The Fat Protective Effect of Fish Oil, Sunflower Seed Oil and Corn Oil on Fluid Rumen Fermentation Parameters

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ABSTRACT: This study was aimed to obtain oil and the exact saponification optimization to protect unsaturated fats, which does not interfere with the fermentation of rumen fluid. The studied the protective effects of unsaturated fat as sheep feed supplement on fermentation parameters of sheep rumen fluid in vitro. Three oil comparising lemuru fish oil (LFO), sunflower seed oil (SSO) and corn oil (CO) each made with a combination of saponification soap and capsulation. Manufacturing unsaturated fatty acids soap was protected by heating and stirring each oil and 20% NaOH solution (caustic soda), added with10% starch solution to form soft and elastic paste (gel). Ratio of oil volume ratio, 20% NaOH solution and starch was 1: 2: 1. Pasta was allowed to solidify within one night (12 hours), then sliced into thin slats with knife and soaked in saturated $CaCl_2$ solution to harden the gel slats which was then crushed by squeezing to form small grains (crystals). Then dried (moisture content of 10 to 20%). Soap samples were taken for in vitro analysis to determine the parameters of the rumen fermentation conditions by observing parameters rumen fluid pH, VFA, NH_3, and rumen microbial protein. The results showed that the three oils were not significantly affecting ($P>0.05$) pH, microbial proteins, VFA, and NH3, except for significantly low digestibility of dry matter (DM) and organic matter (OM) ($P<0.01$) compared with no soap. Conclusively, protection of unsaturated fats did not interfere with rumen microbial fermentation and was safe from degradation in the rumen (shown with a normal pH and in vitro DM digestibility and very low OM).

Keywords: protected fat, fermentation parameters, fish oil, sunflower seed oil, corn oil

INTRODUCTION

Lamb meat has a complete nutritional content of human needs despite the high saturated fatty acids compared to other livestock. Efforts to increase mutton production and to decrease levels of saturated fatty acids and cholesterol by increasing the unsaturated fatty acids have become challenge and demand. The main factors to consider in order to increase the levels of unsaturated fatty acids in sheep meat are the biohydrogenation process from unsaturated into saturated fatty acids in the rumen which causes the fat entering the small intestine mostly in the form of saturated fatty acids. Other property of unsaturated fatty acids is anti-microbial cellulolytic. Supplementation in ruminant diets will coat the fibers then inhibit the action of cellulase enzymes, and inhibits the activity of cellulolytic microbes to degrade the fiber. Efforts to prevent biohydrogenation of unsaturated fatty acids in the rumen consisted of saponification and capsulation to protect the fatty acids.

Fish oil and vegetable oil are expected to be the source of unsaturated fatty acids. Lemuru fish oil (LFO), sunflower seed oil (SSO) and corn oil (CO) were utilized in this research. The third oil contains relatively high unsaturated fatty acids. Lemuru oil is particularly produced in

abundance for fish cannery industry, and is therefore a potential animal feed due to the cheap price and is noncompetitive with food demand.

Different content of unsaturated fatty acids in lemuru oil, sunflower seed oil and corn oil causes possibility of providing different protection outcomes. Protection by saponification and capsulation of unsaturated fats is not expected to interfere with the process of fermentation in the rumen because it will be stable at neutral pH (e.g pH in the rumen), and protection will be released in the abomasum at acidic pH, which eventually will be absorbed in the small intestine. Differences in fatty acids of different raw materials can affect the production, quality chemical and physical quality of the meat. Therefore, it is essential to examine the protective effect of unsaturated fatty acids with different raw materials of vegetable origin and fish oils against biohydrogenation process, fermentation, digestibility in the rumen and cellulolytic microbial activity in the rumen.

Problems expected to be solved in this research was to what extent the protected unsaturated fat affect of unsaturated fatty acids with the raw material origin fish oil and vegetable oil to the process of fermentation, digestibility and microbial activity in the rumen cellulolytic. The sequential study on the protective effects of unsaturated fatty acids was comprehensively tested as seen from the results of rumen fermentation and cellulolytic microbial activity in the rumen. This series of research studies was requisitesince the results were expected to identify and examine the resilience of unsaturated fatty acids in the protected rumen, and not to have negative effect on the result of fermentation in the rumen.

METHOD AND MATERIALS

The material used for fatty acid soap was lemuru oil, sunflower seed oil, and corn oil, while saponification was made of distilled water, caustic soda (NaOH technical), technical $CaCl_2$, and starch served as encapsulation. Material for in vitro test was rumen fluid obtained from one donor sheep's rumen, 40-day old pangola grass, fatty acid soaps are produced from fish oil, sunflower seed oil and corn oil. Other ingredients included CO_2, 5% pepsin, Mc Dougall solution (artificial saliva) and 20% HCl. Tools for manufacturing fatty soap were used, comprising in vitro tools, pH meter, and VFA test kit.

Manufacturing unsaturated fatty acids soap was preceded by heating and stirring each oil and technical 20% NaOH solution (caustic soda), added with10% starch solution to formsoft and elastic paste (gel). Ratio of oil volume ratio, 20% NaOH solution and starch was 1: 2: 1. Pasta was allowed to solidify within one night (12 hours), then sliced into thin slats with knife and soaked in saturated $CaCl_2$ solution to harden the gel slats which was then crushed (still soaked in $CaCl_2$ solution) by squeezing to form small grains (crystals). The grains were remained in $CaCl_2$ solution to solid for approximately 1 hour, then sieved and dried (moisture content of 10 to 20%). Soap samples were taken for in vitro analysis (Tilley and Terry, 1963) to determine the parameters of the rumen fermentation conditions by observing parameters rumen fluid pH, VFA, NH3, and rumen microbial protein.

The data obtained were statistically tested using ANOVA with SPSS version 17 with a One Way completely randomized design. Differences between treatments were tested further by Duncan test (Steel and Torrie, 1991).

RESULT AND DISCUSSIONS

Effect of unsaturated fatty protection as a sheep feed supplement on fermentation parameters of in vitro rumen fluid

The observed parameters of in vitro rumen fluid fermentation were: pH, N-NH_3, microbial protein, VFA, dry matter digestibility, and organic matter digestibility. Saponification process combined with the oil capsulation in lemuru fish oil physically produced better and more viable soap than sunflower oil and corn oil. Protection result of lemuru fish oil, corn oil and sunflower seed oil was not significantly different in moisture content, dry matter, crude protein, crude fat, crude fiber, ash and NFE.

Different treatment of fatty acid soap resulted in not significantly different rumen liquid pH. The average rumen liquid pH in lemuru fish oil-based soap, corn oil soap, and sunflower seed oil soap was 7.44, 7.42 and 7.97, respectively. Komar (1984) stated that the neutral pH was ideal for ruminal microbe development. Chuzhaemi (1994) reported that acid pH or pH under neutral would slower the degradation rate of cellulose cell wall. Research result demonstrated that the activity of cellulolytic microbe was not interfered because ruminal liquid pH was within optimal range. It showed that the fatty acid oil of the three materials (lemuru fish oil, corn oil and sunflower seed oil) was stable in rumen, or not degradable as proven from the optimal pH.

Ammonia (NH_3) in rumen liquid was derived from degradation of feed protein, non-protein feed compound (NPN), N urea and saliva (Egan, 1980). The average NH_3 level in rumen liquid obtaining soap from lemuru fish oil, corn oil, and sunflower seed oil was 2.99 mg/100ml; 3.96 mg/100ml dan 1.99mg/100ml, respectively. According to Rajhan (1981), NH_3 was the main soluble nitrogen amount in rumen liquid needed by ruminal bacteria for protein synthesis as long as carbon frame was available. It was further explained that 20-5-mg/l NH_3 was sufficient for bacteria growth (the value was conversible into the same unit with Egan (1980) or 2-5 mg/100ml).

Rumen NH_3 level was the reflection of degrading activity of feed protein and endogen protein by ruminal microbe through N balance mechanism of cattle body (Kamra, 2005). Treatments of unsaturated fatty acid source types did not cause different NH_3 concentration in rumen. It showed that the activity of proteolytic bacteria as protein degrader to produce nitrogen (N) in rumen was not affected by the supplementation of protected unsaturated fat, therefore the growth of ruminal microbe was not interfered. NH_3 level in rumen in this research demonstrated that fatty acid soap of lemuru fish oil and corn oil did not interfere NH_3 condition in rumen which remained optimal. While supplementing sunflower seed oil-based soap tended to produce a slightly lower NH_3. It was parallel with the level of microbial protein in sunflower seed oil-based soap that was in fact the lowest compared to lemuru fish oil and corn oil. Protein ration level was equally given to treatments in this research but resulted in varied NH_3 rumen liquid. According to Ginting (2005), NH_3 was affected not only by feed protein but also non-protein nitrogen (NPN) degradation, saliva and N of rumen wall. Ranjhan (1981) reported that some amino acids could directly be used by bacteria for protein synthesis, but ammonia was the main soluble protein amount in rumen liquid needed by ruminal bacteria for protein synthesis as long as carbon frame from digestible carbohydrate existed, such as starch or glucose. Widyobroto *et al* (1995) stated that ammonia concentration in rumen liquid depended on the solubility and amount of feed. Result by Tanuwiria *et al* (2011) upon supplementing mineral oil complex (calcium soap) in ration, by comparing corn oil, peanut oil and fish oil, reported that ration protein was less fermentable, as observed from NH_3 product which was less than in 3.57 mM in every treatment.

Table 1. Parameter of in vitro rumen liquid fermentation and digestibility fermentasi cairan based on different fatty acid oil material

Variable	LFO	SSO	CO
pH	7.44	7.97	7.42
Microbial protein (mg/g)	0.008	0.010	0.046
NH3 (mg/100ml)	2.99	1.99	3.96
VFA :			
Acetic acid(ml/Mol)	6.758	6.816	9.588
Propionic acid (ml/Mol)	0.521	0.887	0.875
Butyric acid (ml/Mol)	2.054	1.907	3.060
Dry matter digestibility (%)	22.54	18.98	22.07
Organic matter digestibility (%)	19.16	10.50	12.83

VFA is the carbon source and main energy for ruminants. VFA digestibility in order was first butyric, followed by propionic and acetic. In feedlot, propionic acid concentration is generally higher than the other acids because propionic is the main energy source for meat cattle through gluconeogenesis. Research result showed no different propionic concentration in supplementation of protected lemuru fish oil, corn oil and sunflower seed oil. More propionic acid was absorbed by cell wall in lemuru fish oil than corn oil and sunflower seed oil, so propionic concentration of lemuru fish oil in rumen was low. Chuzaemi (1994) stated that acetic acid and butyric acid were the energy source for ketogenic oxidation, while propionic acid was used for gluconeogenesis or was glucogenic.

Digestibility test on protected fatty organic material by combining saponification and capsulation was not affected by different types of material. Digestibility test on organic matter of dry matter showed low result, indicating saponification combined with capsulation resulted in quite strong protection thereby less degradable by ruminal bacteria.

Supplementing mineral oil complex (calcium soap) in ration, comparing corn oil, peanut oil and lemuru fish oil concluded that the type of oil in making the complex with calcium did not affect fermentability and ration digestibility; however, it was indicated that fermentability and digestibility of ration containing whole oil was lower (Tanuwiria *et al,* 2011). It was reported that the dry matter digestibility of ration supplied with whole corn oil was lower ($P<0.05$) than that with calcium complex oil. It showed that oil saponification process by calcium mineral increased dry matter digestibility.

The results showed that the three oils were not significantly affecting dry matter, crude protein, ether extract, crude fiber, and ash.

Table 2. The results of proximate analysis of soaps

Variable	LFO	SSO	CO
Dry matter (%)	76.65	73.29	74.95
Crude protein (% of DM)	0.42	0.42	0.42
Ether extract(% of DM)	1.30	4.62	4.60
Crude fiber (% of DM)	28.01	22.45	12.78
Ash (% of DM)	45.73	51.98	40,62

CONCLUSION

Conclusion of the study were as follows:

1. Unsaturated fatty acid soap made from lemuru oil was easier to manufacture and deliver the best production results.
2. Rumen fluid conditions (pH, NH_3, VFA) with the addition of fatty acids in protected lemuru oil, corn oil and sunflower seed oil were relatively the same.
3. Protection method with a combination of saponification and capsulation could protect fatty acids from degradation by rumen microbes. This was shown in rumen fluid conditions (pH, NH_3 and VFA) fermented in the normal range, and the results were very low compared to digestibility without fatty acid soaps.

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