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The Effect of Total or Partial Protected Vegetable Oil Supplementation on In Vitro Digestibility, Feed Fermentability and Energy Efficiency

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ABSTRACT

This study was examines the effect of oil (oil palm and corn) combination with the level of protection (total or partial) on feed fermentability, methane production and energy efficiency with in vitro techniques. The experiment was designed using a factorial pattern 2x2, factor A = type of oil (corn and palm) and factor B = oil protection level (total and partial), each treatment combination was repeated 4 times. Data was processed by analyzing various factorial patterns in a randomized block design and if there was an influence between treatments performed by the Dunca test. The results showed that there was no interaction effect (P>0.05) between the types of oil with the level of protection in all parameters, except NH3. Supplementation of palm oil produces total volatile fatty acids (VFA), acetate and methane (CH4) production higher than corn oil (P<0.05), but the efficiency of converting hexose energy to VFA (ECH) was lower (P<0.05) (76.09 vs 77.80%). Supplementation of total protected oil decreased in the protozoa population, resulting in higher dry matter digestibility (DMD) and organic matter digestibility (OMD), but lower ECH yield compared to partial protected oil supplementation (P<0.05), ie 76.68 vs 77.22%. The conclusions of the study are corn oil produce of ECH higher than palm oil. Partial protection produce better feed fermentability and increasing energy efficiency in the form of decreasing A/P ratio and methane production.

Keyword: Energy efficiency, Fermentability, Protection, Type of oil

Introduction

The beginning of lactation is a period that needs to be considered for dairy cattle, because nutrient requirements increased dramatically for milk production. Low supply of energy from feed causes energy for milk production to be unfulfilled and decreased milk production. The cow will also mobilize body fat reserves for additional energy during lactation. It causes an imbalance in energy utilization and a decrease in body weight of livestock or generally called negative energy balance (NEB) (Rehak *et al.*, 2012; Weber *et al.*, 2013).

Oil supplementation is one alternative solution that has been carried out to increase energy density when livestock production is high (Mosley *et al.*, 2007; Benchaar *et al.*, 2006). Oil supplementation will produce energy twice as high as other nutrients when metabolized in the body (Vienna and Susana, 2013). Based on this study, oil can be used to supply energy shortages efficiently. Allen (2000) found that giving oil can increase the efficiency of energy utilization in lactation cattle. Manso *et al.* (2006) reported that the provision of 41g/kg of palm oil concentrate could increase fat digestibility and feed conversion. Oil supplementation is also useful as a defaunation agent and a source of essential unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA). Vegetable oils that contain lots of unsaturated fatty acids are corn oil and palm oil. Both oils have almost the same unsaturated fatty acids, but palm oil has a price that is cheaper and easily accessible when applied on farms.

Oil as a defaunation agent had antimicrobial properties that could limit the activity of rumen microbes, such as protozoa and bacteria. Oil supplementation especially polyunsaturated oils could interfere the activity of fibrolytic bacteria, while the amount of protozoa is affected by saturated and unsaturated oils. It may thereby inhibit the fermentation process in the rumen. Oil supplementation was expected to suppress the protozoa population. Considering the role of protozoa in methane production (in collaboration with the methanogen bacteria), the decrease in the number of protozoa will be followed by a decrease in methane production (Hess *et al.*, 2006; Patra and Yu, 2012). Previous study showed that supplementation of 4% rice bran oil in dairy cow feed increased the

concentration of propionate and can reduce the ratio of acetate/propionate and CH4 (Lunsin *et al.*, 2012; Patra and Yu, 2012). 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 (Jenkins, 1993). It could disrupt the rumen metabolic process, including low feed digestibility, decreased number of rumen microbes and the production of VFA (volatile fatty acids).

Oil protection was a method that can be used to reduce the negative effects of oil supplementation. Oil protection could be done using the saponification method by binding to the carboxyl group free of fatty acids using minerals (Wina and Susana, 2013). Some studies have carried out protection using Ca minerals (Block *et al.*, 2005; Manso *et al.*, 2006), but in this study protection was carried out using Zn minerals. Zn was a micro mineral known as an activator of several enzymes (Underwood and Suttle, 2001). Associated with supplementation of polyunsaturated fatty acids, Zn mainly has a role in the initial level of desaturation and elongation of polyunsaturated fatty acids in tissues. The reaction between minerals and fat from the saponification process forms salts that are inert or slightly dissociated in the rumen at pH 6-7, so that they do not affect the rumen microbial activity. Oil protection could be done in a total or partial. The results of research Widiyanto *et al.* (2008) proved that supplementation of 10% protected kapok seed oil (75%) gave optimal results on ruminal digestibility and energy efficiency. Based on this data, it seemed necessary to study the level of protection of other vegetable oils (palm and corn) which was more applicable in order to obtain optimum feed fermentability and improve feed energy efficiency. The aim of this study was to evaluate the response of the combination of types of oil (palm and corn) with the level of protection (partial or total) on in vitro feed fermentation, methane production and energy efficiency.

Materials and Methods

The experiment was carried out in vitro (Tilley and Terry, 1963). Female Etawah Crossbred Goat with a fistula belonging to the Faculty of Animal and Agricultural Sciences Diponegoro University was used as a source of rumen fluid. Feed consisted of forages and concentrates which were prepared to feed lactating dairy goats (crude protein 15%, total digestible nutrients 60%). The composition and nutrient content of the ration is presented in Table 1, while the fatty acid content of palm oil and corn oil in Table 2.

The oil used was commercial palm oil and commercial corn oil. The preparation of zinc soap was carried out according to Cabatit (1979). Palm oil and corn oil are measured for saponification number, then the soap was made based on saponification rates, KOH was added proportional to the saponification number of each oil. The material used for protection was the mineral $ZnCl_2$. Zn mineral was added based on the results of the reaction stockiometry calculations according to Widiyanto *et al.* (2008), so the addition of $ZnCl_2$ is equal to the KOH needed to soak palm oil or corn. The total protection treatment was by adding 10% protected oil, while partial protection was adding 10% oil consisting of 75% protection and 25% nonprotection.

The study design used was a 2x2 factorial pattern with a basic randomized block design. Factor A was oil type: palm oil (T1) and corn oil (T2), while factor B: oil protection is total (P1) and partial (P2), so there were 4 treatment combinations applied, namely: T1P1: ration + 10% total protected palm oil, T1P2: ration + 10% partially protected palm oil (75% protection), T2P1: ration + 10% total protected corn oil, T2P2: ration + 10% partially protected corn oil (75% protection). The parameters observed were dry matter digestibility (DMD), organic matter digestibility (OMD), crude fiber digestibility (CFD), rumen pH, NH_3, total and partial VFA (acetate, propionate and butyrate), total protozoa, microbial protein, Acetat/Propionate ratio, methane concentration, and energy conversion efficiency hexose becomes VFA (ECH).

Fistula goats were given a dietary trial for 1 week, then rumen fluid was taken. The rumen liquid was filtered using gauze and put into a 39 ° C flask. The fermentation was conducted based on the method of Tilley and Terry (1963). Each treatment ration was weighed in the amount of 0.55-0.56 g, put in a fermentor tube then added 40 ml of McDaugall's solution and 10 ml of rumen liquid. The fermentor cylinder was flowed by CO_2 gas and closed to anaerobic conditions. The fermentor tube was incubated for 4 hours in a 39°C water temperature and stopped after 4 hours by dripping 2 drops of $HgCl_2$ solution. The fermentor tube was centrifuged at 3,000 rpm for 15 minutes and a supernatant was taken to observe the rumen pH value, NH_3, total and partial VFA, microbial protein and protozoa.

The measurement of rumen liquid pH was carried out using a digital pH meter of the brand "ACT" and was repeated 3 times for each sample. NH_3 production was measured using a spectrophotometer with a spectrophotometric technique based on the catalyzed endophenol reaction to produce a stable blue compound (Chaney and Marbach, 1962). Measurement of partial VFA production (acetic acid, propionate, butyrate) was carried out using a gas chromatography (General Laboratory Procedures, 1966). VFA sample production is calculated by the formula:

$$\text{Partial VFA (mM)} = \left(\frac{\text{sample area x standard concentration x 1000}}{\text{standard area x MW}}\right)$$

MW : molecular weight

Table 1. Composition and content of nutrient rations

Feed ingredients	Composition				
	Basic ration	T1P1	T1P2	T2P1	T2P2
	(%)				
Rice bran	17	15.3	15.3	15.3	15.3
Polard	19	17.1	17.1	17.1	17.1
Cassava pulp	13	11.7	11.7	11.7	11.7
Soybean meal	8	7.2	7.2	7.2	7.2
Mollases	3	2.7	2.7	2.7	2.7
Corn straw	30	27	27	27	27
Caliandra	10	9	9	9	9
Total protection of palm oil		10			
Partial protection of palm oil			10		
Total protection of corn oil				10	
Partial protection of corn oil					10
Nutrient content					
	(%)				
Ash	10.28	9.86	9.86	9.86	9.86
Crude protein	15.14	14.44	14.44	14.44	14.44
Crude fat	2.67	12.55	12.54	12.55	12.54
Crude fibe	25.05	24.18	24.18	24.18	24.18
BETN	46.79	44.57	44.47	44.59	44.49
Organic matter	89.72	90.14	90.14	90.14	90.14
TDN	60.41	77.92	77.83	77.94	77.85

Table 2. Fatty acids composition of palm oil and corn oil

Fatty acids	Palm oil*	Corn oil**
	(%)	
Lauric acid (C12:0)	0.2	-
Myristic acid (C14:0)	1	0.1
Palmitic acid (C16:0)	42.9	8 – 12
Stearic acid (C18:0)	4.4	2.5 – 4.5
Oleic acid (18:1)	40.8	19 – 49
Linoleic acid (18:2)	10.2	34 – 62
Caproic (C6:0), linolenic (C18:3), arachidate (20:0)	0.5	1.5
Iod number***	127.08	61.06

Sumber : Lida *et al.* (2002)*
Dwiputra *et al.* (2015)**
Kristianingrum dan Handayani (2005)***

Measurement of rumen fluid microbes using the method of Makkar *et al.* (1982) on the principle of gradual centrifugation. The populations of protozoa were calculated following the procedures of Ogimoto and Imai (1981). The solution used was methil formalin saline which was made from a mixture of 100 ml 35% formaldehyde, 2 g tryhan blue, 9 g NaCl and 900 ml aquades. The number of protozoa was calculated by using a microscope at magnification 100 times. The methane gas concentration was determined by calculating the VFA stockiometry, which was the estimation using the formula Orskov and Ryle (1990). The formula used were:

Methane (mM) = 0.5 A- 0.25 P + 0.5 B

A (asetate)
P (propionate)
B (butyrate)

Energy conversion efficiency was calculated based on the stockyometry of the carbohydrate fermentation reaction from hexose to VFA (acetate, propionate and butyrate) (Orskov and Ryle, 1990). The calculation formula used was:

$$E\ (\%) = \left(\frac{0{,}622\ pA + 1{,}091\ pP + 1{,}558\ pB}{pA + pP + 2\ pB}\right) x\ 100$$

E = Efficiency of hexose energy conversion into VFA
pA = Acetate proportion
pP = Propionate proportion
pB = Butyrate proportion

DMD, OMD and CFD measurements was used the method Tilley and Terry (1963), carried out in the same way as the measurement of VFA and NH_3 production, but the fermentation process was continued for 2x24 hours. The fermentation process was stopped, then the tube was centrifuged and the supernatant was discarded. The HCL pensin solution was added 50 ml and re-incubated for 2x24 hours in a 39°C water temperature under aerobic conditions. The sample was filtered with Whatman No.41 filter paper using a vacuum pump. The filter results were put into a porcelain cup, then it baked at 105˚C for 24 hours to calculate DMD, then the material in the cup was in an electric furnace for 4 hours at 600°C to calculate OMD. DM blank comes from the fermentation residues without samples. DMD and OMD samples are calculated by the formula:

$$\%DMD = \left(\frac{\text{DM sample (g)} - (\text{DM residue (g)} - \text{DM blank (g)})}{\text{DM sample(g)}}\right) x\ 100$$

$$\%OMD = \left(\frac{\text{OM sample (g)} - (\text{OM residue (g)} - \text{OM blank (g)})}{\text{OM sample(g)}}\right) x\ 100$$

CFD measurements were derived from a sample of incubation 2x48 hours centrifuged for 15 minutes at a speed of 3,000 rpm. Supernatant and sludge were separated, then the sludge was added with 50 ml of a solution of 0.3 N H_2SO_4 and cooked for 30 minutes, then added with 1.5 N

NaOH and cooked for 30 minutes. Samples were filtered using Whatman filter paper. 41. Filtering was carried out in suction flasks and washed consecutively using 50 ml of hot water, 50 ml of H_2SO_4, 50 ml of hot water, and 25 ml acetone. Filter paper and its contents were put into porcelain crucible and roasted for 24 hours at 105˚C, then cooled in an excavator for 15 minutes. Samples were weighed and counted as weight after oven. The digestibility of crude fiber is calculated using the formula:

$$\%CFD = \left(\frac{\text{CF sample (g)} - (\text{CF residue (g)} - \text{CF blank (g)})}{\text{CF sample(g)}}\right) x\ 100$$

Data were processed by analyzing factorial patterns in a randomized block design using the SPSS 16. The mean difference test for the treatment was carried out with the Duncan test when there was influence between treatments.

Result and Discussion

Effect of protected oil supplementation on feed digestibility

The effect of total or partially protected vegetable oil supplementation on feed digestibility including DMD, OMD and CFD can be seen in Table 3. The results of the analysis of variance showed that there was no interaction (P>0.05) between the type of oil with the level of protection on DMD and OMD. The level of protection had a significant effect (P<0.05) on DMD and OMD, but it was not significantly different in the treatment of oil types. Total protection gives higher DMD and OMD values (P<0.05) compared to partially protected oils. DMD was 57.33% (P1) and 55.05% (P2), while OMD was 55.52% (P1) and 53.18% (P2). The results of this study was not much different from the results of Dinata *et al.* (2015) that supplementation of 10% oil with high unsaturated fatty acid content resulted in DMD and OMD values of 48.41% and 50.91%.

The results showed that there was no significant difference in DMD and OMD (P>0.05) on oil treatment. This was due to the high portion of fibrous feed in the ration used (Table 1). A high portion of fiber in the feed could create stable rumen environmental conditions for microbial activity in digesting feed. The results of previous studies showed the portion of feed fiber by 60% in the ration can create an ideal environment for rumen microbial activity (Jenkins, 1993; Ueda *et al.*, 2003; Messana *et al.*, 2013). If the portion of the feed fiber is high, the oil will be bound to the particular feed fiber, so that the oil toxicity to microbes decreases. Oil supplementation in rations with balances forage:concentrate of 50:50 also did not have a significant effect on feed digestibility (Adeyemi *et al.*, 2015b). Benchaar *et al.* (2006) found that feed supplemented with essential oils had not a significant effect on DMD and OMD, which was 66.3% (DMD) and 67.95% (OMD).

Protection treatment had a significant effect on DMD and OMD (P<0.05). Total protection (100% protected oil) was the most effective method to reduce the negative effects of oil supplementation, because it gave higher DMD and OMD values than partial protection (Table 3). Wina and Susana (2013) 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. Castillejos *et al.* (2006) states that essential oils have small compounds such as terpenoit and phenolic which are antibacterial and antifungal. Helander *et al.* (1998) stated that oil has hydrophobic antibacterial properties that can penetrate the outer layer of gram-negative bacteria through protein porin, 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 (Pamungkas *et al.*, 2014).

Crude fiber digestibility (CFD) did not provide a significant difference (P>0.05) due to the type of oil treatment and the level of protection component is low and the ability of microbes to degrade the components in DM is higher than OM (Pamungkas *et al.*, 2014).

Crude fiber digestibility (CFD) did not provide a significant difference (P>0.05) due to the type of oil treatment and the level of protection

Table 3. The response of total or partial protected vegetable oil supplementation on digestibility

Digestibility	Protection	Oil type		Average
		T1	T2	
		----------(%)----------		
Dry matter	P1	57.24	57.43	57.33[a]
	P2	55.20	54.90	55.05[b]
	Average	56.22	56.16	
Organic matter	P1	55.38	48.21	55.52[a]
	P2	53.65	44.76	53.18[b]
	Average	54.51	54.18	
Crude fiber	P1	72.11	74.99	73.55
	P2	80.52	83.34	75.54
	Average	72.87	76.22	

T1: palm oil, T2: corn oil, P1: total protection and P2: partial protection.
[a,b] different superscripts at the same column indicate significant differences (P<0.05).

or a combination of both, it's at an average of 74.55%. Based on the research of Adeyemi *et al.* (2015a) that supplementation of a combination of canola oil and palm oil up to 8% had no significant effect on CFD and neutral detergent fiber (NDF), because most of the ration consisted of forage (50:50). The average CFD values in this study were higher than DMD and OMD (Table 3), supported by the average VFA concentration of treatment is high (Table 4).

The high CFD was thought to be due to the presence of calliandra in the ration used as one of the fiber feed ingredients other than corn straw. According of Adebowale and Nakashima (1991) that legumes have higher levels of cell wall degradation (NDF) than forage, due to differences in cell wall characteristics. Legumes have lignin content that is in the cell contents and can bind to soluble carbohydrates that are in it, causing the digestibility of dissolved carbohydrates is lower and result in increased fiber digestibility (Jouany, 1991).

Feed fermentability

The response of a combination of oil type supplementation with protection to in vitro feed fermentability can be seen in Table 4. Supplementation of oil types and protection or interaction of both do not have a significant effect on rumen pH (P>0.05), an average of 6.9 and it's normal. The results were similar to those of Adeyemi *et al.* (2015a), supplementation of 8% combination of canola oil (80%) with palm oil (20%) has a rumen pH of 6.71 and supplementation of 8% carotino oil produces a rumen ph value of 6.95. El-Sherbiny *et al.* (2016) stated that 5% supplementation of several oil nanoemulsions including soybean oil, fish oil and a combination of both had an average pH of 6.83. The pH value was not different because it was assumed that the composition of the treatment ration was the same, so that the difference in the speed of VFA production was not prominent in terms of DMD and OMD which were almost the same (Table 3).

The combination of oils and protection provided a significant interaction on NH_3 concentration (P<0.05). Duncan's test showed that partially protected corn oil (T2P2) gave the highest NH_3 value of 10.43 mM, but did not different from T1P1 (10.36 mM) and T1P2 (9.64 mM). The lowest NH_3 production occurred in the total protected corn oil supplementation of 8.02 mM. Dinata *et al.* (2015) stated that the level combination of cottonseed oil and protection level produced different NH_3, supplementation of 10% oil and protected 25% has highest NH_3 concentration of 6.50 mM. NH_3 concentrations in this study were normal to support microbial protein synthesis. McDonald *et al.* (2002) stated that the optimum concentration of rumen ammonia is around 85-300 mg/l or equivalent to 6-21 mM. Decrease on NH_3 production at the T2P1 (total protected of corn oil) treatment was suspected due to microbial cell polyperation in terms of a decrease in total VFA production and not different rumen fluid microbial protein concentrations in the T2P1 treatment (Table 4).

The total and partial VFA production (acetate, propionate and butyrate) due to the treatment can be seen in Table 4. The combination of oil types and protection has no significant effect on the production of VFA (total or partial), as well as the protection

Table 4. The response of total or partial protected vegetable oil supplementation on fermentability

Rumen fluid profile	Protection	Oil type		Average
		T1	T2	
pH	P1	6.9	7.0	6.96
	P2	7.0	7.0	6.95
	Average	6.95	6.96	
NH_3 concentration (mM)	P1	10.36^{pq}	8.02^{r}	9.19
	P2	9.64^{pqr}	10.43^{p}	10.03
	Average	10.00	9.23	
Total VFA (mM)	P1	157.89	136.77	147.33
	P2	164.38	119.73	142.05
	Average	161.13^{a}	128.25^{b}	
Acetate (mM)	P1	95.80	73.19	84.50
	P2	95.31	61.47	78.39
	Average	95.55^{a}	67.33^{b}	
Propionate (mM)	P1	34.89	35.50	35.20
	P2	39.73	31.13	35.43
	Average	37.31	33.31	
Butyrate (mM)	P1	27.20	28.07	27.64
	P2	29.33	27.13	28.23
	Average	28.27	27.60	
Protozoa (x10^3 cel/ml)	P1	68.33	23.89	46.11^{b}
	P2	83.89	28.89	56.39^{a}
	Average	76.11^{a}	26.39^{b}	
Microbial protein (mg/ml)	P1	11.23	10.79	11.01
	P2	12.37	11.79	12.08
	Average	11.80	11.29	

T1 : palm oil, T2 : corn oil, P1 : total protection dan P2 : partial protection.
^{a,b} different superscripts at the same column or row indicate significant differences (P<0.05).
^{p,q,r} different superscripts at the same column and row indicateof interaction treatment (P<0.05).

treatment (P>0.05). Oil types have a significant effect on total VFA and acetate production (P<0.05). The total VFA production in this study had fulfilled the normal range of 70-150 mM (McDonald *et al.*, 2002).

Duncan's test showed that palm oil supplementation produced a total VFA production of 161.13 mM, higher than corn oil with a high unsaturation degree of 128.25 mM. VFA values in the T1 treatment were high because the acetate concentration was also higher, whereas the propionate and butyrate concentrations did not different between treatments (Table 4). The acetate concentration was lower in the T2 treatment, because the proportion of polyunsaturated fatty acids in the form of linoleic on corn oil was greater, between 34-62% (Table 2). According to O'Brien *et al.* (2014), that linoleic and linolenic acid significantly (P<0.01) decreased the production of acetate, linoleic and linolenic content in oil could reduce the activity of methanogenic bacteria. El-Sherbiny *et al.* (2016) stated that supplementation of nano-encapsulation of fish oil and soybean oil can reduce total VFA and acetate production. The existence of polyunsaturated fatty acids in this study led to an emphasis on the activity of methanogenic bacteria in using H2 for methane production, so that an increase in hydrogen accumulation and cause a reaction change from pyruvate to lactate. Baldwin and Allison (1983) state that if methanogenesis is inactive then H2 in the external environment is high, the thermodynamic reaction of NADH2 H2 + NAD is unfavorable and organisms are forced to reduce pyruvate to lactate or propionate to maintain hydrogen balance.

The concentration of propionate and butyrate was not significantly different (P<0.05) in the treatment of oil type and protection. The average propionate production was 35.31 mM, but the butyrate was 27.94 mM. Propionate concentration was inversely proportional to acetate, but this event did not occur in this study. Decreasing acetate concentration in the treatment of corn oil (T2) did not provide a significant increase in propionate. This was thought to be due to the suppression of methanogenic bacteria, so that the process of methanogenesis was inhibited and it happen an imbalance of the reaction of pyruvate to acetate. Based on this the mechanism of propionate formation through the carboxylic acid pathway was also thought to be inhibited, so it has not shown significant results on propionate concentration in this study. Baldwin and Allison (1983) stated that the conversion of pyruvate to acetate in generally was a pyruvate-format lyase system that produces formate and acetyl-CoA as direct products. The accumulation of formats due to the suppression of the methanogen process will result in an imbalance of the pyruvate reaction to acetate. It will have an impact on the formation of propionate through the carboxylic acid pathway, because in that process pyruvate will be converted to oxaloacetate and end up in the formation of propionate. Based on this mechanism, propionate concentration in this study could not reach maximum due to an imbalance of the reaction from pyruvate to acetate or propinate.

The results of the analysis of variance showed that there was no effect of a combination of oil and protection types on protozoan populations and rumen microbial protein content (P>0.05). Table 4 shows that corn oil significantly affected rumen protozoa population (P<0.05) by 26.39x103 cells/ml, as well as total protection of 46.11x103 cells/ml. This was because corn oil had a high of unsaturation compared to palm oil, a high of unsaturation will be more toxic to rumen microbes. Jenkins (1993) states that lipids have antimicrobial properties that can damage the function of eukaryotic cell membranes such as interfering with intra-oxidative processes. It is thought to suppress the proliferation of rumen bacteria and suspected the protozoa growth also decreases. Protozoa symbiosis with methanogenic bacteria by 70%, so that was decreased in the number of protozoa will be followed by a decrease in methanogenic bacteria (Hess *et al.*, 2003; Patra and Yu, 2012). Inversely with this, the type of oil or the type of protection did not have a significant effect on rumen microbial protein synthesis (P<0.05), was an average of 11.36 mg/ml. This was because the decrease in protozoa will be offset by an increase in bacterial population so that rumen microbial protein was not affected (Table 4). McDonald *et al.* (2002) stated that the optimum concentrations for rumen NH_3 and VFA were 6-21 mM and 70-150 mM.

Efficiency of ruminal energy metabolism

The efficiency of ruminal energy metabolism in the study could be reflected in the ratio of A/P, CH4 concentration and the efficiency of the conversion of hexose energy to VFA (ECH). The results of the analysis of variance showed that the effect of the type of oil had a significant (P<0.05) on the A/P ratio, the concentration of CH4 and EKH. The A/P ratio and methane concentration significantly decreased in the treatment of corn oil by 2.03 and 39.14 mM and an increase in the ECH coefficient of 77.80% (Table 5). The pattern also occured in partial protection treatments, although it was not statistically significant. Adeyemi *et al.* (2015b) stated that supplementation of 8% carotino oil could reduce the A/P ratio by 1.55. According to O'Brien *et al.* (2014), linoleic fatty acid supplementation of 10 ml/l medium in vitro was lower in reducing methane production (6.3 ml/l BK) compared to oleic fatty acid (17.7 ml/l BK), with a ratio A/P of 1.14 vs. 1.54.

Corn oil supplementation increased energy efficiency by changing the ruminal fermentation patterns, in terms of decreasing acetate concentrations while propionate tends to be stable (Table 4). The high of polyunsaturated fatty acids content in corn oil has a function in the

Table 5. The effect of total or partial protected vegetable oil supplementation on conversion energy efficiency

Parameters	Protection	Oil type		Average
		T1	T2	
Ratio A/P	P1	2.76	2.08	2.42
	P2	2.49	1.97	2.23
	Average	2.62[a]	2.03[b]	
CH4 (mM)	P1	52.78	41.76	47.27
	P2	52.39	36.52	44.45
	Average	52.58[a]	39.14[b]	
Energy efficiency (%)	P1	75.76	77.60	76.68[a]
	P2	76.43	78.00	77.22[b]
	Average	76.09[b]	77.80[a]	

T1 : palm oil, T2 : corn oil, P1 : total protection dan P2 : partial protection.
[a,b] different superscripts at the same column or row indicate significant differences (P<0.05).

suppression of methanogenic bacteria, so that the process of methanogenesis is decreased. Related to this, it will cause the partial pressure of hydrogen in the external environment to increase. Increasing the pressure of hydrogen will cause the thermodynamic imbalance of the reaction in the rumen, so the microbes will again use H_2 to reduce pyruvate to propionate. Martin *et al.* (2008) stated that propionate formation requires H_2, while acetate and butyrate produce H_2. Increasing propionate production will require more H_2 so that methane production will fall. Based on this energy efficiency is higher, the number of calories to produce 1 ATP propionate is more efficient than acetate.

Conclusions

Corn oil produced hexose energy conversion efficiency into VFA (ECH) higher than palm oil. Partial protection had a better feed fermentability response and increases energy efficiency in the form of decreasing A/P ratio and methane production.

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