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In Vitro Rumen Fermentation of Ration Supplemented with Protected Vegetable Oils

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ABSTRACT

This experiment was designed to evaluate the effects of protected vegetable oils supplementation on *in vitro* fermentation characteristics, rumen microbial population, and methane production in cattle. The treatments were arranged in a complete randomized block design involving 2 factors i.e. oil type (sesame, canola, and flaxseed) and protection methods (non protected, calcium soap, and microencapsulation). Variables observed were rumen pH, N-NH_3, total and molar proportion of VFA, dry matter and organic matter digestibility, population of protozoa and total bacteria, methane production, and hydrogen balance. Data were tested using Analysis of Variance (ANOVA) and the differences among treatments means were examined by Duncan Multiple Range Test. The oil type did not affect all variables measured. The protection method using microencapsulation significantly increased N-NH_3 concentration. There was an interaction between oil type and protection method on total VFA concentration, molar proportion of VFA, and methane production. The supplementation of calcium soap-flaxseed oil significantly increased total VFA production, while the supplementation of microencapsulated flaxseed oil had the highest propionate concentration and H_2 utilization, the lowest A:P ratio, and methane production. It is concluded that microencapsulated flaxseed oil was the best treatment to optimize rumen fermentation.

Key words: fermentation characteristics, methane, protection methods, vegetable oil, rumen microbe

ABSTRAK

Penelitian ini dirancang untuk menganalisis pengaruh penambahan minyak nabati terproteksi terhadap karakteristik fermentasi, populasi mikroba rumen, dan produksi metan ternak ruminansia secara *in vitro*. Rancangan percobaan yang digunakan dalam penelitian ini adalah rancangan acak kelompok pola faktorial dengan 2 faktor dan 3 ulangan: jenis minyak nabati (wijen, kanola, dan flaxseed) dan jenis metode proteksi (tanpa proteksi, sabun kalsium, dan mikroenkapsulasi). Variabel yang diamati meliputi nilai pH rumen, konsentrasi NH_3, produksi VFA total dan parsial, kecernaan bahan kering dan bahan organik, populasi protozoa dan bakteri total, dan produksi metan. Data dianalisa menggunakan analisis ragam (ANOVA) dan perbedaan nyata antar perlakuan dianalisa menggunakan uji Duncan. Penggunaan jenis minyak nabati yang berbeda tidak berpengaruh terhadap karakteristik fermentasi, populasi mikroba rumen, produksi metan, dan keseimbangan hidrogen. Metode proteksi mikroenkapsulasi nyata meningkatkan konsentrasi NH_3 rumen. Terdapat interaksi antara jenis minyak nabati dan metode proteksi pada produksi VFA total, proporsi VFA parsial, dan produksi metan. Suplementasi minyak flaxseed yang diproteksi dengan metode sabun kalsium sangat nyata meningkatkan VFA total. Suplementasi minyak flaxseed yang diproteksi dengan metode mikroenkapsulasi menghasilkan proporsi propionat dan penggunaan H_2 tertinggi serta rasio A:P dan produksi metan terendah. Kesimpulan dari penelitian ini yaitu bahwa minyak flaxseed dan mikroenkapsulasi merupakan jenis minyak dan metode proteksi terbaik dalam mengoptimalkan fermentasi rumen.

Kata kunci: karakteristik fermentasi, metan, metode proteksi, minyak nabati, mikroba rumen

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INTRODUCTION

National meat production from beef cattle could not fulfill the demand and there is still deficit about 400 000 ton in 2014. Program of increasing beef cattle population and productivity by 23% in 2014 is the Government target to suppress import of beef cattle. The target should be followed with improving of beef quality. Beef is red meat containing high saturated fatty acids (SFA) associated with the risk of cardiovascular and cancer diseases when consumed in high level. High SFA content in beef is normally occurs due to biohydrogenation process in the rumen which transforms the PUFA to SFA. This rumen biohydrogenation process is a detoxification mechanism to avoid bacteriostatic effects of unsaturated fatty acids which could disrupt membrane integrity and decrease growth of microbes. *Butyrivibrio fibrisolvens* is a major microbe which plays role in this biohydrogenation process (Maia *et al.,* 2010).

Previous studies showed that supplementation of vegetable oil (high PUFA content) could decrease saturated fatty acid content and increase unsaturated fatty acid content in beef. Some potential vegetable oils to use are sesame, canola, and flaxseed oil (Manso *et al.,* 2005; Aharoni *et al.,* 2005; Beauchemin *et al*., 2007). Supplementation of 10% flaxseed oil in cattle ration significantly increased PUFA and omega 3 proportion on intramuscular fat (Kim *et al*., 2009). Duckett & Gillis (2010) reported that supplementation of 4% canola oil in ration significantly increased (P<0.001) oleic acid, linoleic acid, and decreased palmitic acid on biohydrogenation process than corn oil. Supplementation of 4% flaxseed oil in the form of non protected, lipase-treated, or soapstock in ration increased linoleic acid concentration in beef tissue (Quinn *et al.* 2008). However, this oil needs to be protected prior to supplementation to avoid biohydrogenation process by rumen microbes, to decrease rumen microbial growth and activity, and to improve feed digestibility. Some protection methods that can be applied are calcium soap (Block *et al.,* 2005; Wynn *et al.,* 2006; Huang *et al.,* 2009) and microencapsulation **(**Kanakdande *et al.,* 2007; Calvo *et al.,* 2010; Agnihotri *et al.,* 2012**).** However, optimization for rumen fermentation, oil type and protection method should be determined. The objective of this research was to evaluate the effects of supplementation of three kinds of vegetable oils (sesame, canola, and flaxseed) protected using calcium soap and microencapsulation on *in vitro* fermentation characteristics, rumen microbial population, methane production, and hydrogen balance.

MATERIALS AND METHODS

Preparation of Calcium Soap and Microencapsulation

The sesame oil, canola oil, and flaxseed oil were produced by MH Farm Bogor Company, Golden Bridge, and Green Tosca, respectively. Calcium soap from these three kinds of vegetable oils was made according to the method by Kumar *et al.* (2006). The main ingredients for calcium soap were NaOH (to hydrolize the fatty acids of vegetable oil) and $CaCl_2$. and $CaCl_2$. Sodium hydroxide solution (in line with saponification value) was added into hot vegetable oil, heated and stirred on a hotplate until the fat was completely dissolved. Calcium chloride (2.35 g) were dissolved in 4.7 mL of water and then added slowly into the water soluble soap while being stirred to aid the precipitation of calcium soap. Calcium soap was then dried overnight in a oven at 60 ^oC.

The microencapsulation was done according to the method by Calvo *et al.* (2010). Sodium caseinate (protein source) from Sigma Aldrich (Singapore) and lactose (carbohydrate source) of commercial grade were used for microencapsulation wall. The process included making emulsion, mixing the wall materials, and mixing it with oil until homogenous. The ratio of oil and wall materials used was 1% (oil): 2% (1% sodium caseinate and 1% lactose). The emulsion prepared was spray dried using a laboratory scale Buchi spray drier (Mini Spray drier B-190) with 100 mesh or 0.149 mm of nozzle diameter. The pressure of compressed air for the flow of the spray was adjusted to 5 bars. The inlet and outlet air temperatures were maintained at 175±5 ^oC and 55±5 ^oC, respectively.

In Vitro Fermentation

In vitro fermentation was conducted according to the method of Tilley and Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate, 40 mL McDougall buffer, and 10 mL rumen fluid were added at conducted at 39 ^oC. The substrate contained 60% king grass and 40% concentrate mixture (cassava by product, wheat pollard, soybean meal, coconut cake meal, molasses, $CaCO_3$, premix, urea, and 4% vegetable oil either non-protected, calcium soap, or microencapsulated) with 15%-17% CP and 69%-74% TDN (Table 1). The rumen fluid for this experiment was collected after 3 h morning feeding from the 3 rumens fistulated Ongole crossbred beef cattle with Ethical Approval from Animal Care and Use Committee (AUAC) 01-2013b IPB. Samples from aliquol were taken after 4 h incubation for pH, VFA, NH_3, protozoa, total bacterial analysis and after 48 h incubation for dry matter and organic matter digestibility analysis.

Sampling and Measurement

The rumen's pH was measured with pH meter. Ammonia (N-NH3) concentration was measured by microdiffusion conway method. Total VFA concentration and molar proportion of VFA were analyzed using gas chromatography (GC 8A, Shimadzu Crop., Kyoto, Japan, Capillary column type containing 10% SP-1200, 1% H_3PO_4 on 80/100 Cromosorb WAW and nitrogen as gas carrier). Prior to analysis, the pH of rumen liquid from *in vitro* incubation was adjusted to 3-4 with H_2SO_4. The dry matter digestibility (DMD) and organic matter digestibility (OMD) were measured using Tilley & Terry (1963) method. Protozoa population was determined using *Fuch Rosenthal Counting Chamber* (4 x 4 x 0.2 mm) under a microscope (40×). The 0.5 mL liquid sample from 4 h incubation tubes were mixed with 2 mL methyl green formaldehyde saline solution. Population of total bacteria were quantified by Ogimoto & Imai (1981) method used BHI media and roller tube method. Methane pro-

Table 1. Chemical composition of experimental subtrate (dry metter basis) with 60% king grass forage and 40% concentrate mixture

Nutrient (%)	R1	R2	R3	R4	R5	R6	R7	R8	R9
Ash	7.67	7.26	6.78	7.51	7.42	7.54	8.08	7.24	9.09
EE	5.43	5.60	5.41	4.92	4.59	5.40	4.43	3.87	3.56
CP	15.66	16.10	16.52	16.69	16.05	15.15	16.67	17.93	17.58
CF	22.89	23.86	24.17	23.33	24.02	23.04	22.74	23.65	23.46
BETN	48.35	47.18	47.12	47.55	47.93	48.88	48.08	47.31	46.31
TDN	74.05	72.60	72.70	73.04	71.08	73.78	72.66	71.34	69.12

Note: 1) Estimation of TDN by Hartadi (1980) formula: TDN= 92.464 - (3.338 x CF) - (6.945 x EE) - (0.762 x Beta-N) + (1.115 x CP) + (0.031x CF^2) - (0.133 x EE^2) + (0.036 x CF x Beta-N) + (0.207 x EE x Beta-N) + (0.1 x EE x CP) - (0.022 x EE x CP); 2) R1= 4% non protected sesame oil; R2= 4% non protected canola oil; R3= 4% non protected flaxseed oil; R4= 4% calcium soap-sesame; R5= 4% calcium soap-canola; R6= 4% calcium soap-flaxseed; R7= 4% microencapsulation-sesame, R8= 4% microencapsulation-canola; R9: 4% microencapsulation-flaxseed.

duction was estimated from molar proportions of VFA according to Moss *et al.* (2000) (CH_4 = 0.45 C_2 – 0.275 C_3 + 0.40 C_4), meanwhile hydrogen balance was estimated from molar proportion of VFA according to Mitsumori *et al.* (2012) [2HP (Hydrogen production)= 2 x C_2 + C_3 + 4 x C_4 + 2 x Ci_5 + 2 x C_5] and [2HUS (Hydrogen utilization)= 2 x C_3 + 2 x C_4 + C_5].

Statistical Analysis

The experiment was conducted in a factorial randomized block design with 2 factors and 3 replicates. The first factor was kinds of vegetable oil (sesame, canola, and flaxseed) and the second factor was protection methods (non protected, calcium soap, and microencapsulation). Data were tested using Analysis of Variance (ANOVA) and the differences among treatments' means were examined by Duncan Multiple Range Test (Steel & Torrie, 1995).

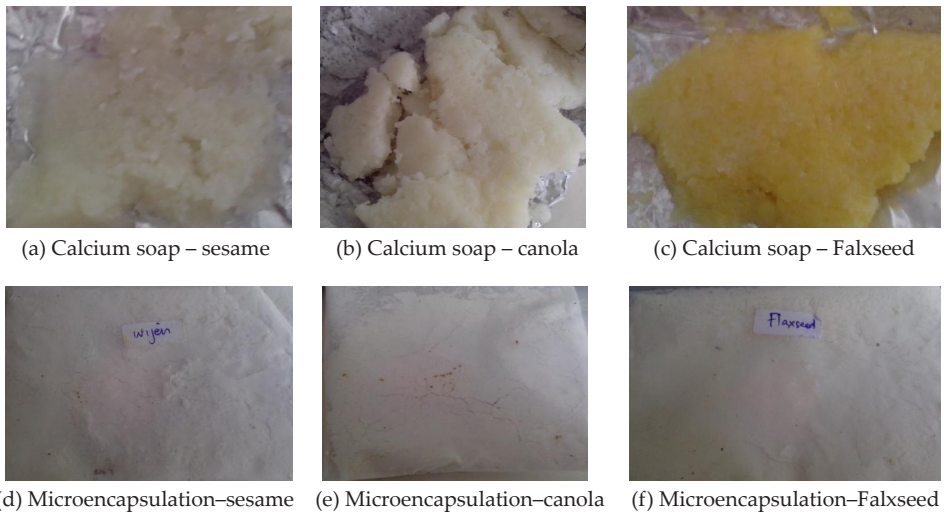
RESULTS AND DISCUSSION

Characteristics of Calcium Soap and Microencapsulation Products

The calcium soap products were a little mushy so a carrier was needed to be able to use it, while the microencapsulation product was in the form of powder and was very small in size (Figure 1). The yield of calcium soap product from sesame, canola, and flexseed oils were 95.60%, 96.81%, and 97.30%, respectively, while microencapsulation of sesame, canola, and flexseed oils were 64.17%, 53.74%, and 51.64%, respectively. Similar finding was reported by Calvo *et al.* (2010) in which microencapsulation yield was reported at 49.49% with the ratio of the coating material from combination of carbohydrates (lactose) and protein (sodium caseinate) of 1:1 and 1:2 for core material and coating material. Microencapsulation yield was lower than calcium soap yield because proportion of oil and coating used in microencapsulation was 1:2 while calcium soap was 9:1.

Fermentation Characteristics and Population of Rumen Microbes

There were no interaction between type of vegetable oils and the protection method on rumen pH, ammonia (NH_3) concentration, dry matter digestibility (DMD) and organic matter digestibility (OMD), protozoa and total bacterial population. Kinds of vegetable oil did not affect fermentation characteristic and rumen microbe population. Supplementation of non protected vegetable oil significantly decreased (P<0.05) rumen pH. Supplementation of microencapsulated vegetable oil



(a) Calcium soap – sesame (b) Calcium soap – canola (c) Calcium soap – Falxseed

(d) Microencapsulation–sesame (e) Microencapsulation–canola (f) Microencapsulation–Falxseed

Figure 1. Characteristic of calcium soap and microencapsulation product from sesame oil, canola oil, and flaxseed oil.

significantly increased (P<0.05) N-NH_3 concentration. Supplementation of vegetable oil without and with protection using calcium soap or microencapsulation methods did not affect dry matter digestibility (DMD) and organic matter digestibility (OMD), protozoa and total bacterial population (Table 2).

Decrease in rumen pH value after supplementation of non-protected vegetable oil was presumably due to less rumen microbial activity, especially protozoa. Jenkins (1993) stated that supplementation of fat on feed can disturb membrane and cellular function, activity and expression of microbial hydrolytic enzymes. Decrease in protozoa activity will reduce its ability to stabilize rumen pH. Protozoa has ability to stabilize rumen pH value and decrease redox potential of rumen digestibility. Similar finding was reported by Bhatt *et al.* (2011) in which supplementation of coconut oil at 7.5% on feed of Malpura sheep *in vivo* tend to decrease the rumen pH value (P= 0.108) linearly compared to supplementation of coconut oil at 2.5% and 5% (6.31 to 6.62). Different result was reported by Jalc *et al.* (2007) in which supplementation of PUFA (oleic, linoleic, and α-linolenic) at 3,5% on diet containing 80% *lucerne* and 20% *barley* did not influence rumen pH value (6.73–6.93). The differences in these responses might be due to differences of the level and profile concentration of fatty acid sources used in these researches. Rumen pH value in this research was 6.00-6.33 which was still considered normal. Dehority (2005) reported that normally rumen pH value was 5.4-7.8.

Increasing ammonia (N-NH_3) concentration in response to supplementation of microencapsulated vegetable oil was caused by sodium caseinate as a coating material that increased crude protein content of microencapsulation product (27.64%-30.22%). This microencapsulation product would increase protein content of the ration (19.49%-22.64%), so that linearly increasing ammonia production. McDonald *et al*. (2002) stated that the amount of protein in ration is one of the factors that influence ammonia production. Concentration of ammonia with addition of non protected oil lower than microencapsulation protection. This was presumble because addition at 4% non protected oil has started disturbing rumen microbial activity, especially proteolytic bacteria and protozoa. Hristov *et al.* (2004) reported that concentration of ammonia is highly correlated with the total number of protozoa and bacterial activity in the rumen. The addition of fat especially MCFA (Medium Chain Fatty Acid) decreased the growth and activity of the protozoa, proteolysis and ammonia concentrations *in vitro* and inhibit the activity of polysaccharide degradation.

Supplementation of non protected vegetable oil at 4% did not disturb feed digestibility (DMD and OMD) and rumen microbial population (protozoa and bacteria), but it started to show a decrease in the activity. This might be due to the low level of vegetable oil added on concentrate. Purushothaman *et al.* (2008) reported that addition at 6% calcium salt of palm oil in the concentrate mixture of lactating crossbred cows did not decreased dry maetter and organic metter digestibility. Supplementation of red palm oil in the form

Table 2. Fermentation characteristic and rumen microbe population with different types of vegetable oil and protections method

Variables	Types of vegetable oil	Not protected	Calcium soap	Microencapsulation	Mean types of vegetable oil
Rumen pH	Sesame	5.89 ± 0.16	6.25 ± 0.27	6.27 ± 0.25	6.14 ± 0.22
	Canola	6.03 ± 0.09	6.24 ± 0.28	6.33 ± 0.28	6.20 ± 0.20
	Flaxseed	6.09 ± 0.04	6.25 ± 0.26	6.38 ± 0.27	6.24 ± 0.19
	Mean of Protections Method	6.00 ± 0.10[b]	6.25 ± 0.27[a]	6.33 ± 0.26[a]	
N-NH_3 (mM)	Sesame	8.26 ± 0.64	8.28 ± 0.55	9.90 ± 1.40	8.81 ± 1.15
	Canola	7.23 ± 1.53	7.87 ± 1.84	9.12 ± 1.02	8.07 ± 1.55
	Flaxseed	7.96 ± 0.58	9.06 ± 0.86	10.08 ± 1.07	9.03 ± 1.18
	Mean of Protections Method	7.81 ± 0.99[b]	8.40 ± 1.17[b]	9.70 ± 1.11[a]	
DMD (%)	Sesame	62.30 ± 5.10	64.10 ± 1.57	65.68 ± 3.75	64.03 ± 3.47
	Canola	66.31 ± 1.38	64.64 ± 5.22	63.93 ± 4.85	64.96 ± 3.82
	Flaxseed	63.67 ± 0.20	66.01 ± 1.76	64.22 ± 4.50	64.64 ± 2.15
	Mean of Protections Method	64.09 ± 2.22	64.92 ± 2.85	64.61 ± 4.36	
OMD (%)	Sesame	60.50 ± 5.41	61.43 ± 3.62	62.13 ± 0.34	61.35 ± 3.33
	Canola	65.49 ± 1.80	65.40 ± 1.51	62.85 ± 4.34	64.58 ± 2.79
	Flaxseed	61.68 ± 1.51	64.66 ± 2.14	63.23 ± 4.71	63.19 ± 2.99
	Mean of Protections Method	62.56 ± 3.71	63.83 ± 2.89	62.74 ± 3.24	
Protozoa (Log Cell/mL)	Sesame	3.91 ± 0.45	4.03 ± 0.03	3.98 ± 0.06	3.97 ± 0.23
	Canola	4.00 ± 0.17	4.20 ± 0.05	4.28 ± 0.05	4.16 ± 0.16
	Flaxseed	4.10 ± 0.30	4.40 ± 0.29	4.13 ± 0.54	4.21 ± 0.37
	Mean of Protections Method	4.00 ± 0.30	4.21 ± 0.22	13.13 ± 0.30	
Bakteri (Log Cell/mL)	Sesame	7.28 ± 1.93	6.66 ± 1.47	7.25 ± 2.45	7.06 ± 1.75
	Canola	6.87 ± 0.68	7.49 ± 1.26	6.92 ± 0.82	7.09 ± 0.88
	Flaxseed	7.29 ± 0.66	7.79 ± 0.54	6.69 ± 0.98	7.26 ± 0.80
	Mean of Protections Method	7.14 ± 1.10	7.31 ± 1.12	6.95 ± 1.40	

Note : Means in the same row with different superscript differ significantly (P<0.05).

calcium soap at 15% on ration contained 59.5 parts sorghum straw and 40.5 parts concentrate mixture did not disturb dry metter and organic metter digestibility on Decani sheep (Ramana *et al.,* 2003). Bhatt *et al*. (2011) reported that protozoa population decreased linearly (P= 0.006) alongside with the increase in coconut oil added (0% : 99.7x10^4 cells/ml), (2.5% : 74.6x10^4 cells/ml), (5% : 57.7x10^4 cells/mL), and (7.5% : 8.6x10^4 cells/ml). In this study, supplementation of non protected vegetable oil at 4% did not significantly decrease protozoa population, but the population was still lower compared to that of vegetable oil protected with calcium soaps or microencapsulation. Sitoresmi *et al.* (2009) reported that supplementation of oil at 5.0% gave significant effect in decreasing protozoa population. Different response was reported by Adawiyah (2007) in which supplementation of non-protected fish oil at 1.5% was highly significant in decreasing (P<0.01) total bacterial population, but the population did not decrease in response to 3% supplementation of fish oil protected with calcium soaps (1.71 and 3:53 x 109/mL). The supplementation of non protected fish oil at low level significantly decreased total bacterial population. Fish oil contains EPA and DHA which are the most toxic component in disturbing the growth of rumen bacteria. Maia *et al.* (2007) stated that EPA and DHA were not metabolized by bacteria so that EPA and DHA were more toxic than linoleic (LA) and linolenic acid (LNA). The addition sesame oil decreased the lowest protozoa population than others. This was presumably the highest lauric acid content in sesame oil can reduced protozoa. Hristov *et al.* (2004) reported that lauric acid was the most toxic of MCFA to the protozoa. Lauric acid increased the sensitivity of microbial cell wall structure so can inhibit of the activity ciliate protozoa and gram-positive archaea (Machmuller, 2006).

Total VFA Concentrations, Molar Proportion of VFA, Methane Production, and Hydrogen Balance

There was interaction (P<0.01) between the kinds of vegetable oils and protection methods on total VFA concentration, molar proportion of VFA, methane production, and H_2 utilization (Table 3, 4, and 5). The supplementation of calcium soap flaxseed oil significantly increased total VFA production, on the other hand the supplementation of microencapsulated flaxseed oil produced the highest propionate concentration and H_2 utilization, the lowest A:P ratio and methane production.

The high total VFA production with supplementation of calcium soaps indicated that flaxseed oil protected with calcium soap method was able to contribute the highest energy source for ruminants. This was presumably because of flaxseed oil is high linolenic acid content (C18 : 3) compared to other oils, so that need high amount of calcium to be bound. High availability of calcium might be stimulate of the growth rumen bacterial population and their activities that will lead to increase feed fermentation. Ruckebusch & Thivend (1980) stated that calcium plays a role in the synthesis and stability of the microbial cell wall structure and able to activate a wide range of microbial enzymes such as α-amylase and is needed by the rumen microbes to digest cellulose. Additionally, high TDN in the ration with calcium soap flaxseed oil supplementation was potential to increase the availability of nutrients for rumen bacterial degradation process. Bhatt *et al*. (2013) reported that the addition of 4 % rice bran oil in the form of calcium soaps *in vivo* can increase (P<0.05) total VFA production, body weight gain, body weight, dry matter intake, and lower feed conversion ratio (FCR) compared to the addition of oil in non protected or control (without the addition of oil) .

Supplementation microencapsulated flaxseed oil resulted in the lowest proportion of acetate and the ratio of A : P, and the highest proportion of propionate. This was presumably because microencapsulated flaxseed oil supplementation can stimulate the growth of bacteria propionate producers in the rumen system so that the ruminal propionate production increased. High production of propionate was correlated with low methane production and high used of H_2. This was presumably because the propionate formation pathway was a ruminal metabolic pathway that used H_2 (Moss *et al.,* 2000). Thus,

Tabel 3. Total VFA production and molar proportion of VFA with different types of vegetable oil and protections method

Variables	Types of vegetable oil	Protections method		
		Non protected	Calcium soap	Microencapsulation
Total VFA (mM)	Sesame	47.65 ± 7.67^{DEF}	55.79 ± 3.67^{CD}	52.67 ± 10.62^{CDE}
	Canola	42.84 ± 4.84^{EF}	69.84 ± 6.15^{AB}	62.75 ± 6.25^{BC}
	Flaxseed	42.02 ± 6.19^{EF}	79.90 ± 2.48^{A}	39.03 ± 5.53^{F}
Acetate (%)	Sesame	62.17 ± 3.02^{A}	62.24 ± 2.78^{A}	58.94 ± 4.81^{AB}
	Canola	55.64 ± 1.26^{BC}	58.49 ± 4.44^{AB}	61.40 ± 3.86^{A}
	Flaxseed	57.84 ± 5.54^{ABC}	62.07 ± 1.51^{A}	53.34 ± 2.79^{C}
Propionate (%)	Sesame	26.26 ± 4.01^{C}	27.25 ± 2.71^{BC}	30.04 ± 4.96^{ABC}
	Canola	32.39 ± 0.73^{A}	29.29 ± 2.99^{ABC}	27.27 ± 3.03^{BC}
	Flaxseed	30.86 ± 4.10^{AB}	27.10 ± 1.37^{BC}	33.22 ± 2.31^{A}
Butirat (%)	Sesame	8.14 ± 1.57	8.11 ± 0.59	8.68 ± 0.36
	Canola	9.25 ± 0.92	9.07 ± 1.13	8.42 ± 0.76
	Flaxseed	8.67 ± 1.45	8.12 ± 0.28	9.81 ± 0.53
A : P	Sesame	2.40 ± 0.35^{A}	2.31 ± 0.32^{AB}	2.02 ± 0.53^{ABC}
	Canola	1.72 ± 0.08^{C}	2.02 ± 0.37^{ABC}	2.28 ± 0.37^{AB}
	Flaxseed	1.92 ± 0.47^{BC}	2.30 ± 0.17^{AB}	1.61 ± 0.19^{C}

Note : Means with different superscript differ significantly (P<0.01).

Tabel 4. Methane gas production from different types of vegetable oil and protections method (mol/100mol)

Estimation model	Types of vegetable oil	Protections method		
		Non protected	Calcium soap	Microencapsulation
Moss *et al.* (2000)	Sesame	24.01 ± 1.55^{A}	23.76 ± 1.82^{A}	21.74 ± 3.40^{ABC}
	Canola	19.83 ± 0.49^{BC}	21.89 ± 2.40^{AB}	23.50 ± 2.27^{A}
	Flaxseed	21.01 ± 3.09^{ABC}	23.73 ± 0.94^{A}	18.79 ± 1.73^{C}

Note: Means with different superscript differ significantly (P<0.01).

Tabel 5. Hydrogen balance from different types of vegetable oil and protections method (mol/100mol)

Estimation model	Types of vegetable oil	Protections method		
		Non protected	Calcium soap	Microencapsulation
H_2 Production	Sesame	187.27 ± 2.16	188.98 ± 2.00	187.32 ± 4.53
Mitsumori *et al.* (2012)	Canola	186.10 ± 1.32	188.85 ± 1.36	188.18 ± 2.00
	Flaxseed	186.48 ± 1.73	189.13 ± 0.83	187.34 ± 1.88
H_2 Utilization	Sesame	69.43 ± 6.76^{D}	71.45 ± 6.16^{CD}	78.24 ± 10.26^{ABCD}
Mitsumori *et al.* (2012)	Canola	84.05 ± 2.99^{AB}	77.76 ± 8.24^{BCD}	72.33 ± 7.52^{CD}
	Flaxseed	79.80 ± 10.99^{ABC}	71.33 ± 3.27^{CD}	87.19 ± 5.44^{A}

Note: Means with different superscript differ significantly (P<0.01).

it would reduce the bond of H_2 and CO_2 that caused CH_4 production to decrease. Besides, the effect of linolenic fatty acid (C18 : 3) from microencapsulated flaxseed oil was of slow release which will decrease the activity of methanogenic archae. Dan Li *et al.* (2012) reported that the addition of linoleic fatty acid (C18 : 2) and linolenic fatty acid (C18 : 3) was highly significant (P<0.01) in decreasing *Methanobacterium formicicum* population compared to the addition of oleic fatty acid. Dan Li *et al.* (2012) stated that anti methanogenic activity of fatty acids can be caused by the toxic effects of fatty acids on methanogens and by methanogenic competition in using H2 in the process of biohydrogenation of unsaturated fatty acids. Zhang *et al.* (2008) stated that the decrease in methane production increased with the increase of degree of unsaturated fatty acids. Czerkawski *et al.* (1966) reported that oleic acid with one double chain / mole could decrease methane production by 1.70 moles / mole of fatty acid. Linoleic acid with 1.72 double chain / mole could decrease methane production by 1.79 moles / mole of fatty acid and linolenic acid with 2.4 double chains / mole could decrease the methane production by 2:05 moles / mole fatty acid.

CONCLUSION

Flaxseed oil and microencapsulation method are the best vegetable oil and protection method to optimize rumen fermentation. Supplementation of microencapsulated flaxseed oil produces the highest propionate concentration and H_2 utilization, the lowest A:P ratio and methane production, and did not disturb rumen microbial activity.

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