Effect of Crude Palm Oil Protection on Fermentation Parameter and Rumen Microbial Activity of Male Local Lamb

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Abtstract. The experiment was conducted to determine the effect of protected crude palm oil (CPO) protection in the ration on in vivo fermentation parameters and microbial activity. Fifteenth local male lambs aged 9-12 months weighing 14-17 kg, were divided into 3 groups ration treatment. The first group received only the basal ration (R0), the 2nd group received the basal ration and 3% CPO (R1), while the 3rd group received the basal ration and 3% CPO protected with 2% formaldehyde (R2). The data were analyzed by completely random design with oneway pattern. The different of treatments were tested by Duncan's New Multiple Range Test. The results showed that addition CPO protected with formaldehid (R2) in sheep ration increasing microbial protein in the rumen fluid.

Keywords: fermentation parameters, rumen microbial activit. CPO

Abstrak. Penelitian ini dilakukan untuk mengetahui pengaruh proteksi kelapa sawit mentah (CPO) dalam ransum terhadap parameter fermentasi in-vivo dan aktivitas mikroba. Lima belas ekor domba lokal muda jantanumur 9-12 bulan dengan bobot 14-17 kg, dibagi menjadi tiga kelompok perlakuan ransum. Kelompok pertama hanya diberi ransum basal (R0), kelompok kedua diberi ransum basal dan 3% CPO (R1), kelompok ketiga diberi ransum basal dan 3% CPO terproteksi dengan 2% formaldehid (R2). Rancangan Acak Lengkap dan dan anova satu arah digunakan pada penelitian ini dengan uji lanjut jarak ganda Duncan. Hasil penelitian menunjukkan bahwa penambahan CPO terproteksi dengan formaldehida (R2) pada ransum domba meningkatkan protein mikroba dalam cairan rumen.

Kata kunci: parameter fermentasi, aktivitas mikroba rumen, CPO

Introduction

The use of fats rich in unsaturated fatty acids in ruminant rations is limited, because it would interfere with rumen fermentation and reduce the use of fiber (Hristov et al., 2009; Vafa et al., 2009). It is caused fats can wrap feed particles and close access to the microbial cell membrane surface in contact with the feed, so undisturbed production of enzymes to degrade the feed. Addition of linoleic acid (*cis*-9,*cis*-12-18: 2) and linolenic (*cis*-9,*cis*-12,*cis*-15-18:3) in the ration will the hydrogenated by microbes in the rumen becomes saturated fatty acid, primarily stearic (Jenkins et al., 2008; Wang et al., 2010), so that only about 10% left and joined in the lipid tissues (Wood et al., 2008) while 90% hydrogenated to saturated fatty acids.

The fat feed sources may be protected with formaldehyde to prevent microbial rumen hydrogenation on unsaturated fatty acids, and increased use of fat in the ration (de Veth et al., 2005; Kitessa et al., 2001). It principle, the oil particles will be surrounded by a bond between protein and formaldehyde which prevents hydrogenation of unsaturated fatty acids in the rumen at neutral conditions (pH 6-7), but the acidic conditions in the abomasum (pH 2-3) bond between protein and formaldehyde was split, unsaturated fatty acids can be digested and absorbed in the small intestine.

Studies have been carried out in vitro using CPO as much as 0, 1.5, 3, 4.5, 5 and 6% of the

dry matter are mixed with milk powder expired (1:2) and protected with a 0, 1, 2 and 3% formaldehyde from the mixture, obtained results that the CPO as much as 3% of dry matter that is protected with 2% formaldehyde can protect unsaturated fatty acids from rumen microbial hydrogenation and no negative effect on fermentation parameters and rumen microbial activity (Tiven et al., 2011). This research is very important, because the application of in vitro studies, with the aim to determine the effect of the addition of CPO protected with formaldehyde in the ration on parameters fermentation and rumen microbial activity of sheep.

Materials and Methods

Animals. As much as 15 local male lambs aged 9-12 months with a body weight of about 14-17 kg maintained in individual cages shaped stage equipped with places to eat and drink. Sheeps were randomly divided into 3 groups according to the treatment of feed, each group consisted of 5 animals.

Feed. Used basal ration consisting of forage and concentrate (60:40). Forage used was elephant grass, while the concentrates used consisted of 30% bran and 10% soybean meal. Nutrient content of basal ration was 62.98% TDN, 45.5% DM, 14.48% CP, 4.70% CF and 21.93% crude fiber. The first group received only the basal diet (R0), the 2nd group received the basal diet and 3% CPO (R1), while the 3rd group received the basal diet and 3% CPO protected with 2% formaldehyde (R2).

Fermentation parameters and microbial activity testing. Sheeps are maintained for 3 months and given a ration of twice a day, ie morning at 08.00 and the afternoon at 15.00, while the drinking water provided ad libitum. During the maintenance recorded amount of consumption daily of each animal. Sheeps were weighed once a week to find out his weight and to adjust the amount of rations provided. At the end of the collection, taken sheep rumen fluid using trocar and put in a thermos. Rumen fluid was taken to the laboratory and then centrifuged at 500 g for 15 min to separate the feed particles. Most of the filtrate obtained from the centrifuge is used to calculate the number of protozoa (Abreu et al., 2004), while others again centrifuged at 10,000 g for 15 min to separate the sediment microbes. The filtrate was taken for determination of CMC-ase activity by reducing the amount of sugar that is formed from the substrate CMC (Halliwel et al., 1985), is expressed in specific enzyme activity with measuring protein enzymes content using Lowry method (Niamke et al., 2005), N-NH3 (Weatherburn, 1967), total VFA by gas chromatography method and pH (Abreu et al., 2004). The microbial precipitate was dissolved by adding 0.5 ml NaOH 1 N and then heated at 90°C for 10 minutes. After soluble added distilled water so its total volume of 1 ml. Determination of the amount of biomass by microbial cell protein by the Lowry method in a spectrophotometer (Niamke et al., 2005).

Statistical analysis. Used one way complete randomized design with 3 ration treatments, namely the basal ration (R0), the basal ration and 3% CPO without protected by formaldehyde (R1) and the basal ration and 3% CPO protected with 2% formaldehyde (R2), each three replications. The data obtained were statistically tested using the analysis of variance and the differences between treatments were tested further by Duncan's New Multiple Range Test. Data processing is done with the SPSS program 17.0 for Windows Evaluation Version (Oramahi, 2008).

Results and Discussion

Rumen Fermentation Parameters

Ammonia. Effect addition of CPO protected in the ration on sheep rumen fluid ammonia content, can be seen in Figure 1. The results of statistical analysis showed that the addition of

CPO (R1) decrease in ammonia ($P<0.01$) of 10.38 mg/100 than sheep which were given only the basal ration (R0). The decrease in ammonia (NH_3) becomes larger ($P<0.01$), namely 10.38 mg/100 ml when given the addition of CPO protected with formaldehyde (R2). The compared with R1 treatment, the decreased NH_3 content of R2 treatment not significant. The decrease of NH_3 content in R1 treatment influenced of fat in the diet can inhibit the ability of microbial metabolism, because the fat can be wrapped around the feed and close access to the microbial cell membrane surface in contact with the feed, so disrupting the production enzymes to feed degrade. The decreased of NH_3 content in R2 treatment caused by microbial degradation was reduced due to formaldehyde protection, so decreased protein solubility. The NH_3 content in this study ranged from 28.53 to 45.10 mg/100 ml (285.3 to 451.0 mg/l). According to McDonald et al. (2002), the concentration of NH_3 in the rumen cattle range 85-300 mg/l, while Satter and Slyter (1972) states that the NH_3 content to 80 mg/100 ml does not interfere rumen microbial growth, so it can be stated that the NH_3 content in this study are normal range.

pH. Effect addition of CPO protected in the ration on sheep rumen fluid pH, can be seen in Figure 1. The results of statistical analysis showed that the addition of CPO (R1) and CPO are protected with formaldehyde (R2) in the ration not significant on sheep rumen fluid pH. The range rumen fluid pH is 6.79 to 6.98 with the average of 6.89. It takes a good pH for the normal rumen fermentation. The range optimal pH for microbial activity is 6.70 to 7.00, while the optimal pH for the digestibility of cellulose and starch, respectively 6.90 and 6.80 (Johnson, 1966). When the associated with fermentation products, the VFA does not affect the pH value due to the absorption in the rumen wall that regulates the pH of the rumen in order to stay in optimal condition for the process of fermentation. Based on the pH range, it can be stated that the rumen fluid pH in this study are within the normal range.

Volatile Fatty Acid. Effect addition of CPO protected in the ration on sheep rumen fluid VFA content, can be seen in Figure 2. The results showed that the addition of CPO (R1) and CPO protected with formaldehyde (R2) in the ration not significant on acetate, propionate, butyrate, total VFA and C2/C3 ratio in rumen fluid. Average value of VFA in the R2 treatment tends to be lower than the R1 treatment. Its caused the feed protection with formaldehyde to reduce feed degradation by rumen microbes. VFA tends to be lower in the R2 treatment can be associated with decreased of NH_3 content (Figure 1), which may be used as a framework of C for microbial protein synthesis when available N source, so increased microbial protein content (Figure 3). Total VFA content obtained in this study ranged from 15.49 to 16.63 mM/l. According to Mc Donald et al. (2002), the concentration of VFA in the rumen varied between 0.2 to 1.5 g/100 ml (10-70 mM/l), so it can be said that the VFA content obtained are still within normal limits.

Rumen Microbial Activity

Carboxymethyl cellulase activity. Effect addition of CPO protected in the ration on sheep rumen fluid carboxymethyl cellulase **(**CMC-ase), can be seen in Figure 3. The results of statistical analysis showed that the addition of CPO (R1) in the ration decrease rumen fluid CMC-ase activity ($P<0.05$) at 2.37 U/g compared to sheep which were given only the basal ration (R0). The decrease CMC-ase activity to be greater ($P<0.05$), amounting to 3.80 U/g when the addition of CPO protected with formaldehyde (R2). CMC-ase activity on R2 treatment not significantly compared to the R1 treatment. The decrease in CMC-ase activity in the R1 treatment caused the fat that is not protected in the ration, which can act as an

defaunating agent and inhibits the enzymes production to feed degrade. The decreased CMC-ase activity of R2 treatment caused fat protection with formaldehyde in the ration have not been able to fix the decrease in the number of protozoa (Fig. 4). Protozoa have a relatively large role in degrading fiber than bacteria, so the decrease in the number of protozoa will decrease fiber degradation. The decrease in CMC-ase activity is not disruptive VFA production, so it can be stated that its decrease is still normal.

Microbial protein. Effect addition of CPO protected in the ration on sheep rumen fluid microbial protein, can be seen in Figure 3. The results of statistical analysis showed that the addition of CPO (R1) in the ration not significant than the sheep that were given only the basal ration (R0). The addition CPO protected with formaldehyde in the ration (R2), increased microbial protein ($P<0.05$), respectively by 0.61 mg/ml and 0.51 mg/ml compared to sheep

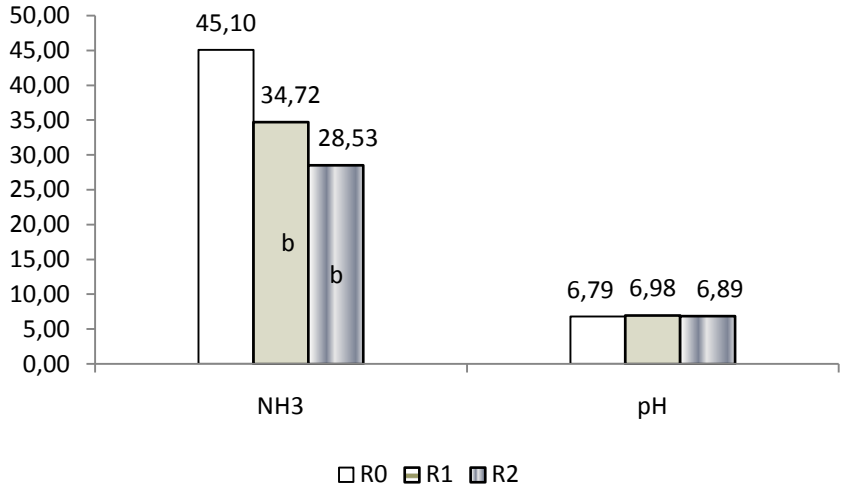

Figure 1. NH_3 content (mg/100 ml) and pH of sheep rumen fluid which give the basal ration (R0), basal ration with the addition of CPO (R1) and basal ration with the addition of CPO are protected with formaldehyde (R2)

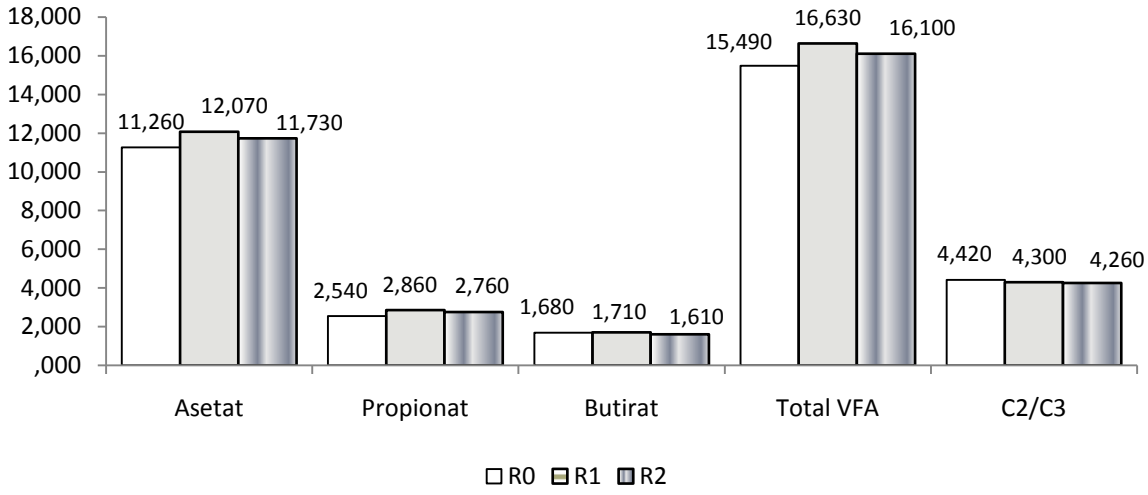

Figure 2. VFA content (mM) of sheep rumen fluid which give the basal ration (R0), basal ration with the addition of CPO (R1) and basal ration with the addition of CPO are protected with formaldehyde (R2).

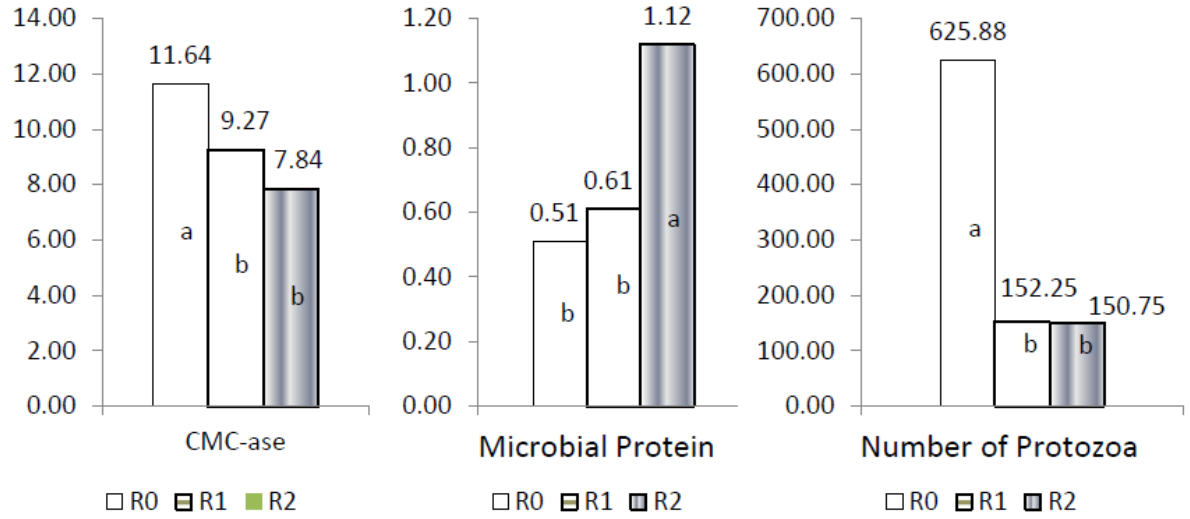

Figure 3. CMC-*ase* activity (U/g), microbial protein and the number of protozoa (x10^3) of sheep rumen fluid which give the basal ration (R0), basal ration with the addition of CPO (R1) and basal ration with the addition of CPO are protected with formaldehyde (R2)

which were given only the basal ration (R0) and the basal ration with the addition of CPO (R1). The increase microbial protein caused increase in rumen bacteria due to the decrease in the number of protozoa which having the predator character due to defaunating by fat feeding, so providing an opportunity for bacteria to grow and synthesize microbial protein. The increase in microbial protein is also associated with decreased of NH_3 and VFA content in R2 treatment (Figures 1 and 2), which serve as sources of N and C framework for microbial protein synthesis, so increasing microbial protein.

Number of protozoa. Effect addition of CPO protected in the ration on sheep rumen fluid number of protozoa, can be seen in Figure 3. The results of statistical analysis showed that the addition of CPO (R1) in the ration decrease the number of protozoa ($P<0.01$) in the sheep rumen fluid amount to 473.63 x10^3, compared sheep were given only the basal ration (R0), but not significant compared the addition of CPO protected with formaldehyde (R2). The decrease the number of protozoa in the R1 and R2 treatment caused to the high fat content in CPO. Rumen protozoa number will decrease with the addition of oil which contains saturated fatty acids (Ueda et al., 2003; Hristov et al., 2004). According to Jordan et al. (2006), the addition of 1.0% (DM) of coconut oil in the ration grass silage and soybean meal (50:50) will defaunating rumen protozoa in the significantly (62.5%). The addition of rapeseed oil (62% oleic acid) and linseed oil (53% linolenic acid) as much as 7% (DM) in hay and grain (60:40), decrease number of protozoa amounted to 30.77% and 36.15% (Cieślak et al., 2006). The decrease the number of protozoa in the R2 treatment indicates that CPO protection with formaldehyde has not been effective in reducing the negative effects of fat on rumen protozoa. When associated with a product of fermentation, the decrease in the number of protozoa has not disrupt the VFA production, so the decrease is still normal.

Conclusions

Crude palm oil protection with formaldehyde in the male local lamb ration, increased microbial protein on rumen fluid.

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