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In Vitro Ruminal Fermentation, Microbial Population, Fatty Acid Profile in Cattle in the Presence of *Sapindus rarak* Extract Combined With Oils Microencapsulation

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Abstract | The aim of this study was to analyze the effect of *Sapindus rarak* extract combined with canola/sesame oils microencapsulation on *in vitro* rumen microbial, fermentation characteristics and unsaturated fatty acid. The research was conducted using rumen fluid obtained from fistulated Ongole grade cattle. This research used randomized complete block design with 6 treatments and 5 replications. The treatments were T0 = control (forage:concentrate = 70:30), T1 = T0 + *S. rarak* extract 1 mg/ml, T2 = T1 + sesame oil 10%, T3= T1 + sesame oil microencapsulation 10%, T4= T1 + canola oil 10%, T5=T1 + canola oil microencapsulation 10%. The difference in time for taking rumen fluid is used as a block/group. The result showed that the addition of *S. rarak* extract 1 mg/mL and its combination with canola oil microencapsulation significant decreased (P<0.01) protozoal population and increased (P<0.05) bacterial population especially *Anaerovibrio lipolytica*, NH_3-N concentration, dry matter and organic matter digestibility. The pH value, total and proportion of VFA, CH_4 production, saturated and unsaturated fatty acids were similar among treatments. It is concluded that the combination of *S.rarak* extract and oils microencapsulation could modify rumen fermentation but did not affect rumen fatty acid profile.

Keywords | Rumen fermentation, Rumen microbe, *Sapindus rarak extract,* Oil Microencapsulation, Fatty acid profiles

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INTRODUCTION

The main problem behind low productivity of beef cattle in tropical regions of the world is less availability of feed resources and based on high forage ration with low quality (native grass). The efficiency of feed utilization based on high forage ration is relatively low resulting in low beef cattle productivity and tends to produce high methane gas. In addition, high forage based ration is usually deficient in nitrogen (protein), thereby decreasing the synthesis of microbial proteins which are the primary source of protein in ruminants (Suharti et al., 2015). Meanwhile, the protein supply from microbial protein will decrease with the presence of protozoa in the rumen because of bacteria predation by protozoa (Gutierrez, 1958). Our previous study showed that defaunation of rumen protozoa by using *Sapindus rarak* extract increased the growth of rumen bacteria. The extract of *S. rarak* contains high saponin up to 84.5% and proven to suppress the growth of protozoa, as well as increase the proportion of *Ruminococcus albus* and *Prevotella ruminicola* (Suharti et al., 2011).

An appropriate strategy needs to be done to enhance beef cattle production which is fed high forage ration. Moreover, the increasing of beef cattle production strategy should be balanced by an improvement of meat quality especially unsaturated fatty acid content. Therefore, efforts should be made to reduce the content of saturated fatty acids in ruminant meat. Supplementation of oils as unsaturated fatty acid sources could improve the quality of meat which have

high content of unsaturated fatty acid (Chang and Nickerson, 2018). However, the used of oils need to be protected to avoid rumen biohydrogenation by rumen microbe that convert unsaturated fatty acid to saturated fatty acid. Maia et al. (2010) stated that biohydrogenation occurs to enable *B. fibrisolvens* to survive from the bacteriostatic effects of polyunsaturated fatty acid (PUFA). Microencapsulation of oils that contain high PUFA is one of strategy to protect unsaturated fatty acid of feed from rumen microbe biohydrogenation (Baroso et al., 2014).

Very little information is available about the study on the effect of *S.rarak* extract combined with oils microencapsulation on rumen microbe, fermentation and fatty acid profiles. Combination of *S.rarak* extract and oil microencapsulation hopefully could increase rumen fermentation activity and level of unsaturated fatty acid. This research aimed to analyze the combination effect of *S. rarak* extract and microencapsulation of canola/sesame oils on rumen fermentation *in vitro* including population of bacteria and protozoa, concentration of NH_3-N, production of VFA total and its proportion, dry matter and organic matter degradability, methane production, and rumen fatty acid profile.

MATERIALS AND METHODS

Preparation of *S. rarak* extract and Canola/Sesame Oils Microencapsulation

Extraction of *S. rarak* was done using maseration method according to Wina et al. (2006). The *S. rarak* fruits were obtained from Central Java. Preparation of Sesame/Canola oil microencapsulation according to the Carneiro et al. (2013) method. The sesame oil obtained from CV. MH Farm and Marine, Cibinong, Bogor, Indonesia and canola oil obtained from MOI foods Malaysia SDN BHD. Each of oils as a core material about 5.88%,, then it was mixed with coating materials and water with the proportion of 11.76% and 82.36% respectively. The coating consisted of arabic gum and maltodextrin with proportion 50%:50%. The mixture was stirred until homogeneous for 10 minutes at a constant speed of 10000 rpm and dried using a spray dryer with an inlet temperature of 180 ± 5 °C and outlet 80 ± 5 °C with a pressure of 5 atm to produce encapsulation with size 0.2 - 5000 µm.

In vitro Fermentation

In vitro fermentation was conducted according to Tilley and Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate according to the treatment, 40 mL McDougall buffer, and 10 mL rumen fluid were added and incubated at shaker water bath with temperature 39° C. The rumen fluid for this experiment was collected at 3h after morning feeding from 3 rumens fistulated Ongole crossbred beef cattle with Ethical Approval from Animal Care and Use Committee (AUAC) 01-2013b IPB, Bogor Agricultural University.

The experiment was designed as a 6 × 5 randomized complete block design with 6 treatments include:T0= Napier grass: concentrate=70:30 (control); T1 = control + *S.rarak* extract 1 mg/ml; T2=T1+Sesame oil 10%; T3=T1+microencapsulation sesame oil 10%, T4=T1+Canola oil 10%, and T5= T1+microencapsulation Canola oil 10%. The difference in time for taking rumen fluid is used as a block/group. The *S. rarak* extracts and oils were mixed with concentrate. Concentrate mixture with 15-17% CP and 69-74% TDN (Table 1). Variables observed were populations of bacteria and protozoa, concentration of NH_3, production of VFA total and its proportion, dry matter and organic matter degradability, methane production, protozoa population, bacterial population (total bacteria, *Ruminococcus albus, Butirivibrio fibrisolvens, Ruminococcus flavefaciens, Anaerovibrio lipolytica,* and *Fibrobacter succinogenes)* and fatty acid profile.

Sampling and Measurement

Samples from aliquot were taken after 4 h incubation for pH, VFA, NH_3-N, protozoa, total bacterial analysis and after 48 h incubation for dry matter and organic matter digestibility analysis. The numbers of protozoa and total bacteria in the rumen fluid was counted under a microscope according to Ogimoto and Imai (1981).

Ammonia (N-NH_3) concentration was determined by using the micro diffusion method (Conway 1962). Analysis of total VFA concentration and proportion of VFA by using gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, Capillary column type WCOT Fused Silica 25 m × 0.32 mm, oven temperature: conditioning at 60°C and running at 115°C and nitrogen as gas carrier). Methane production was calculated from molar proportion of VFA according to Moss et al. (2000) by using the formula: 0.45(C2) - 0.275(C3) + 0.4(C4) which is C2= acetate, C3= propionate and C4= butyrate.

The specific bacterial population (*Ruminococcus albus, Butirivibrio fibrisolvens, Ruminococcus flavefaciens, Anaerovibrio lipolytica,* and *Fibrobacter succinogenes)* was quantified by using real time PCR. A total of 200 ml rumen liquid at 4 h incubation were taken for DNA extraction by using *QIAmp Stool Mini Kit.* The primers of those specific bacteria were designed according to Denman and McSweeney (2006) for total bacteria and *F.succinogenes*; Tajima et al. (2001) for *R. flavefaciens* and *A. lipolytica*, Koike and Kobayashi (2001) for *R. albus,* and Fernando et al. (2010) for *B. fibrisolvens.* The nucleotide sequence of targeted primers used for real time PCR analysis is was shown in Table 2. PCR amplification for standard prepar-

Table 1: Concentrate formulation and nutrient composition of the treatment

Ingredient	Treatments					
	Control (C)	C+S.rarak extract (T1)	T1+SO	T1+MSO	T1+CO	T5+MCO
Soybean meal (%)	4	4	9	6	8	5
Coconut cake (%)	18	18	16	35	19	18
Cassava waste (%)	40	40	32	40	33	35
Pollard (%)	35	35	31	5	28	30
Molasses (%)	1	1	1	2	1	1
Urea (%)	2	2	1	2	1	1
Sesame Oil (SO) (%)	0	0	10	0	0	0
Microencapsulation- Sesame Oil (MSO) (%)	0	0	0	10	0	0
Canola oil (%)	0	0	0	0	10	0
Microencapsulation- Canola Oil (MCO) (%)	0	0	0	0	0	10
S. rarak extract (mg/mL)	0	1	1	1	1	1
Nutrient Composition (%)						
Crude Protein	14.06	14.06	14.06	14.06	14.06	14.05
Crude Fiber	2.76	2.76	4.73	2.99	5.13	2.84
Total Digestible Nutrient	70.00	70.00	69.00	69.00	69.00	67.00
Ca	0.54	0.54	0.52	0.52	0.53	0.52
P	0.26	0.26	0.29	0.24	0.31	0.29

Table 2: Nucleotida sequence of targeted primers used for *RT-PCR*

No	Spesies	Size (bp)	No acces of Genbank	Forward primer	Reverse primer
1	Total bacteria[a]	114	KX26061	5'CAA CGA GCG CAA CCC3'	5'CCA TTG TAG CAC GTG TGT AGC3'
2	*Fibrobacter succinogenes*[a]	121	KY463941	5'- GTT CGG AAT TAC TGG GCG TAA A -3'	5'- CGC CTG CCC CTG AAC TAT C -3'
3	*Ruminococcus albus*[c]	175	NR.074399	(5'-CCC TAA AAG CAG TCT TAG TTC G-3')	(5'-CCT CCT TGC GGT TAG AAC A-3')
4	*Ruminococcus flavefaciens*[b]	835	KP689131	5'-GGA CGA TAA TGA CGG TAC TT-3'	5'-GCA ATC Y*GA ACT GGG ACA AT-3'
5	*Butiryvibrio fibrisolvens*[d]	625	AM039822	5'- CGC ATG ATG CAG TGT GAA AAG CTC -3')	5'- CCT CCC GAC ACC TAT TAT TCA TCG -3')
6	*Anaerovibrio lipolytica*[b]	597	AB034191	5'-TGG GTG TTA GAA ATG GAT TC-3'	5'-CTC TCC TGC ACT CAA GAA TT-3'

[a]Denman dan McSweeney (2006); [b] Tajima et al. (2001). [c] Koike and Kobayashi (2001) ; [d]Fernando et al. (2010)
* IUPAC for nucleotide t or c

preparation was performed by using Mastercycler personal (Eppendorf 5332) with the following condition: 5 min at 94°C, 30 s at 94°C, 45 s at 55°C, 1 min at 72°C for 40 cycles and a final extension of 5 min at 72°C. The qPCR conditions for bacteria specific targets were based on qPCR machine (Rotor gene, Qiagen) protocol with the following conditions: initial denaturation at 95°C for 5 minutes, amplification at 95°C for 10 seconds and different annealing temperatures such as total bacteria at 55°C, *R. albus* and *B. fibrisolvens* at 51 °C, *A. lipolytica* at 57 °C, *F. succinogenes* and *R. flavefaciens* at 62 °C for 1 minute. There were 40 cycles of amplification. The population of different microbial groups was determined as relative to the total bacterial populations.

Rumen fatty acid profile measured at 8h incubation by using Gas Chromatography (GC type Claurus 580, polyetilen glycol column (30m × 0.25 mm × 0.25µm). Sample preparation was done using Soxhlet extraction according Weibull-Stoldt method (AOAC, 2005). A total of 2-3 g

Table 3: Fatty acid profile of sesame and canola oils microencapsulation (%)

Variables	Sesame Oil[1)]	Sesame Oil Microencapsulation	Canola Oil[2)]	Canola Oil Microencapsulation
SFA	14.85	43.67	4.07	17.36
UFA	85.15	56.36	95.93	82.64
Stearic Acid (C18:0)	5.43	4.53	1.36	2.73
Oleic Acid (C18:1)	38.81	44.07	32.73	55.53
Linoleic Acid (C18:2)	46.34	23.61	14.35	39.19

Note : [1)] Nzikou et al. (2009), [2)] Chang (2018), SFA =Saturated fatty Acid, UFA=Unsaturated Fatty Acid

Table 4: Rumen microbial population with the addition of *S. rarak* extract and its combination with pure Sesame/Canola Oil or microencapsulated

Variables	Treatments					
	Control (C)	C+S.rarak extract (T1)	T1+SO	T1+MSO	T1+CO	T5+MCO
Protozoa (log cell/ml)	4.52 ± 0.02^{A}	4.03 ± 0.04^{B}	3.89 ± 0.05^{E}	3.98 ± 0.03^{C}	3.75 ± 0.03^{F}	3.92 ± 0.03^{D}
Total Bacteria (log cell/ng)	16.50±0.35a	14.29 ± 0.62bc	15.13 ± 0.14b	12.74 ± 0.60d	13.06 ± 1.19d	13.85 ± 0.75cd
R. Albus (log cell/ng)	10.45 ± 0.59	10.32 ± 0.27	10.32 ± 0.36	10.74 ± 0.21	10.46 ± 0.17	10.34 ± 0.14
F. succinogenes (log cell/ng)	11.79 ± 0.62	11.48 ± 0.24	11.46 ± 0.33	11.64 ± 0.88	11.11 ± 0.81	11.13 ± 0.77
R.flavefaciens (Log cell/ng)	9.15 ± 0.78	9.30 ± 0.83	8.97 ± 0.61	9.07 ± 0.72	8.57 ± 0.35	9.18 ± 0.71
B. fibrisolvens (log cell/ng)	9.53 ± 0.30	9.55 ± 0.37	9.13 ± 0.22	9.12 ± 0.46	8.90 ± 0.04	9.36 ± 0.57
A.lipolytica (log cell/ng)	9.33±0.74^{ab}	9.50 ± 0.74^{ab}	8.94 ± 0.40^{bc}	9.27 ± 0.72^{abc}	8.67 ± 0.47^{c}	9.66 ± 0.43^{a}

Note : SO=Sesame oil, MSO=Microencapsulation Sesame Oil, CO=Canola Oil, Microencapsulation Canola Oil. The different superscripts with capital letters indicates very significant different (P<0.01) and small letters indicates significant different (P<0.05).

rumen liquid after incubated for 8h mixed with 10 ml H_2O and 15 ml HCl 25% and boiled for 15 minutes. The solution then filtered during heat and washed with hot water until it did not react with acid again. Samples that have been wrapped in filter paper dried at 100-105°C for 30 min until dry then extracted in soxhlet with hexane solvents ± 5-6 h at 80°C. Fat extract obtained was dried at 105°C for 1 h and cooled in the exicator until it reaches a fixed weight.

Analysis of crude protein, crude fiber, calcium and phospor of the ration was done according to to Association of Official Analytical Chemists (AOAC) method (2005).

Data Analysis

The data obtained were tested using analysis of variance and the differences among treatments means were examined by Duncan Multiple Range Test (Steel and Torrie, 1997). The statistical analysis was performed by using IBM SPSS v23.

RESULTS

Fatty Acid Profile of Plant Oil's Microencapsulation

Fat protection using the microencapsulation method changed the proportion of unsaturated fatty acids. The percentage of unsaturated fatty acids reduction in sesame oil was 33.81%, while in canola oil was 13.85% during the microencapsulation process. However, the proportion of oleic acid increased in the oils microencapsulation product compared to pure oils (Table 3).

Rumen Microbe Population

The addition of *Sapindus rarak* extract and its combination with canola/sesame oil microencapsulation or pure canola/sesame oil significantly reduced (P<0.01) protozoal population compared to the control treatment. Furthermore, population of total bacteria also decreased (P<0.05) with the addition of *S. rarak* extract combined with canola oil microencapsulation compared to the control treatment (Table 4).

Table 5: *In vitro* rumen fermentation characteristic with the addition of *S. rarak* extract and its combination with pure Sesame/Canola Oil or microencapsulated

Variables	Treatments					
	Control	C+S.rarak extract (T1)	T1+SO	T1+MSO	T1+CO	T5+MCO
pH	6.60±0.45^{c}	6.56±0.43^{abc}	6.56±0.43^{abc}	6.52±0.46^{a}	6.58±0.44^{bc}	6.54±0.47^{ab}
N-NH_3 (mM)	4.88 ± 0.46^{A}	4.23 ± 0.46^{B}	3.21 ± 0.44^{C}	5.26 ± 0.45^{A}	3.07 ± 0.46^{C}	5.00 ± 0.44^{A}
VFA total (mM)	52.61 ± 4.43	57.77 ± 9.83	54.11 ± 12.51	54.67 ± 4.34	61.81 ± 15.99	68.17± 17.52
Proportional VFA (mM/100mM)						
Acetate (C2)	60.12 ± 5.20	61.32 ± 7.44	64.65 ± 2.38	59.11 ± 5.31	60.94 ± 5.83	59.71 ± 8.12
Propionate (C3)	25.43 ± 2.98	26.22 ± 5.27	21.52 ± 2.38	26.90 ± 2.85	25.60 ± 4.07	27.26 ± 5.43
Butyrate ($_n$C4)	11.04 ± 2.37	9.51 ± 2.23	10.65 ± 4.27	10.81 ± 2.31	9.79 ± 2.19	9.95 ± 2.64
Iso Valerate ($_i$C5)	1.84 ± 0.29	1.59 ± 0.41	1.75 ± 0.64	1.57 ± 0.18	1.84 ± 0.21	1.61 ± 0.41
Valerate ($_n$C5)	1.57 ± 0.32	1.36 ± 0.30	1.43 ± 0.46	1.61 ± 0.35	1.83 ± 0.22	1.48 ± 0.34
$C_2 : C_3$	2.40 ± 0.51	2.45 ± 0.86	3.02 ± 0.24	2.23 ± 0.41	2.45 ± 0.63	2.30 ± 0.79
CH_4 (mM) estimation	12.82 ± 0.46	13.72 ± 0.26	12.80 ± 0.27	14.93 ± 4.00	14.71 ± 2.12	15.47 ± 2.24
Dry matter digestibility (%)	55.07± 2.90^{C}	59.04 ± 3.26^{B}	53.56 ± 3.87^{D}	59.18 ± 2.97^{B}	56.03 ± 3.27^{C}	67.32 ± 2.87^{A}
Organic matter digestibility (%)	54.62± 2.61^{C}	58.38 ± 3.15^{B}	52.84 ± 3.90^{D}	58.70 ± 2.99^{B}	55.42 ± 3.34^{C}	66.40 ± 2.46^{A}

Note : SO=Sesame oil, MSO=Microencapsulation Sesame Oil, CO=Canola Oil, Microencapsulation Canola Oil. The different superscripts with capital letters indicates very significant different (P<0.01) and small letters indicates significant different (P<0.05).

Table 6: Rumen fatty acid profiles with the addition of *S. rarak* extract and its combination with pure Sesame/Canola Oil or microencapsulated at 8h incubation

Variables	Treatments					
	Control	C+S.rarak extract (T1)	T1+SO	T1+MSO	T1+CO	T5+MCO
Concentration (ppm)						
SFA	27.23 ±15.56	29.97±16.65	70.77±22.73	40.58±20.34	33.57±15.52	46.30±1.84
UFA	9.62±8.99	7.32±3.08	21.83±5.93	6.33±2.27	13.55±7.55	11.73±4.63
Stearic acid	9.38±9.39	11.93±8.74	22.70±5.38	17.35±10.52	12.60±8.78	21.68±4.70
Oleic Acid	5.08±4.29	5.92±3.96	13.75±5.25	4.18±2.13	9.88±5.54	7.80±3.11
Linoleic Acid	2.80±2.05	2.43±0.11	6.98±5.69	1.15±0.65	3.18±1.18	1.68±0.18
Proportion (%)						
SFA	76.68±6.63	78.72±5.07	76.23±2.96	85.06±5.94	72.17±2.97	75.96±7.02
UFA	23.32±6.63	21.28±5.07	23.77±2.96	14.94±5.94	27.83±2.97	20.04±7.02
Stearic acid	22.48±11.31	29.32±12.11	27.22±12.69	35.01±13.78	24.92 ±10.34	37.71±7.01
Oleic Acid	13.45±4.46	15.76±4.71	15.18±2.34	10.63±7.53	21.24±3.73	13.53±5.00
Linoleic Acid	15.11±5.20	10.24±0.39	16.66±12.43	5.01 ±1.40	13.60±2.97	5.84±0.43

Note : SFA = Saturated fatty acid, UFA=Unsaturated fatty acid

Rumen Fermentation Characteristic

The used of *S. rarak* extract and its combination with sesame/canola oil with or without microencapsulation significantly decreased (P<0.05) rumen pH. In addition, the used of sesame/canola oil microencapsulation tended to lower pH value compared to sesame/canola oil without protection (Table 5). Concentration of NH_3 significantly decreased (P<0.05) with the addition of *S. rarak* extract and its combination with sesame/canola oil without protection. In contrast, the used of *S.rarak* extract and Sesame/Canola oil microencapsulation significantly increased (P<0.05) NH_3 concentration. Total VFA production, proportional VFA and methane estimation were similar among treatments. Dry matter and organic matter digestibility very significantly increased (P<0.01) with the addition of *S. rarak* extract and its combination with sesame/canola oil

without protection or microencapsulation. In addition, the used of canola oil microencapsulation resulted the highest (P<0.01) dry matter and organic matter digestibility (Table 5).

Rumen Fatty Acid Profile

The used of *S. rarak extract* and its combination with sesame/canola oils microencapsulation did not increase concentratiom of rumen unsaturated fatty acids at 8h incubation compared to the control treatment. The proportion of stearic acid, linoleic acid and linolenic acid were similar among treatments (Table 6).

DISCUSSION

Fatty Acid Profile of Plant Oil's Microencapsulation

This slight decreasing of unsaturated fatty acid after microencapsulation process may be due to the accumulation of the linolenic oxidation process (C18:3) during the spray drying process. However, the combination of arabic gum and maltodextrin maintain the fatty acid composition (Gabriela et al., 2018). Gawad et al. (2015) reported that there was no significant difference between linseed oil fatty acids content before and after encapsulation process. The encapsulation process didn't have a significant effect on PUFA fraction and other linseed oil fatty acids.

Rumen Microbe Population

The reduction of protozoal population due to saponin content in the *S. rarak* extract reached to 84.5% (Suharti et al., 2011). Saponin could inhibit the growth of protozoa because it will bind the steroid membrane of protozoa cell wall and caused cell lysis. The addition of sesame/canola oil without protection also decreased protozoal population because those oil contain unsaturated fatty acid which toxic for protozoa (Newbold and Chamberlain, 1988; Machmuller and Kreuzer, 1999). In addition, microencapsulation of sesame/canola oil had lower defaunation activity compared to those oil without protection because the coating material in the microencapsulation product blocked the interaction between unsaturated fatty acid with protozoa.

The decreasing of total bacterial population with the addition of *S. rarak* extract and its combination with sesame oil and canola oil may be due to the toxic effect of unsaturated fatty acid in those oils especially linoleic and linolenic acids. This result in contrast with Suharti et al. (2011) which stated that the used of *S. rarak* decreased protozoa population and increased bacterial growth. However, the addition of *S. rarak* extract combined with microencapsulation of canola oil (MSO) slightly increased total bacteria population might be caused by microencapsulation of canola oil which could protect the unsaturated fatty acid content in the oil so that it was not toxic for rumen bacteria anymore. Maia et al. (2010) reported that linoleic and linolenic acids had toxic effect and could alter the growth of rumen bacteria.

Microencapsulation of sesame/canola oils by using maltodextrin and arabic gum as coating material might be degraded by rumen microbe. This caused sesame/canola oils had toxic effect to the rumen bacteria. This result is in contrast with the study reported by Kanakdande et al. (2007) who suggested that Arabic gum as a coating material could protect volatile compound from oxidation and evaporation. In addition, arabic gum has capability as emulsifier caused by protein content. However, the combination of arab gum with maltodextrin did not seem to have a strong enough bond to resist from rumen microbe degradation as maltodextrin has low emulsifier activity. When the bond of arabic gum and maltodextrin are broken, the unsaturated fatty acid will be released to the rumen and caused toxicity to the rumen bacteria.

Number of *B. fibrisolvens* also similar among treatments indicating that the growth of those bacteria was not altered by the toxic effect of unsaturated fatty acid compound. In contrast, number of *A. lipolytica* decreased in the presence of sesame/canola oils but increased when added by those oils in the form microencapsulation. Maia et al. (2010) suggested that *B. fibrisolvens* and *A. lipolytica* had important role in the rumen lipid degradation and biohydrogenation. Tajima et al. (2001) reported that there were no change DNA concentration of *A. lipolytica* with the addition of lipid in the ration.

Rumen Fermentation Characteristic

This increasing of rumen fermentation performances indicating that the used of *S. rarak* extract and its combination with sesame/canola oil microencapsulation could enhance rumen fermentation activity by rumen microbes so that the pH value decreased. The increasing of rumen fermentation activity will reduce rumen pH. The reduction of NH_3 with the addition of *S. rarak* saponin also reported by Patra and Yu (2015) and its combination with sesame/canola oil without protection (Atikah et al., 2018) indicating that the decreasing of feed protein degradation. This might be due to toxic effect of unsaturated fatty acid in the oil to rumen microbes and caused the reduction of feed protein degradation by rumen microbes (bacteria and protozoa).

The addition of *S. rarak* extract significantly increased dry matter and organic matter digestibility might be due to the reduction of protozoa since protozoa could inhibit the growth of some bacteria and decrease the nutrient digestibility in the rumen (Patra et al., 2012; Suharti et al., 2005). The contrast result when we combined *S. rarak* extract with

canola/sesame oil without protection decreased dry matter and organic matter digestibility caused by the reduction of protozoa and bacterial population. Moreover, the addition of *S. rarak* extract combined with canola/sesame oil microencapsulation improved dry matter and organic matter digestibility because microencapsulation of canola/sesame oil could protect those oil in the rumen so can reduce the toxic effect of unsaturated fatty acid of those oil to rumen microbes. Our previous research suggest that supplementation of canola and flaxseed oil protected by calcium soap at level 6% did not affect pH level, dry matter digestibility, rumen protozoa and bacteria total, but did increase organic matter digestibility and N-NH_3 concentration (Suharti et al., 2015).

The similar total and partial VFA production as wel as methane production among treatments indicating that the used of *S. rarak extract* and its combination with sesame/canola oils microencapsulation did not alter fermentation by rumen microbes in the rumen. The addition of canola/sesame oils which contain unsaturated fatty acid may be still low and did not toxic for rumen microbe.

Rumen Fatty Acids Profile

The similar fatty acid profiles among treatments indicating that microencapsulation by using maltodextrin and arabic gum less effective to coat the unsaturated fatty acid content in the oil. The bond between the coating material used in microencapsulation is less potent to protect the oil from microbial degradation of rumen. Our previous result suggested that sesame oil is the most resistant to the rumen microbe biohydrogenation process compared to flaxseed or canola oils (Hidayah, 2014). Although the fat protection using microencapsulation had not avoided the rumen microbe biohydrogenation effectively, but microencapsulation could increased the digestibility of dry and organic matter. It is indicating that the oil microencapsulation can eliminate the negative effects of oil on rumen microbes. As we know that the used of pure oils can trapped rumen microbes and inhibit the binding of rumen microbes to the feed so that it will lower feed degradation. Khattab et al. (2015) reported that supplementation of vegetable oils (olive oil and sunflower oil) and monensin increased vaccenic acid and conjugated linoleic acids in ruminal cultures without affecting the rumen dry matter and organic matter digestibilty.

CONCLUSION

The addition of *S. rarak* extract 1 mg/mL combined with canola oil microencapsulation 10% in the concentrate ration decreased protozoa population, increased *Anaerovibrio lipolytica* growth, NH_3 concentration, dry matter and organic matter digestibility but did not affect VFA total and partial and rumen fatty acid profile.

CONFLICT OF INTEREST

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper. This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

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AUTHORS CONTRIBUTION

All authors were involved in the design and carried out the research, preparing and proofreading the manuscript.

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