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Effects of Protected Unsaturated Fatty Acids Addition on *In Vitro* Digestibility and Rumen Microbes

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ABSTRACT

Unsaturated fatty acid supplementation in beef cattle rations is required to improve the quality of meat products. In order to avoid rumen biohydrogenation of unsaturated fatty acids, need the proper protection techniques. This research was aimed to investigate the effectiveness of two protection techniques on unsaturated fatty acids from plant oils to prevent from rumen biohydrogenation. This experiment was conducted *in vitro* and used fistulated Ongole grade as a rumen liquid donor. The experimental design was a factorial block with 2 factors i.e. protection techniques (no protection/control, microencapsulation, calcium-soap) and source of unsaturated fatty acids (sesame oil, canola oil, flaxseed oil). Rumen pH, total bacteria and protozoa were measured at 4 h incubation, while dry matter and organic matter digestibility were measured at 48 h incubation. The results show that there was no interaction between protection techniques and sources of unsaturated fatty acids on rumen pH, total bacteria, protozoal number, dry matter and organic matter digestibility. The protection of unsaturated fatty acids using microencapsulation and calcium-soap significantly increased ($P<0.05$) rumen pH compared to the control. Different sources of unsaturated fatty acids did not affect rumen pH. Total bacteria and protozoal number did not significantly increase ($P>0.05$) with the addition of different sources of unsaturated fatty acids either protected by using microencapsulation or calcium-soap. However, there was a trend of increasing protozoal number and total bacteria with the addition of protected fatty acids by using calcium soap. *In vitro* dry matter and organic matter digestibility was similar among treatments. It is concluded that the protection of unsaturated of fatty acid from plant oils did not interfere the growth of rumen microbes and could stimulate the fermentation activity. The protection of unsaturated of fatty acids by using calcium-soap technique is more effective to stimulate the growth of rumen microbes.

Key Words: Unsaturated fatty acid, Calcium-soap, Microencapsulation, Total bacteria, Protozoa

INTRODUCTION

The strategy to improve beef cattle production should be balanced with an increase in the quality of the meat, especially the unsaturated fatty acid content. Saturated fatty acid content in beef is high enough, therefore efforts should be made to reduce the content of saturated fatty acids in beef thus is safer for health. High saturated fatty acids content in beef meat occurred as a result of biohydrogenation process in the rumen which transform the unsaturated fatty acids to saturated fatty acids by rumen bacteria. This process is a detoxification mechanism to avoid the bacteriostatic effects of unsaturated fatty acids and decrease the growth of rumen microbes. *Butyrivibrio fibrisolvens* is a major bacteria which plays role in the rumen biohydrogenation process. Previous studies showed that supplementation of vegetable oil (high unsaturated fatty acids content) could decrease saturated fatty acid content and increase unsaturated fatty acid content in beef. Some potential vegetable oils being used are sesame, canola, and flaxseed oil. However, these oils need 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 (Jenkins and Palmquist 1984) and microencapsulation (Calvo et al. 2010). The objective of this research is to evaluate the effects of supplementation of three kinds of vegetable oils (sesame, canola, and flaxseed) protected using calcium soap and microencapsulation on dry and organic matter digestibility and protozoa and bacteria population.

MATERIALS AND METHODS

In vitro Fermentation. The sesame oil, canola oil, and flaxseed oil were produced by CV. MH. Farm Bogor, Golden Bridge, and Green Tosca respectively. Calcium soap from these three kinds of vegetable oils was made according to method by Kumar et al. (2006). The microencapsulation was done according to method by Calvo et al. (2010). *In vitro* fermentation was conducted according to method of Tilley and Terry (1963). The rumen fluid for this experiment was obtained before morning feeding from the rumen of fistulated Ongole crossbred beef cattle using commercial concentrate and elephant grass as substrate. The substrate contained 60% king grass forage and 40% concentrate mixture (cassava by product, wheat pollard, soybean meal, coconut cake meal, molasses, CaCO3, premix, urea, and oil) with 15-17 % CP and 69-74% TDN (Table 1). The design of experiment was factorial block design with 2 factors which were: protection methods (no protection, calcium soap and microencapsulation) as the first factor and kind of vegetable oils (sesame, canola and flaxseed oil) as the second factor. Protozoa and bacteria population were measured from liquid sample taken at 4 h incubation. Dry matter and organic matter digestibility were evaluated after 48 h incubation. After 4 h of incubation, samples of rumen aliquot were taken for protozoa counting under a microscope bacteria population measurement (Ogimoto and Imai 1981).

Table 1. Chemical composition of subtrate experiment on dry matter 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
Ether Extract	5.43	5.60	5.41	4.92	4.59	5.40	4.43	3.87	3.56
Crude Protein	15.66	16.10	16.52	16.69	16.05	15.15	16.67	17.93	17.58
Crude Fiber	22.89	23.86	24.17	23.33	24.02	23.04	22.74	23.65	23.46
NFE	48.35	47.18	47.12	47.55	47.93	48.88	48.08	47.31	46.31
	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 NFE)+(1.115 x CP)+(0.031x CF^2)-(0.133 x EE^2)+(0.036 x CF x NFE)+(0.207 x EE x NFE)+(0.1 x EE x CP)-(0.022 x EE x CP)

2) R1: 4% sesame oil without protection; R2: 4% canola oil without protection; R3: 4% flaxseed oil without protection; 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

Statistical Analysis. All data were analyzed by ANOVA using the GLM procedures (SPSS 13.0 for windows, 2004). The differences among all treatments were analyzed by Duncan's multiple range test to compare the means of the treatments.

RESULTS AND DISCUSSION

There was no interaction between protection techniques and sources of unsaturated fatty acids on rumen pH, total bacteria, protozoal number, dry matter and organic matter digestibility. The protection of unsaturated fatty acids using microencapsulation and calcium-soap significantly increased ($P<0.05$) rumen pH compared to the control. Different sources of unsaturated fatty acids did not affect rumen pH. Total bacteria and protozoal number did not significantly increase ($P>0.05$) with the addition of different sources of unsaturated fatty

acids either protected by using microencapsulation or calcium-soap. However, there was a trend of increasing protozoal number and total bacteria with the addition of protected fatty acids by using calcium soap. *In vitro* dry matter and organic matter digestibility was similar among treatments (Table 2).

Table 2. Digestibility and microbal population of the ration supplemented with different kind of vegetable oils and protection methods

Parameters	Kind of vegetable oil	Protection Methods: Not protected	Calcium soap	Micro-encapsulation	**Mean**
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	**6.00 ± 0,10[b]**	**6.25 ± 0.27[a]**	**6.33 ± 0.26[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	**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	**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	**4.00 ± 0.30**	**4.21 ± 0.22**	**4.13 ± 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	**7.14 ± 1.10**	**7.31 ± 1.12**	**6.95 ± 1.40**	

Means in the same row with different letters are significantly different (P<0.05)

The increase of rumen pH with the addition of vegetable oils which protected by using calcium soap and microencapsulation methods will have positive effect on rumen microbial activity in the feed degradation and fermentation. This result indicates that the addition of protectted vegetable oils was more advantageous for rumen condition than control.

Supplementation of vegetable oil at 4% did not alter feed digestibility (DMD and OMD) and rumen microbe population. This might be due to the low level of vegetable oil added on concentrate. Supplementation of protected vegetable oil by using calcium soap slightly increased protozoa and bacteria population compared to the control treatment, indicating that vegetable oil-calcium soap could stimulate the growth of microbial population. This might be due to the calcium content of vegetable oil-calcium soap which important for microbial growth. Previously, Sitoresmi et al. (2009) stated that oil supplementation without protection at level 5.0% significantly decreased protozoa population. The same response was reported by Adawiyah (2007) in which supplementation of non-protected fish oil at 1.5% significant decreased total bacteria population, but the population did not decrease in response to 3% supplementation of fish oil protected with calcium soaps.

IMPLICATION

The protection of unsaturated fatty acid from plant oils did not affect the growth of rumen microbes and could stimulate the fermentation activity. The protection of unsaturated fatty acids by using calcium-soap technique is more effective to stimulate the growth of rumen microbes.

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