDMD 2 (%)*                    | 55.17 ± 0.57        | <0.001  |
| IVOMD 1 (%)*                    | 53.46 ± 0.30        | 0.008   |
| IVOMD 2 (%)*                    | 54.12 ± 0.54        | <0.001  |
| IVCPD 1 (%)*                    | 7.96 ± 1.54         | 0.038   |
| IVCPD 2 (%)*                    | 23.29 ± 2.65        | 0.002   |
| pH ruminal fluid †              | 7.27 ± 0.71         | 0.739   |

*Shows a significant effect (P<0.05). † superscript show no significant effect (P>0.05).

In the first stage (rumen digestion), digestibility of DM, OM, and CP on ration with protected feed substitution (T2) showed a significant decrease (P <0.05) when compared with ration without protected feed substitution (T1). The opposite occurred in post-rumen digestion (second stage), DM, OM, and CP digestibility in T2 ration showed a significant increase (P <0.05) compared with T1 ration. This can be interpreted that some nutrients of feed are protected from the degradation of rumen microbes, and can be digested and absorbed in post-rumen so that it can be of direct benefit to the ruminant.

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Decreasing nutrient digestion of T2 rations in the rumen was caused by the substitution of protected feed. The protection method used was saponification and formaldehyde. Saponification of CPO with calcium salts causes fatty acid bonds with calcium salts which are thought to be inert (not easy to change) in the rumen because calcium soap is insoluble in rumen pH but soluble in the abomasum which has a pH of 2-3 [10]. This is in accordance with the results of the study, that the digestibility post rumen of T2 ration had increased compared to the T1 ration. The results of the formation of fat saponification are precipitate of calcium soap which can be used as supplements or mixed in concentrated components of the ration [11].

The success of the protection method was also evidenced by a decrease in crude protein digestibility of the T2 ration in the rumen and increased the digestibility of post-rumen CP. Formaldehyde can form chemical bonds with proteins that are stable at rumen pH and become labile at acidic pH (post rumen) so that the bond is released and then proteins can be digested in the intestine [12]. Protein protection is intended to increase the amount of feed protein that enters the digestive tract behind the intestine [13]. Amino acids that are digested in the intestine in dairy cows are derived from synthesized microbial proteins in the rumen and feed proteins that are not degraded in the rumen so that the substitution of protected feed is expected to increase amino acid intake for ruminant and increase feed efficiency.

3.2. Rumen pH level
The ruminal fluid pH in this study was in the normal range and there was no significant difference between the rations T1 and T2 (P > 0.05). The pH value of the rumen fluid in this study was 7.24 - 7.28 (Table 2), this value included the normal rumen pH of Bali cattle, this is in accordance with the research on the use of bio-supplement (consists of a mixture of rumen waste, molasses, rice bran, corn bran, coconut flour, tapioca flour, soybeans, NaCl, limestone (CaCO3), and the multivitamin mineral 'Pignox') which produced normal pH of rumen fluid (7.04 - 7.34) and showed no significant difference between treatment [14]. This can be interpreted that the substitution of protected feed in dairy cattle rations has no effect on the rumen microbial environment, so it is expected that the substitution of protected feed is beneficial for livestock without disturbing the rumen ecology.

4. Conclusion
The substitution of protected feed in dairy cattle rations can be used as a way to increase nutrient absorption of feed for ruminants. The ration substituted with protected feed decreased DM, OM, and CP digestibility in the rumen, but increased in post-rumen digestibility. Protected feed substitution can improve the efficiency of the beneficiaries of good quality feed without affecting the environmental conditions of the rumen.

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