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Changes in *in Vitro* Methane Production and Fatty Acid Profiles in Response to *Cakalang* Fish Oil Supplementation

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ABSTRACT

This experiment was conducted to determine the effect of *cakalang* fish oil addition in ruminant feed on *in vitro* methane production and fatty acid profiles. This experiment consisted of four treatments which were R0 : feed composing of forage and concentrate at a ratio of 60% : 40% without *cakalang* fish oil (CFO) addition as control feed; R1: R0 added with CFO at 2.5%; R2: R0 added with CFO at 5%, and R3: R0 added with CFO at 7.5%. Fermentation with rumen fluid was done using the Hohenheim Gas Test (HGT); feeds were incubated at 39 °C for 72 hours. At the end of fermentation, samples were obtained and methane production and fatty acid profiles were determined. The experiment was conducted in completely randomised design with four replications. Data were analysed using analysis of variance and differences among treatment means were analysed using Duncan multiple range test. Results showed that CFO supplementation affected (P<0.05) methane production, protozoa numbers and NH_3 concentration; whereas the other parameters, i.e. VFA concentration, pH, and microbial protein were not affected. Some fatty acid profiles were influenced by treatments, such as palmitic, stearic, oleic, linoleic, and linolenic (P<0.05), while others, i.e. lauric and miristic were not affected. It is concluded that the best level of CFO supplementation is 5% as this level reduces methane production and increases unsaturated fatty acids without any negative effects on other variables measured.

Keywords: cakalang fish oil, fatty acids, in vitro fermentation, methane, gas test

ABSTRAK

Penelitian ini dilaksanakan untuk menguji pengaruh suplementasi minyak ikan cakalang ke dalam ransum ternak ruminansia terhadap produksi gas metan dan profil asam lemak *in vitro*. Perlakuan terdiri atas empat perlakuan ransum, yaitu R0: ransum yang terdiri atas hijauan dan konsentrat dengan rasio 60% : 40% tanpa penambahan minyak ikan cakalang (MIC) sebagai ransum kontrol; R1: R0 yang disuplementasi dengan 2,5% MIC; R2: R0 yang disuplementasi dengan 5% MIC; R3: R0 yang disuplementasi dengan 7,5% MIC. Fermentasi *in vitro* dilakukan dengan menggunakan tes gas Hohenheim (TGH); ransum perlakuan difermentasi pada suhu 39 °C selama 72 jam. Pada waktu akhir fermentasi, dilakukan pengambilan sampel untuk pengukuran produksi gas metan dan profil asam lemak. Rancangan percobaan yang digunakan adalah rancangan acak lengkap dengan empat replikasi. Data dianalisis dengan analisis ragam, dan perbedaan di antara perlakuan diuji dengan uji jarak berganda Duncan. Hasil percobaan menunjukkan bahwa perlakuan mempengaruhi produksi gas metan, jumlah protozoa, dan konsentrasi NH_3 (P<0,05); sedangkan peubah lainnya, seperti konsentrasi VFA, pH, and sintesis protein mikrobia tidak memberikan pengaruh yang nyata. Beberapa profil asam lemak yang dipengaruhi oleh perlakuan adalah palmitat, stearat, oleat, linoleat, dan linolenat, serta konsentrasi SAFA dan UFA (P<0,05). Beberapa asam lemak lainnya, seperti asam laurat dan miristat, menunjukkan tidak berbeda nyata. Dapat disimpulkan bahwa taraf terbaik suplementasi MIC adalah 5 % karena dapat menurunkan produksi gas metan dan meningkatkan asam lemak tak jenuh tanpa menyebabkan efek negatif pada variabel lainnya.

Kata kunci: minyak ikan cakalang, asam lemak, fermentasi in vitro, metan, tes gas

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INTRODUCTION

Methane is gas compound produced as a result of feed fermentation by rumen microbes. Methane is produced by protozoa as one of microbes in the rumen (Dohme *et al*., 1999). Methanogenic bacteria are the other rumen microbes producing methane and are found to attach to ciliate protozoa to obtain a constant supply of hydrogen for producing methane (Kamra, 2005). In their symbiosis, the protozoa released the hydrogen which were then transferred to the methanogenic bacteria to produce methane. To reduce methane production needs to inhibit growth of protozoa (Machmuller, 2006; Bhatta *et al*., 2013; Sondakh *et al*., 2015). However, several researchers indicated that reduction of methane production through protozoal defaunation caused some problems. This was because of the role of protozoa in fibre fermentation and in maintaining rumen pH at the normal pH (Bhatta *et al*., 2013). In addition, protozoa was capable of using fermentable carbohydrate to sustain its life, and the protozoa was able to slow down the conversion of fermentatble carbohydrate into lactic acid by the bacteria; as a result, the rumen pH could be controlled or maintained at normal pH.

Methane formation in the rumen was influenced not only by the methanogenic microbes, but also by the presence of hydrogen to react with carbon which was then oxidized to produce methane (Morgavi *et al*., 2010). The hydrogen was also used by the propionic bacteria producing propionate (Sondakh *et al*., 2015). These resulted a competition between methanogenic bacteria and propionic bacteria in using the hydrogen; and this was known as hydrogen - sink mechanism (HSM).

Competition for hydrogen use was not only for methane production in which methanogenic microbes competed with propionic bacteria, but also for hydrogenation of unsaturated fatty acid in the rumen. Rumen bacteria (lipolitic bacteria) the rumen hydrolyzed fats yielding saturated and unsaturated fatty acids. The unsaturated fatty acids were then hydrogenated to C:18 saturated fatty acids. This hydrogenation process needs hydrogen and enzymes. This situation caused competitions for hydrogen among the methanogenic microbes, propionate bacteria and the bacteria hydrogenating unsaturated fatty acids. It is expected methane production can be reduced when cattle are fed with feed containing unsaturated fatty acids.

Cakalang fish oil containing fatty acid consists of capric acid 1.62%, oleic acid 3.15%, stearic acid 21.12%, palmitic acid 0.63%, elaidic acid 13.98%, palmitoleinic acid 0.48%, linoleic acid 1.33%, arachidonic acid 24.78%, and lignoceric acid 0.49%. Among these fatty acids, unsaturated fatty acid dominated content of fatty acid in fish oil for about 43.72%. Therefore, it can be probable for hydrogenazing process by supplementation of cakalang fish oil. Therefore, the objective of this experiment is to reduce methane production through hydrogenation process.

MATERIALS AND METHODS

Materials

Materials used in this experiment were ruminal fluid, *cakalang* fish oil (CFO), feed consisting of forage (napier grass) and concentrate (rice bran), and materials (solutions) for fermentation gas test, and analysis of gas production and fatty acid profiles. Ruminal fluid was taken from the rumen of ruminant. The CFO was taken from the extraction of CFO industry. Feeds composing of napier grass and rice bran at a ratio of 60 : 40.

Treatments

Treatments applied in this experiment were R0: feed composing of forage and concentrate at a ratio of 60% : 40% with 0% CFO as control ration; R1: R0 supplemented with 2.5% CFO; R2: R0 supplemented with 5% CFO; and R3: R0 supplemented with 7.5% CFO. All treatments of CFO were in dry matter (DM) and every treatments were conducted in four replications. Composition of experimental ration was listed in Table 1.

Procedure

Medium for fermentation was prepared by mixing 474.0 mL of distilled water, 0.12 mL of trace mineral, 237 mL of buffer, 1.22 mL of resazurin, and 49.5 mL of reducer solution. All the materials were homogenized and flowed with CO_2 to achieve anaerobic conditions. The medium solution was then mixed with ruminal fluid at a ratio of 2 : 1 (v.v) (Menke & Steingass, 1988).

Each treatment feed (300 mg) was placed into a fermentation syringe which was subsequently added with a mixture of rumen fluid and fermentation medium (30 mL). All syringes containing treatement feeds were incubated at 39 °C for 72 hours. At the end of fermentation time, the fluids were filtered to separate the fluids

Tabel 1. Composition of experimental rations

Variables	Treatments			
	R0	R1	R2	R3
Feedstuff				
Forage (%)	60.00	60.00	60.00	60.00
Concentrate (%)	40.00	40.00	40.00	40.00
Nutrient ingredients				
Crude protein (%)	12.16	12.16	12.16	12.16
Crude fat (%)	4.28	4.28	4.28	4.28
Crude fiber	21.60	21.60	21.60	21.60
Nitrogen free extract (%)	41.45	41.45	41.45	41.45
Ash (%)	8.10	8.10	8.10	8.10
Adding CKO (%)	0.00	2.50	5.00	7.50

Note: CFO: cakalang fish oil; R0: feed added with 0% CFO; R1: R0 added with 2.5% CFO; R2: R0 added with 5% CFO; and R3: R0 added with 7.5% CFO; CFO in dry matter.

from the solid matter. The fluids were then used for analysing methane gas production, pH, fatty acid profile, and protozoal numbers.

Methane gas was measured with gas chromatography (GC, Hitachi 263 - 50). Sample of fermentation fluid (1 mL) was injected into GC using a spoit. The GC was conditioned as follows: 1 mm height of active carbon column, 0.5 m diameter, 190 °C detector temperature, 190 °C injector temperature, 150 °C column temperature, and 50 mL/min nitrogen gas. The methane gas production was measured using this formula: (area of standard x area of sample)/area of standard (Lopez *et al*., 1996).

pH was measured using pH meter. Fatty acid profiles were analyzed with GC. Protozoal numbers were counted using 1 mL of samples that were mixed previously with formal ssaline solution (1 : 9 ratio). Formal saline solution was prepared by mixing 0.8 mL formaldehyde (37 % v.v) with 0.9 % (w/v) NaCl solution up to 100 mL. Each sample was then put in a hemacytometer using a Pasteur pipet, and was placed under a microscope with 40 x magnification (Diaz *et al*., 1993).

After taking the fluid samples for measuring methane production and fatty acid profiles, the samples were then centrifuged at 3,000 g for 15 minutes. Supernatants were used to determine VFA and NH_3 concentrations. The filtrates were then centrifuged at 10,000 g for 15 minutes; supernatants were discarded, and the filtrates were used to measure microbial protein synthesised.

Concentration of NH_3 was analysed using 0.5 mL of samples centrifuged at 3,000 g following Charney & Marbach (1962) method. That sample was centrifuged at 10,000 g for 10 min, then 20 µL supernatant was added with 2.5 mL LC and 2.5 mL LD, and mixed homogenously. That mixture was incubated in a waterbath at 40 °C for 30 minutes. After blue colour was formed, the samples were taken to cool down in room temperature, then the absorbance of samples were measured using a spectrophotometer at λ 630 nm. LC was a mixture of Na - nitroprusside (50 mg) and phenol crystal (10 g) added with distilled water up to 1 L volume. LD was made up with NaOH pellet (5 gram), Na_2HPO_4 $2H_2O$ solution (36.7125 g diluted in 100 mL of distilled water) and 25 mL of 5% sodium hypochloride, which was then mixed homogenously and added with distilled water up to 1 L volume.

VFA concentrations were determined using GC (Doreau *et al*., 1993). Samples previously prepared by centrifuging at 3,000 g for 15 minutes were used as much as 0.2 mL which were added with metaphosphoric acid (1 mL). After mixing homogenously, the samples were centrifuged at 10,000 g for 10 minutes. Supernatants were used as samples injected into GC with 1 µL and was then read VFA after 6 minutes.

Filtrates produced after centrifuging at 10,000 g were used for analysing microbial protein synthesis measured with Lowry method (Plummer, 1987). Sample (0.5 mL) was put into a test tube which was then added with 2.5 mL of Lowry I solution and was kept at room temperature for 10 minutes. This mixture was then added with 0.25 mL of Lowry II solution. After keeping the smaples at room temperature for 30 minutes, the sample absorbances were then read using spectrophotometer at λ 750 nm. Lowry I solution contained 2% Na_2CO_3 in 0.1 N NaOH solution, 2% sodium tartrate, and 1% $CuSO_4 5H_2O$ and was mixed in a ratio of 100 : 1 : 1. Lowry II solution composed of 1 N Folin mixed with distilled water at a ratio of 1 : 1.

Experimental Design, Variables Measured, and Data Analysis

The experiment was carried out in completely randomized design with four treatments as described above and four replications. Variables measured were methane production, fatty acid profiles, pH, protozoal numbers, NH_3 concentration, VFA concentration and microbial protein synthesis. Analysis of variance (ANOVA) was used for analyzing the data. Differences among treatment means were examined with Duncan multiple range test (Steel & Torrie, 1980).

RESULTS

Table 2 shows effects of treatments on methane production, protozoal numbers, total and partial VFA concentrations, NH_3 concentration, microbial protein synthesis and pH of rumen fluid. Treatments affected significantly protozoal numbers ($P<0.05$). Protozoal numbers reduced linearly from 0% to 2.5% and 2.5% to 5% and 7.5% CKO additions. There were no differences in protozoal numbers when CFO was added at 5% and 7.5%.

Methane productions was reduced significantly with the addition of CFO in feeds at different levels ($P<0.05$). Methane production was the highest in feed added with 0% CFO ($P<0.05$), but this did not differ from that of feed added with 2.5% CFO. Methane production then decreased significantly ($P<0.05$) at 5% and 7.5% CFO addition without any significant differences in methane production between 5% and 7.5% CFO addition.

Small fluctuations were observed in acetic, propionic, butyric, and total VFA concentrations when feeds were added with 0%, 2.5%, 5%, and 7.5% CFO. However, differences among the levels of CFO additions were not statistically significant. These results did not cause any significant differences in acetate : propionate ratios among the treatments.

NH_3 concentrations were influenced by addition of CFO at different levels into feeds ($P<0.05$). The highest NH_3 concentration was produced by feed containing 0% CFO, and the lowest NH_3 concentration was yielded by feed added with 7.5% CFO. NH_3 concentrations did not differ significantly when CFO was added at 0%, 2.5% and 5%, or when CFO was added at 2.5%, 5% and 7.5%.

There were no significant effects of adding CFO at 0%, 2.5%, 5%, and 7.5% into the feeds on microbial protein synthesis. The same results were also observed in rumen fluid pH.

Effects of addition of CFO at 0%, 2.5%, 5%, and 7.5% on fatty acid profiles are demonstrated in Table 3. The addition of CFO at 0%, 2.5%, 5% and 7.5% did not affect productions of lauric (C:12) and myristic (C:14) acids. On the other hand, the treatments affected other

Table 2. Methane production, protozoal numbers, total and partial VFA concentrations, NH_3 concentration, microbial protein synthesis, and rumen fluid pH treated with cakalang fish oil addition

Variables	Treatments			
	R0	R1	R2	R3
Protozoal number (x 10^3 mL)	23.57 ± 0.87^c	21.57 ± 0.25^b	18.77 ± 0.50^a	18.67 ± 0.18^a
Methane production (mL/g)	23.77 ± 0.75^c	22.44 ± 0.27^c	20.83 ± 0.78^{ab}	20.37 ± 0.93^a
VFA concentration (mMol)				
Acetic acid	22.87 ± 0.31	23.24 ± 0.91	22.02 ± 0.67	23.19 ± 0.55
Propionic acid	7.93 ± 0.54	8.97 ± 0.54	8.51 ± 0.12	8.67 ± 0.09
Butyric acid	2.85 ± 0.18	3.02 ± 0.05	3.04 ± 0.17	3.18 ± 0.23
Total VFA	33.65 ± 0.71	35.23 ± 1.39	33.57 ± 0.91	35.04 ± 0.42
Acetate/propionate ratio	2.89 ± 0.18	2.59 ± 0.07	2.58 ± 0.05	2.67 ± 0.07
Microbial protein synthesis (mg/mL)	0.50 ± 0.08	0.48 ± 0.07	0.42 ± 0.03	0.41 ± 0.06
NH3 concentration (mg/100 mL)	15.19 ± 0.48^b	14.08 ± 0.20^{ab}	13.12 ± 0.42^{ab}	12.40 ± 0.21^a
Rumen fluid pH	6.28 ± 0.06	6.47 ± 0.17	6.49 ± 0.07	6.41 ± 0.15

Note: Means in the same row with different superscript differ significantly (P<0.05). R0: feed added with 0% CFO; R1: R0 added with 2.5% CFO; R2: R0 added with 5% CFO; and R3: R0 added with 7.5% CFO.

Table 3. Fatty acid profile as affected by cakalang fish oil addition at different levels

Fatty acids	Treatments			
	R0	R1	R2	R3
	---------- g/100 g crude fat ----------			
Lauric acid (C12:0)	0.25 ± 0.02	0.22 ± 0.01	0.24 ± 0.01	0.32 ± 0.01
Miristic acid (C14:0)	2.35 ± 0.06	2.50 ± 0.16	2.48 ± 0.08	2.33 ± 0.09
Palmitic acid (C16:0)	18.75 ± 0.20^a	18.91 ± 0.33^a	19.41 ± 0.31^{ab}	20.39 ± 0.55^b
Stearic acid (C18:0)	24.15 ± 1.90^a	26.09 ± 1.14^b	28.11 ± 0.92^b	28.31 ± 1.22^b
Oleic acid (C18:1)	13.88 ± 0.59^a	14.09 ± 0.69^a	15.35 ± 0.64^{ab}	16.74 ± 0.79^c
Linoleic acid (C18:2)	5.91 ± 0.24^a	6.18 ± 0.23^a	7.08 ± 0.19^b	7.30 ± 0.30^b
Linolenic acid (C18:3)	0.37 ± 0.03^a	0.41 ± 0.03^{ab}	0.61 ± 0.02^b	0.74 ± 0.04^c
Total of fatty acids	65.66 ± 1.01^a	69.23 ± 2.05^b	73.24 ± 0.63^{bc}	76.06 ± 1.94^c
SFA	45.50 ± 1.30^a	48.54 ± 1.89^b	50.20 ± 0.57^b	51.28 ± 1.70^c
UFA	20.16 ± 0.82^a	20.69 ± 0.85^a	23.04 ± 0.45^b	24.69 ± 0.58^c
SFA/UFA ratio	2.26 ± 0.15	2.35 ± 0.13	2.18 ± 0.05	2.07 ± 0.07

Note: SFA: saturated fatty acids; UFA: unsaturated fatty acids; Means in the same row with different superscript differ significantly (P<0.05). R0: feed added with 0% CFO; R1: R0 added with 2.5% CFO; R2: R0 added with 5% CFO; and R3: R0 added with 7.5% CFO.

fatty acid productions (P<0.05) by increasing productions of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids.

Production of palmitic acid (C16:0) were not different when feeds were added with 0%, 2.5%, and 5%. This palmitate production then increased to the highest level when 7.5% CFO was added into the feed (P<0.05); however, there were no significant differences in palmitate productions between 5% and 7.5% CFO additions. The same effects of CFO addition at different levels as those found in palmitic acid production were also observed in oleic acid (C18:1) production.

Stearic acids (C18:0) were produced at the lowest level when feed was added with 0% CFO. Adding CFO from 2.5% up to 7.5% in feed increased stearic acid productions (P<0.05), but the differences in stearic acid productions among the three treatments were not significant. The trends in stearic acid productions as affected by CFO addition were also observed in linoleic acid (C18:2) productions.

Addition of CFO at different levels affected significantly (P<0.05) linolenic acid (C18:3) production. The lowest linolenic acid production was obtained when CFO was added at 0%. Adding 2.5% CFO increased linolenic acid production, but this increase was not different from that of 0% CFO addition. A further increase in linolenic acid production was obtained by increasing the CFO level to 5%, but there were no differences in linolenic acid production between CFO addition at 2.5% and at 5%, or between CFO addition at 5% and 7.5%. Differences in linolenic acid production were observed between CFO addition at 2.5% and that at 7.5% (P<0.05).

Addition of CFO from 0%, to 2.5%, 5%, and up to 7.5% affected total fatty acid production (P<0.05). The lowest production of total fatty acid was obtained from feed added with 0% CFO (P<0.05), this production

then increased when feed was added with 2.5% CFO (P<0.05). Further increases in total fatty acid production were obtained by adding 5% and 7.5% CFO, but there were no differences in total fatty acid production obtained from 5% and from 7.5% CFO addition.

Saturated fatty acid (SFA) production was affected by adding CFO at different levels (P<0.05). Adding 0% CFO produced the lowest SFA. SFA production increased with CFO addition levels, but differences in SFA production between 2.5% and 5%, or between 5% and 7.5% CFO additions were not significant.

Unsaturated fatty acid production (UFA) was also affected by addition of CFO at different levels (P<0.05). The lowest UFA was produced when CFO was added at 0%, and the highest was obtained by adding CFO at 7.5%. Similar UFA productions were obtained between 2.5% and 5%, or between 5% and 7.5% CFO additions.

Although there were effects of CFO addition of SFA and UFA productions. these did not cause any significant differences in the ratio between SFA and UFA among the levels of CFO addition.

DISCUSSION

Reduction in protozoal numbers by adding CFO at different levels had been indicated in this experiment meaning that supplementing with CFO as unsaturated fatty acids from animal products had reduced the protozoal numbers, and this became another attempt of defaunation. The effects of CFO supplementation as unsaturated fatty acid source were similar to those obtained by Hristov *et al*. (2004) and Cieslak *et al*. (2006) using unsaturated fatty acids from plant sources.

Hristov *et al*. (2004) stated that the use of unsaturated fatty acid containing more double bonds was capable of reducing protozoal numbers in the rumen. Those researchers also reported that supplementationwith monounsaturated fatty acid (MUFA) such as oleic acid, and polyunsaturated fatty acid such as linoleic acid, had reduced protozoal numbers, respectively, by 10.74% and 14.90%. In addition, Cieslak *et al*. (2006) showed that protozoal numbers also reduced by 30.77% and 36.15%, respectively, by supplementing feed at a level of 7.5% with rapeseed oil containing 62% oleic acid or linseed oil containing 53% linolenic acid. Reductions in protozoal numbers were due to ability of unsaturated fatty acid to inhibit protozoal growth (Gao *et al*., 2016), and the unsaturated fatty acids containing C18 in high amount could be toxic for protozoa in the rumen (Varadyova *et al*., 2007). These reductions in protozoal numbers may contribute to the reductions in methane productions.

Reductions in methane productions was due to the effects of CFO additions. This CFO contained high amounts of unsaturated fatty acids composing of oleic, linoleic and linolenic acids having double bonds in its structures. These double bonds became the site for hydrogenation after lipids were hydrolyzed into fatty acids. Hydrogenation of double bonds in unsaturated fatty acids produced hydrogens that were used for saturated fatty acid formation. However, hydrogens were not only used for saturated fatty acid formation, but also for producing methane gas by methanogenic bacteria and for propionate production by propionic bacteria (Sondakh *et al*., 2015). This may cause a competition for hydrogen utilization.

Additions of CFO at different levels as the source of unsaturated acids did not affect concentrations of total and partial VFA. Ratios of acetate to propionate in this study were in the range of 2.58–2.89 which were less than 3.125 for normal rumen condition as suggested by Hungate *et al*. (1975). Low ration in this study can be due to the increase of propionic acid after adding of feed substrate in CFO. The effect of unsaturated fatty acid addition on total and partial VFA concentrations was not in an agreement with Sitoresmi *et al*. (2009) and Harwanto *et al*. (2014). This was because addition of unsaturated fatty acids, types, and substrate proportion affected total VFA concentrations. Other factors, such as the ratio of napier grass and concentrate in 60% : 40%, may also affect total and partial VFA concentrations.

Microbial protein synthesis in this study were in the range of 0.41–0.50 mg/mL indicating no effects of CFO addition on microbial protein synthesis. Those microbial protein synthesis were still higher than those obtained by Sondakh *et al*. (2015) which were 0.24–0.27 mg/mL by supplementing with VCO (a medium-chain fatty acid source) at 8%. Higher microbial protein synthesis (0.39–0.40 mg/mL) were obtained by using vegetable oil supplementation at 7.5% (Sitoresmi *et al*., 2009). The precursors needed for microbial protein synthesis were carbon, NH_3, and energy in sufficient amount (Orskov, 1992).

The NH_3 concentration in this study were 12.40–15.19 mg/100 mL. Although, it was indicating that there was decrease of NH_3 after adding CFO, the range of NH_3 in this study were still in normal range. This agreed with Harfiah (2006) stating that normal activity of microbia needed ammonia concentration of 8.5 to 30 mg/100 mL. These NH_3 concentrations were less than that obtained by Sitoresmi *et al*. (2009) which were in the range of 33.24–34.53 mg/100 mL by supplementing with coconut oil, palm oil, and sunflower oil at 7.5%.

Addition of CFO at increasing levels could still manage rumen fluid pH at its normal range. Owen & Zinn (1988) stated that normal rumen fluid pH was 5.5-7.6 for supporting normal activity of rumen micobes in degrading and fermenting the feeds. Sung *et al*. (2007) stated that the range of normal pH to ruminal metabolism were 6-7. This could relate to the pH for optimum enzyme activity in the rumen, such as 5.5–7.0 for peptidase and 6.2–7.0 for cellulose, and other processes, such as VFA productions at 6.8–7.0 (Keidane & Birgele, 2003), and increase in unesterified fatty acids at 6.97 and 7.35 (Hristov *et al*., 2004).

In this study, the addition of 5% CFO has been able to reduce methane production, whereas the number of protozoa decreased at the addition of 7.5%. Highest decrease in the number of protozoa will exacerbate rumen metabolism process. It was also indicated by NH_3 concentration which was decreased at addition 7.5% CFO. Addition of 7.5 CFO indicated a bad condition due to low amount of NH_3.

CFO addition, especially at the highest levels (7.5%), had changed the fatty acid profiles. The changes

were in relation to the contents and amounts of unsaturated fatty acids that affected biohydrogenation in the rumen. Wasowska *et al*. (2006) stated that the presence of unsaturated fatty acids inhibited biohydrogenation.

CONCLUSION

The addition of CFO reduced protozoal numbers, methane gas production, NH_3 concentration and fatty acid profile without any effects on total and partial VFA concentrations, microbial protein synthesis and rumen fluid pH. The greater effects were produced by CFO addition at 7.5%, and the effects were similar to those obtained by CFO addition at 5%. Therefore, it is recommended to use CFO at 5% as unsaturated fatty acid source in feed composing of napier grass and concentrate at 60% : 40% ratio.

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