Effect of Combination of Protected and Non-Protected Corn Oil Supplementation on *In Vitro* Nutrient Digestibility

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Abstract. This research was done to study the effect of supplementation of the combination of corn oil (CO) and protected corn oil (PCO) using formaldehyde on *in vitro* nutrient digestibility in the rumen and post rumen. Protection of corn oil is carried out by mixing skim milk powder and corn oil (2:1) using formaldehyde 1.5%. Feed for fermentation substrate consists of *Penisetum purpureum* and wheat pollard (60:40). The combination of CO and PCO in several rations was added as the supplement to feed substrate (dry matter basis). The ratios of CO: PCO supplementation that given were T0 (0%:0%) as a control, T1 (5%:0%), T2 (3.75%:1.25%), T3 (2.5%:2.5%), T4 (1.25%:3.75%), and T5 (0%:5%). Their effects on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) and crude fiber digestibility (CFD) were studied using the two-stage *in vitro* digestibility method of Tilley and Terry (1963) modified by Utomo (2012). Data were statistically analyzed using one-way analysis of variance continued by Duncan's Multiple Range Test. The results showed that T1 and T2 significantly (P<0.05) decreased DMD, OMD and CFD at 48 h and 96 h incubation and did not affect rumen CPD. In conclusion, the supplementation of corn oil 1.25% combined with protected corn oil 3.75% had no negative effect on DMD, OMD, CPD and CFD on rumen and post-rumen digestibility.

1. Introduction

One of the alternatives that has been studied for manipulation the ruminants diets is the utilization of feeds rich in lipids, which can contribute to the supply the energy levels closer to those required by high producing animals, in addition to promoting a more adequate balance between structural and non-structural carbohydrates of the diet, and even optimizing the utilization of the digestible energy [1]. Moreover, oil supplementation on feed has the function to reduce methane emission from the rumen. Oil can inhibit methane formation due to toxicity effect on protozoa and methanogens and some other bacteria that digest fiber [2]. The oil containing high unsaturated fatty acid also has the double bond which can act as a hydrogen sink in the hydrogenation process [3].

Oil supplementation has the disadvantage in inhibiting feed fermentation in the rumen because the oil will coat the feed particles. As a result, enzymes produced by bacteria was difficult to penetrate the feed particles. Oil also has antimicrobial properties [4]. It could disrupt the rumen metabolic process, including low feed digestibility, decreased number of rumen microbes and the production of volatile fatty acids. Also, oils containing polyunsaturated fatty acids will undergo the biohydrogenation process in the rumen to produce saturated fatty acids [5].
Oil protection was a method that can be used to reduce the negative effects of oil supplementation. Oil protection could be done using formaldehyde. Formaldehyde forms a cross-link with the amino acid in the protein, called methylene bridge (–CH₂), which make protein resistance from microbial degradation [6]. Therefore this study was conducted to determine the effect of supplementation of the combination of corn oil and protected corn oil in several ratios on in vitro nutrient digestibility.

2. Materials and methods

2.1. Materials
The materials used in this study were corn oil, skim milk powder, and formaldehyde 37% pro analysis. Feed substrate consisted of elephant grass (Pennisetum purpureum) and wheat bran (60:40), thoroughly homogenized and ground to pass 1 mm screen. Rumen fluid was taken from fistulated Bali cattle.

2.2. Capsulation of corn oil
The capsulation procedure of polyunsaturated fatty acid in corn oil (CO) was based on the method described by [7]. The corn oil was mixed evenly with skim milk powder with the ratio of 1:2. Formaldehyde 37% pro analysis was added to the mixture with the level of 1.5% by weight of the mixture, mixed evenly to form a protected corn oil (PCO). Then, CO and PCO combination was added as the supplement to feed substrate based on the dry matter (DM basis). There were six treatments of the combination of CO:PCO which were evaluated using in vitro nutrient digestibility: T0 (0%:0%) as a control, T1 (5%:0%), T2 (3.75%:1.25%), T3 (2.5%:2.5%), T4 (1.25%:3.75%), and T5 (0%:5%).

2.3. Incubation and analytic methods
Fistulated Bali cattle were given a dietary trial for 1 week, then rumen fluid was collected before morning feeding. The rumen liquid was filtered using gauze and put into a 39°C flask. Determinations of nutrient digestibility were conducted by using in vitro [8] method modified by [9]. The in vitro digestibility analysis was carried out using the first stage (48 h) and two-stage (96 h) in vitro method. Rumen liquor-buffer mixture with ratio 1:4 (v/v). Twenty-five millimeters of buffered-rumen solution were dispensed in a 50 ml glass tube was used to determine DMD and OMD and 50 of buffered-rumen solutions were dispensed in a 100 ml glass tube to determine CPD and CFD. Triplicate fermentor for each treatment was incubated at 39°C for 48 h and 96 h. Chemical analysis including DMD, OMD, CPD, and CFD by using [10] method.

2.4. Statistical analysis
One-way ANOVA design was used to analyze the data. The differences found among means were tested by Duncan's multiple range tests.

3. Result and discussion
The effect of protected and non-protected corn oil supplementation in several ratios on feed digestibility including DMD, OMD, CPD, and CFD can be seen in Table 1. The results of the analysis of variance showed that T1 and T2 reduced the DMD and OMD significantly (P<0.05). It might be a result because of the supplementation corn oil without protection in T1 and T2 was higher than the other treatments. It has been suggested that dietary fats may coat the fiber and interfere with microbial attachment thus leading to depressed fiber digestibility [11]. [12] reported that fatty acids inhibited the growth of the cellulolytic organism in vitro, but had no effect on other organisms that produced propionate. [13] also reported that 2.4% of corn oil supplementation on feed can decrease DMD 7.18%. The greater the level of corn oil protection gives higher DMD and OMD values. This result is similar to previously reported by [5] in which the DMD and OMD increased by supplementation of total oil protection rather than oil without and partial oil protection. [14] stated that unprotected oil can reduce feed digestibility higher than protected oil. Oil protection is a solution to prevent negative effects in the form of direct toxic
effects on rumen microbes. [15] added that essential oils have small compounds such as terpenoid and phenolic which are antibacterial and antifungal. [16] stated that oil has hydrophobic antibacterial properties that can penetrate the outer layer of gram-negative bacteria through protein, so that the compound will damage the performance of enzymes in bacteria. The DMD value in this study was higher than OMD, presumably because the ration had a low insoluble ash content, a high degree of lignification from feed fiber and high cellulose crystallinity. DMD was higher than OMD due to ash degradation in the dry material component is low and the ability of microbes to degrade the components in DM is higher than OM [17].

**Table 1.** DMD, OMD, CPD and CFD in the rumen (48 h) and total DMD, OMD, CPD and CFD (96 h) of feed with supplementation of corn oil (CO) and protected corn oil (PCO) in several ratios

| Digestibility (%) | T0 | T1 | T2 | T3 | T4 | T5 |
|-------------------|----|----|----|----|----|----|
| IVDMD             |    |    |    |    |    |    |
| 48 h              | 57.97±3.87c | 45.14±2.14a | 48.88±0.65a | 49.70±2.11ab | 51.86±1.73bc | 56.17±0.81bc |
| 96 h              | 66.09±0.81c | 50.64±3.48a | 56.19±2.79ab | 59.25±3.68bc | 63.23±2.03bc | 64.56±1.93bc |
| IVOMD             |    |    |    |    |    |    |
| 48 h              | 56.08±2.73d | 42.74±2.87a | 47.80±0.56ab | 48.88±2.29abc | 50.10±1.79bcd | 54.91±0.24cd |
| 96 h              | 62.03±0.86c | 48.68±0.49a | 53.47±2.14ab | 56.70±4.75bc | 60.98±2.40bc | 62.32±1.68c  |
| IVCPD             |    |    |    |    |    |    |
| 48 h              | 54.29±3.48c | 54.74±1.87c | 53.76±3.47c | 53.79±5.01c | 53.75±0.68c | 52.64±1.33c  |
| 96 h              | 59.22±5.19c | 59.18±0.47c | 61.05±2.64c | 62.71±4.02c | 65.76±0.29c | 68.66±0.67c  |
| IVCFD             |    |    |    |    |    |    |
| 48 h              | 56.06±2.38ab | 47.04±1.13a  | 49.49±1.02a  | 55.23±0.65b  | 55.20±2.76b  | 56.70±1.27b  |
| 96 h              | 60.12±3.35b  | 50.52±2.02a  | 51.32±1.24a  | 56.78±0.41b  | 57.15±2.62b  | 59.06±1.26b  |

1DMD: Dry matter digestibility, OMD: Organic matter digestibility, CPD: Crude protein digestibility, CFD: Crude fiber digestibility

2T0: control, T1: CO 5% and PCO 0%, T2: CO 3.75% and PCO 1.25%, T3: CO 2.5% and PCO 2.5%, T4: CO 1.25% and PCO 3.75%, T5: CO 0% and PCO 5%.

abc superscripts within the same row indicate significant difference (P<0.05)

c not significant

The study found that there was no singnificant difference between corn oil and protected corn oil supplementation on the CPD at 48 h incubation. It indicated that formaldehyde has the ability to bind protein from skim milk that will eventually protect the corn oil on the feed ration. The protein-formaldehyde complex is stable at neutral condition, while labile on acidic condition. The neutrality of rumen liquid enables to maintain the stability of the protein-formaldehyde complex from the hydrolytic enzyme produced by rumen microorganism [18]. In 96 incubation, also there was no singnificant difference between corn oil and protected corn oil supplementation on the CPD. It might be a result because of the low proportion of protein from skim milk that added on diet.

The result showed that T1 and T2 reduced the CFD significantly (P<0.05) compared to control group at 48 h and 96 h incubation. The lowest CFD was T1 (5.05% at 48 h and 5.17% at 96 h) compared to the control group. Inclusion of fat in the diet caused a linear depression intake and fiber digestibility [19]. [20] stated that lipids in the form of soybean grains and soybean oil and verified that soybean oil negatively affected fiber digestibility. The addition of oil can suppress the ability and inhibit microbial metabolism because oil can wrap the feed and close the surface access of the microbial cell membrane in contact with the feed, which can further disrupt the production of enzymes to degrade feed [21]. [22] added that the provision of fat in the feed will reduce the utilization of fiber in the rumen.
4. Conclusion
Supplementation of corn oil and protected corn oil at a certain ratio on feed can affect the nutrient digestibility of the rumen and post-rumen fermentation. The ratio of 1.25% corn oil and 3.75% protected corn oil had no negative effect on dry matter digestibility, organic matter digestibility, crude protein digestibility and crude fiber digestibility.

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