

Unsaturated fat supplemented in the form of Ca-soap and prill fat in dairy cattle ration: in vitro study

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Abstract. Supplementing vegetable oils in dairy ration containing polyunsaturated fatty acid (PUFA) as an energy source tend to increased fatty acid profiles. This study aimed to identify the effect of supplementing vegetable oil encapsulated in Ca-soap and prilled on rumen fermentability and digestibility. The experiment used a 4x4x2 factorial randomized block design with three replications. Rumen liquor was obtained from fistulated dairy cow Frisien Holstein. The first factor was the vegetable-based oil (sunflower, soybean, sesame, and canola), the second factor was the level of protected vegetable oils supplementation (0%, 1%, 2%, and 3%) and the third factor was the protection forms. The results showed that supplementing Ca-soap and prill fat significantly decreased ($p < 0.05$) in rumen fermentability and ration digestibility. The variation of oil used, protection forms and level supplementation were significantly influenced pH score and protozoa population. Interaction effects between two factors were found in volatile fatty acid, ammonia, dry matter digestibility (DMD) and organic matter digestibility (OMD). Interaction effects between three factors were found in total rumen bacteria. It is concluded that Ca-soap and prilled fat-protected vegetables oil addition in dairy ration can be used up to 3% to reach the optimum result and less adverse effect on rumen microbial.

1 Introduction

The productivity of dairy cattle still needs to improve due to insufficient feed availability for livestock productivity. Farmed dairy cows are traditionally fed through agricultural by-products with relatively low nutrient content and digestibility. The increased need for nutrients in producing dairy cows can only be fulfilled with high-quality forage as

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an energy source. The decline in the quality of forage occurs primarily during the dry season, which causes livestock to lack of energy [1]. Low quality forage has a high heat increment, causing reduced feed consumption and causing an increased risk of heat stress [3].

The low-quality ration can be modified by adding vegetable oil to overcome the risk of severe heat stress in dairy cows. Providing rations with the addition of vegetable oil can enrich the fatty acid content of milk and function as a source of energy for livestock [2]. Fat can be given as a good strategy for enhancing low-energy ration [3]. Fat supplementation can be used as a source of high energy with low heat increment [4], and as a post-rumen energy to overcome the low quality-roughage and increase milk yield [5]. However, levels and supplementation techniques should be considered to avoid rumen bacteria and metabolic disturbance.

Manipulation of the use of protected oil is directed at making oil as a source of energy that is protected from rumen biohydrogenation or a source of rumen bypass fat. Agnihotri et al. [6] explained that the encapsulation method not only aims to avoid the process of biohydrogenation in the rumen, but it can also avoid a decrease in microbial activity and feed digestibility. Research by Hidayah et al. [7] reported that PUFA-rich oil protected by microencapsulation could increase the content of oleic acid (C18:1) due to the accumulation of the biohydrogenation process of linoleic acid (C18:2), which derived from vaccenic acid. Encapsulation through the saponification process is also known to have a beneficial effect on ruminal activity. Research by Suharti et al. [8] reported that Ca-soap of canola and flaxseed oil could increase the volatile fatty acid (VFA) and had no effect on dry matter digestibility. Research through ration supplementation needs to be carried out by modifying protected fats from vegetable oils rich in polyunsaturated fatty acids (PUFA) in the form of calcium soap (Ca-soap) and prill fat through spray drying process. Therefore, this study aims to identify the rumen's optimum fermentation activity and feed digestibility without interfere the rumen microbial.

2 MATERIALS AND METHODS

2.1 Ethical approvals

Rumen fluid as a source of inoculant was collected from three fistulated Frisian Holstein bulls in the dairy stall belonging to the laboratory Dairy Nutrition, Department of Animal Nutrition, Animal Science Faculty, IPB University. Ethical principles on the use of animal and dairy fistula surgery have followed the regulation of Animal Ethics Committee in IPB University.

2.2 *In vitro* preparation

The research was conducted at the Dairy Nutrition Laboratory, Department of Animal Nutrition and Feed Technology, IPB University. The research was assigned for 12 weeks trial, from December 2023 to March 2024. *In vitro* dairy cattle ration consisted of 40.27% Napier grass and 59.73% local concentrate. In addition, Ca-soap and prill fat were added at 0% (C0), 1% (C1), 2% (C2), and 3% (C3). Data on each feedstuff and ration nutritional content are shown in Table 1 and Table 2, respectively.

Table 1. Nutrient composition of feedstuffs for dairy cows (%DM)

Feed	Dry Matter	Ash	Crude Protein	Ether Extract	Crude Fiber	NFE ^b	TDN ^c
Napier grass ^a	24.94	17.78	13.05	1.70	36.36	31.11	44.99
Concentrate ^a	89.53	7.16	15.12	4.70	11.23	61.79	69.27

^aResult using NIRS

^bNitrogen free extract (NFE)

^cTotal digestible nutrient (TDN) = -17.2649+1.212(%CP: Crude Protein) + 0.8352(%NFE: Nitrogen Free Extract) + 2.4637(%EE: Ether Extract) + 0.4475(%CF: Crude Fiber) [9]

Table 2. Nutrient content and composition of dairy cow ration

Feed	Percentage (%)			
	C0	C1	C2	C3
Napier grass	40.27	39.87	39.46	39.06
Concentrate	59.73	59.13	58.54	57.94
Supplementation level (%)	0.00	1.00	2.00	3.00
Nutrient composition (%DM)				
Ash	11.44	11.32	11.21	11.09
Crude Protein	14.29	14.14	14.00	13.86
Ether Extract	3.49	4.46	5.42	6.39
Crude Fiber	21.35	21.14	20.92	20.71
NFE ^a	49.43	48.94	48.45	47.95
TDN ^b	59.49	61.19	62.89	64.58

^aNitrogen free extract (NFE)

^bTotal digestible nutrient (TDN) = -17.2649+1.212(%CP: Crude Protein) + 0.8352(%NFE: Nitrogen Free Extract) + 2.4637(%EE: Ether Extract) + 0.4475(%CF: Crude Fiber) [9]

2.3 Preparation of Ca-soap and spray-dried

The production of Ca-soap was prepared using a saponification modification method, conducted by Kumar et al. [10]. The oil was preheated up to 100°C and mixed with NaOH solvent until it well-dissolved. The calcium chloride (CaCl₂) solvent was slowly poured to the mixture and stirred into a solid phase.

The spray drying procedure refers to the method by Calvo et al. [11]. The manufacturing process includes emulsification, mixing ingredients, and homogenization. The ratio used is oil and coating material of 1:2. Encapsulation uses a laboratory mini spray drier (BUCHI-190) with nozzle diameter of 0.149 (100 mesh). The pressure on the spray drier compressor is set at 5 bars with an inlet temperature of 130° C-140° C and an outlet of 70° C-80° C.

2.4 Fermentability Analysis

The fistulated Frisian Holstein bulls breed was used as an inoculant source. The basal diet used was the conventional diet contained roughage and concentrates. The rumen fluid was taken before feeding. Ration fermentability analysis was conducted following Tilley

and Terry [12] method. The pH value was measured using Hanna pH meter. The steam distillation method is used to measure the total volatile fatty acid (VFA), and ammonia (NH₃) was measured using the method of Conway microdiffusion.

2.5 Microbes Population Sampling

The number of protozoa is counted under a binocular microscope, according to Ogimoto and Imai [13]. The 1.0 mL of rumen fluid was mixed with 1.0 mL of Trypan Blue Formalin Saline (TBFS). The population of protozoa were counted by using a logical count through counting chamber (0.0025 mm²).

The total of rumen bacteria was measured by Ogimoto and Imai [13] method. The total bacteria population was counted using the roller tube. Anaerobic dilution (4.5 mL) and 0.5 mL of rumen fluid were placed in the Hungate tubes. The sample was diluted in four times dilution. The 0.1 mL of samples from dilutions 1 to 4 placed into the BHI (Brain Heart Infusion) media, then rolled to form a solid phase.

2.6 Digestibility Analysis

Digestibility analysis was carried out following a two-stage method [12]. The first was digestibility fermentation, which follows a procedure similar to the fermentability analysis that lasts up to 48 hours. After the fermentation, tubes were centrifuged at 4000 rpm for 10 minutes, and the supernatant were collected. In the second phase, 50 ml of 2% HCl-pepsin was poured to the tube and incubated aerobically for 48 hours in a 39 °C shaker water bath. Subsequently, the sample was filtered using No.41 of Whatman paper. The residue was dried at 60 °C in the oven for 48 hours. Incineration occurred in a 650 °C oven for 4 hours to determine ash residue.

2.7 Study Design and Data Analysis

The study used a 4 x 4 x 2 factorial block design with three replications. Factor A was oil types consisting of F1 = Sunflower oil, F2 = Soybean oil, F3 = Sesame oil, and F4 = Canola oil. Factor B was oil addition levels of C0 = 0%, C1 = 1%, C2 = 2% and C3 = 3%. Factor C was the protection method, Ca-soap and prill fat. The data were analyzed and tested by Tukey's test using the Minitab ver. 18.

3 RESULTS

3.1 Fermentation characteristics and rumen microbial population

Protected fat addition significantly influenced fermentation activity. This study showed that supplementing Ca-soap and prill fat significantly decreased ($p < 0.05$) in rumen fermentability and ration digestibility. The variation of oil used, protection forms and level supplementation were significantly influenced pH score and protozoa population. Interaction effects between two factors were found in volatile fatty acid (VFA) and ammonia (NH₃). Interaction effects between three factors were found in total ruminal bacteria. The effect of Ca-soap and prilled fat supplementation on fermentability showed in Table 3 and the population of rumen microbes were shown in Tabel 4.

Table 3. Effect of protected-vegetable oil supplementation on ruminal fermentability values

Variables	Oils	Protection form	Level (%)				Mean ± SD
			0	1	2	3	
pH	Sunflower		6.94 ± 0.10	6.90 ± 0.07	6.90 ± 0.16	6.86 ± 0.03	6.90 ± 0.03 ^{ab}
	Soybean	<i>Ca-soap</i>	6.91 ± 0.05	6.96 ± 0.16	7.05 ± 0.03	7.02 ± 0.09	6.98 ± 0.06 ^a
	Sesame		6.98 ± 0.08	6.87 ± 0.09	6.85 ± 0.05	6.85 ± 0.06	6.89 ± 0.06 ^b
	Canola		6.96 ± 0.05	6.95 ± 0.05	6.92 ± 0.06	6.91 ± 0.09	6.93 ± 0.04 ^b
		Mean ± SD	6.94 ± 0.03	6.92 ± 0.05	6.93 ± 0.09	6.92 ± 0.07	6.93 ± 0.05 ^B
	Sunflower		6.96 ± 0.08	6.91 ± 0.09	6.96 ± 0.12	7.01 ± 0.07	6.96 ± 0.04 ^{ab}
	Soybean	<i>Prilled</i>	6.98 ± 0.04	6.93 ± 0.12	6.96 ± 0.07	6.98 ± 0.08	6.96 ± 0.06 ^a
	Sesame		6.91 ± 0.01	7.02 ± 0.08	7.03 ± 0.10	7.03 ± 0.08	7.00 ± 0.03 ^b
	Canola		6.94 ± 0.04	6.83 ± 0.10	6.93 ± 0.08	6.94 ± 0.26	6.91 ± 0.04 ^b
		Mean ± SD	6.96 ± 0.03	6.93 ± 0.08	6.97 ± 0.05	6.99 ± 0.04	6.96 ± 0.04 ^A
VFA (mM)	Sunflower		114.63 ± 2.54 ^{cd}	117.87 ± 4.82 ^{abc}	125.03 ± 5.75 ^a	132.93 ± 1.54 ^{ab}	122.62 ± 8.14
	Soybean	<i>Ca-soap</i>	113.75 ± 9.78 ^{cd}	102.42 ± 5.75 ^d	123.90 ± 1.54 ^{abc}	122.15 ± 5.02 ^{abcd}	115.56 ± 11.05
	Sesame		110.23 ± 2.12 ^{bcd}	117.92 ± 5.02 ^{abcd}	126.89 ± 7.43 ^{ab}	135.76 ± 1.57 ^a	122.70 ± 9.81
	Canola		109.63 ± 1.27 ^{bcd}	108.71 ± 7.43 ^{bcd}	112.94 ± 1.57 ^{bcd}	108.13 ± 2.23 ^{cd}	109.85 ± 6.38
		Mean ± SD	112.77 ± 2.24	113.20 ± 6.31	122.98 ± 5.03	126.81 ± 10.39	118.94 ± 5.19
	Sunflower		109.63 ± 4.18 ^{cd}	121.79 ± 2.23 ^{abc}	124.54 ± 2.84 ^a	115.69 ± 6.00 ^{ab}	117.91 ± 6.64
	Soybean	<i>Prilled</i>	110.23 ± 2.91 ^{cd}	114.30 ± 2.84 ^d	117.11 ± 6.00 ^{abc}	113.05 ± 6.45 ^{abcd}	113.67 ± 3.76
	Sesame		111.48 ± 2.45 ^{bcd}	109.70 ± 6.54 ^{abcd}	118.41 ± 9.12 ^{ab}	112.82 ± 8.50 ^a	113.10 ± 2.86
	Canola		112.44 ± 2.09 ^{bcd}	115.38 ± 9.12 ^{bcd}	115.75 ± 8.50 ^{bcd}	111.44 ± 4.22 ^{cd}	113.75 ± 5.71
		Mean ± SD	110.61 ± 1.12	120.69 ± 11.18	124.02 ± 11.61	120.03 ± 13.67	118.84 ± 8.91
NH ₃ (mM)	Sunflower		8.81 ± 2.97 ^b	9.21 ± 2.67 ^{ab}	9.03 ± 3.03 ^{ab}	8.60 ± 2.50 ^b	8.91 ± 0.27
	Soybean	<i>Ca-soap</i>	9.39 ± 3.55 ^b	8.93 ± 3.03 ^{ab}	8.53 ± 2.50 ^{ab}	7.84 ± 3.04 ^b	8.67 ± 0.77
	Sesame		9.57 ± 1.14 ^b	8.69 ± 3.04 ^{ab}	8.16 ± 3.41 ^{ab}	7.80 ± 2.61 ^b	8.56 ± 0.66
	Canola		9.44 ± 1.48 ^b	9.73 ± 3.41 ^{ab}	9.50 ± 2.61 ^{ab}	8.39 ± 2.83 ^b	9.26 ± 0.09
		Mean ± SD	9.24 ± 0.34	9.00 ± 0.41	8.75 ± 0.48	8.22 ± 0.37	8.80 ± 0.25
	Sunflower		9.44 ± 0.71 ^a	7.85 ± 2.83 ^b	7.76 ± 2.97 ^b	7.73 ± 3.80 ^b	8.20 ± 0.83
	Soybean	<i>Prilled</i>	9.57 ± 0.57 ^a	9.32 ± 2.97 ^b	8.87 ± 3.80 ^b	8.14 ± 3.55 ^b	8.97 ± 0.67
	Sesame		9.39 ± 0.89 ^a	8.79 ± 3.55 ^b	8.04 ± 2.60 ^b	7.99 ± 1.65 ^b	8.56 ± 0.63
	Canola		8.81 ± 0.35 ^a	8.50 ± 2.60 ^b	8.33 ± 1.65 ^b	8.57 ± 2.71 ^b	8.55 ± 0.87

Mean ± SD	9.37 ± 0.29	8.63 ± 0.53	8.15 ± 0.40	7.99 ± 0.34	8.54 ± 0.25
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VFA= volatile fatty acid; NH₃= ammonia

Table 4. Effect of protected-vegetable oil supplementation on rumen microbial population

Variables	Oils	Protection form	Level (%)				Mean ± SD
			0	1	2	3	
Protozoa (Log cell/mL)	Sunflower	<i>Ca soap</i>	6.69 ± 0.19	6.74 ± 0.20	6.61 ± 0.15	6.57 ± 0.28	6.65 ± 0.08
	Soybean		6.68 ± 0.16	6.67 ± 0.15	6.63 ± 0.18	6.65 ± 0.21	6.66 ± 0.09
	Sesame		6.78 ± 0.09	6.74 ± 0.17	6.64 ± 0.09	6.57 ± 0.25	6.68 ± 0.02
	Canola		6.76 ± 0.10	6.80 ± 0.17	6.65 ± 0.19	6.65 ± 0.27	6.71 ± 0.01
	Mean ± SD		6.71 ± 0.04 ^a	6.74 ± 0.06 ^a	6.64 ± 0.02 ^b	6.62 ± 0.04 ^b	6.68 ± 0.02
	Sunflower	<i>Prilled</i>	6.76 ± 0.08	6.67 ± 0.20	6.65 ± 0.27	6.64 ± 0.30	6.68 ± 0.05
	Soybean		6.78 ± 0.04	6.70 ± 0.26	6.66 ± 0.24	6.51 ± 0.41	6.66 ± 0.07
	Sesame		6.68 ± 0.04	6.69 ± 0.27	6.60 ± 0.28	6.54 ± 0.38	6.63 ± 0.11
	Canola		6.69 ± 0.04	6.69 ± 0.23	6.62 ± 0.26	6.49 ± 0.23	6.62 ± 0.07
	Mean ± SD		6.74 ± 0.04 ^a	6.69 ± 0.02 ^a	6.63 ± 0.03 ^b	6.54 ± 0.06 ^b	6.65 ± 0.02
Bacteria (Log CFU/mL)	Sunflower	<i>Ca-soap</i>	9.57 ± 0.04 ^{abcd}	10.34 ± 0.21 ^{abc}	10.25 ± 0.14 ^{abcd}	9.75 ± 0.60 ^{abcd}	9.98 ± 0.38
	Soybean		10.92 ± 0.05 ^{abcd}	9.19 ± 0.01 ^{bcd}	9.35 ± 0.10 ^{abcd}	9.62 ± 0.26 ^{abcd}	9.77 ± 0.53
	Sesame		9.39 ± 0.31 ^{abcd}	10.59 ± 0.11 ^a	10.42 ± 0.21 ^{abc}	10.16 ± 0.09 ^{abcd}	10.14 ± 0.79
	Canola		8.98 ± 0.02 ^{abcd}	9.11 ± 0.01 ^{cd}	9.70 ± 0.04 ^{abcd}	9.54 ± 0.14 ^{abcd}	9.33 ± 0.08
	Mean ± SD		9.89 ± 0.83	9.90 ± 0.68	9.97 ± 0.44	9.70 ± 0.26	9.87 ± 0.35
	Sunflower	<i>Prilled</i>	8.98 ± 0.28 ^{cd}	9.23 ± 0.03 ^{bcd}	9.19 ± 0.11 ^{bcd}	9.10 ± 0.20 ^{cd}	9.13 ± 0.11
	Soybean		9.39 ± 0.15 ^{abcd}	9.31 ± 0.04 ^{abcd}	9.24 ± 0.19 ^{bcd}	9.12 ± 0.03 ^{cd}	9.27 ± 0.86
	Sesame		10.92 ± 0.01 ^{abcd}	9.30 ± 0.02 ^{bcd}	9.19 ± 0.03 ^{bcd}	9.11 ± 0.04 ^{bcd}	9.63 ± 0.11
	Canola		9.57 ± 0.14 ^{ab}	9.24 ± 0.05 ^{abcd}	9.22 ± 0.03 ^{abcd}	9.17 ± 0.03 ^{cd}	9.30 ± 0.18
	Mean ± SD		9.54 ± 0.72	9.31 ± 0.07	9.24 ± 0.06	9.16 ± 0.07	9.31 ± 0.17

3.2 Nutrient digestibility

Protected fat supplementation can suppress or reduce the adverse effects on fibre digestibility. Data on feed digestibility showed in Table 5. Based on this finding, interaction between supplementation level and protection form in dairy ration did affect on the dry matter and organic matter digestibility (DMD) and (OMD).

Table 5. Effect of protected-vegetable oil supplementation on digestibility values

Variables (%)	Oils	Protection form	Level (%)				Mean ± SD
			0	1	2	3	
DMD	Sunflower		63.98 ± 4.12 ^a	63.88 ± 8.36 ^{ab}	61.52 ± 5.65 ^b	63.15 ± 5.99 ^{ab}	63.13 ± 1.14
	Soybean	<i>Ca-soap</i>	62.83 ± 8.79 ^a	63.01 ± 4.55 ^{ab}	62.30 ± 5.65 ^b	60.14 ± 6.03 ^{ab}	62.07 ± 0.56
	Sesame		62.56 ± 3.64 ^a	61.31 ± 6.30 ^{ab}	61.52 ± 8.59 ^b	61.58 ± 5.62 ^{ab}	61.74 ± 1.32
	Canola		63.48 ± 2.06 ^a	60.92 ± 5.01 ^{ab}	61.44 ± 7.72 ^b	62.46 ± 5.65 ^{ab}	62.08 ± 0.83
	Mean ± SD		63.28 ± 0.62	62.49 ± 1.21	61.75 ± 0.48	61.69 ± 1.18	62.30 ± 0.64
	Sunflower		63.48 ± 3.92 ^b	64.59 ± 8.48 ^{ab}	61.89 ± 8.77 ^{ab}	60.39 ± 7.49 ^{ab}	62.59 ± 1.84
	Soybean	<i>Prilled</i>	62.56 ± 1.82 ^b	64.16 ± 5.48 ^{ab}	62.88 ± 8.99 ^{ab}	63.46 ± 9.30 ^{ab}	63.27 ± 1.63
	Sesame		62.83 ± 2.20 ^b	62.31 ± 5.20 ^{ab}	59.50 ± 4.45 ^{ab}	60.13 ± 5.70 ^{ab}	61.19 ± 0.70
	Canola		63.98 ± 1.96 ^b	63.94 ± 6.79 ^{ab}	62.64 ± 3.79 ^{ab}	63.98 ± 3.94 ^{ab}	63.63 ± 0.68
	Mean ± SD		63.15 ± 0.58	64.05 ± 1.24	61.99 ± 1.31	62.09 ± 1.58	62.82 ± 0.88
OMD	Sunflower		62.37 ± 4.60 ^a	62.53 ± 7.80 ^{ab}	59.59 ± 6.89 ^b	61.26 ± 9.10 ^{ab}	61.44 ± 1.35
	Soybean	<i>Ca-soap</i>	61.37 ± 9.38 ^a	61.45 ± 6.89 ^{ab}	60.89 ± 9.10 ^b	59.51 ± 5.61 ^{ab}	60.80 ± 1.43
	Sesame		60.87 ± 4.16 ^a	59.01 ± 5.61 ^{ab}	57.41 ± 6.39 ^b	59.50 ± 4.94 ^{ab}	59.20 ± 0.90
	Canola		61.92 ± 2.30 ^a	58.86 ± 6.39 ^{ab}	60.41 ± 4.94 ^b	60.45 ± 7.30 ^{ab}	60.41 ± 0.88
	Mean ± SD		61.71 ± 0.61	60.70 ± 1.50	59.78 ± 1.33	59.94 ± 0.94	60.53 ± 0.86
	Sunflower		61.92 ± 4.42 ^b	62.96 ± 7.30 ^{ab}	59.79 ± 6.74 ^b	58.45 ± 7.52 ^{ab}	60.78 ± 2.04
	Soybean	<i>Prilled</i>	60.87 ± 2.08 ^b	62.56 ± 6.74 ^{ab}	60.91 ± 7.52 ^b	61.67 ± 6.96 ^{ab}	61.50 ± 1.95
	Sesame		61.37 ± 2.35 ^b	60.33 ± 6.96 ^{ab}	57.15 ± 7.22 ^b	58.11 ± 6.63 ^{ab}	59.24 ± 0.79
	Canola		62.37 ± 2.21 ^b	61.96 ± 7.22 ^{ab}	61.24 ± 6.63 ^b	62.91 ± 8.75 ^{ab}	62.12 ± 1.13
	Mean ± SD		61.55 ± 0.61	62.48 ± 1.40	60.02 ± 1.54	60.33 ± 1.86	61.09 ± 1.01

DMD= Dry matter digestibility; OMD= Organic matter digestibility

4 DISCUSSION

4.1 Fermentation characteristics and rumen microbial population

Using different types of plant oil in the form of Ca-soap and prill fat does not disturb the pH conditions in the rumen, proving that every kind of oil is well protected and maintained. The rumen pH in this study was around 6.9, which indicates that the rumen pH range is still in normal conditions [14], so it does not inhibit the feed fermentation process by rumen microbes.

Providing levels of up to 3% high PUFA oil in the form of Ca-soap and prill fat did not affect the pH value in the rumen. This study was in line with research by Suharti et al. [8] who said that supplementation of Ca-soap-protected canola and flaxseed oil at a high level of 6% in the diet did not affect the rumen pH value. According to Suharti et al. [8] protected-canola oil is inert in the rumen, so it would not change the rumen pH. The protected fat in the rumen will be neutral and pass through the rumen without disturbing its

microbial activity, which will continue in the post-rumen area. This result is in accordance with Suharti et al. [15] that the bonds in Ca-soap and prill fat can be separated under acidic conditions. The Ca-soap is stable in rumen fluid, which has a neutral pH. However, when the Ca and fatty acids are bound when they pass via abomasum with a very acidic pH, they will be detached and produce Ca ions and free fatty acids, and be absorbed in the small intestine.

The results of protozoa populations in all treatments were in the range of $6 \log \text{ cell mL}^{-1}$, indicating that these values were in the normal value. According to McDonald et al. [14], the normal protozoa population is $5\text{-}6 \log \text{ cells mL}^{-1}$. Providing up to 3% Ca-soap and prill fat significantly reduces the protozoa population. The number of protozoa decreases as the increasing in long-chain unsaturated fatty acids double bonds [16].

In a different study, the addition of oil without protection by Despal et al. [17] reported that the addition of canola, soybean, and palm oil without protection at a level of more than 1% significantly reduced the protozoa population. This shows that the protection treatment is quite good compared with no protection in protecting protozoa from defaunation in the rumen.

This study predicted that protozoal would have no lipolytic activities in digested fat. It causes a decrease in metabolic activity when treatments in higher levels are added in the ration. The defaunation effect will be decreased the protozoa and made a breakage of protozoa [18]. It also assumed that protected-vegetable oil could partially cover the existence of protozoa and cause a protozoa suppression in the rumen [19].

The protected oil supplementation significantly influenced the bacterial population. The population of total rumen bacteria were affected by the interaction of level infusion, oil type, and protection forms. The addition of more than 3% of prill fat will reduce the bacteria population. Declining in the bacteria population was caused by the oil inhibition on the feed particle and prevented bacteria from contacting and digesting the feed [20]. The association of oil to the feed particle surface inhibited the digestion and metabolism process of the feed by rumen bacteria. The use of coating materials also affects the protection strength of vegetable oils. According to Balasubramani et al. [21], maltodextrin has low emulsifying ability so that the protective layer of the core material is not thick which causes the release of the core material may not be on the target and undergone biohydrogenation. Maltodextrin has a low viscosity at high solubility.

Results of this study showed that adding varying oil high in PUFA in the ration did not affect the NH_3 concentration. However, there was an interaction between protected fat form and level of supplementation in decreasing the NH_3 concentration. This condition assumed due to the material used in protecting the fat and lead to the decrease of ammonia concentration result. This study showed, the higher level of protected fat added to the ration, the lower concentration of ammonia formed. According to Suharti et al. [15], the use of Ca-soap canola oil had a higher result in ammonia concentration, which in line with this study. Findings of Montgomery et al. [22] reported that lipid addition did not affect the N metabolism in the rumen.

Adding protected fat derived from vegetable oil is predicted to augment the total VFA concentration due to the high energy density in the dietary ration. This finding was also showing an increase of the total concentration of VFA by the addition of protected-oil, and there was an interaction between level and oil type. Sesame and sunflower oil-Ca-soap showed the highest value in total VFA concentration. This condition assumed that the PUFA content in the protected fat, particularly linoleic acid which cannot be well protected using Ca-soap and have undergone partial biohydrogenation. Enhancing total VFA through oil supplementation which contains rich linoleic acid, has been reported [23] and this study predicted that sesame oil is rich in linoleic and oleic acid [24]. Lipids in the rumen will

undergo lipolysis, lead the fat to be hydrolyzed into glycerol and fatty acids [25] and glycerol will be converted into VFA.

4.2 Nutrient digestibility

The study showed that protected fat can interfere with ruminal bacteria activity due to the extensive biohydrogenation and intensive physical nature of fat that can be shrouded by rumen bacteria and suppress feed digestion. This finding can be seen in total bacteria and protozoa, which decrease significantly by the infusion of protected fat in the ration, mainly prill fat.

The addition of minerals, especially Ca, can be increase the digestibility of the ration in large quantities supplemented with fat. It is further stated that the use of insoluble calcium soap is capable negates the effect of fatty acids on bacteria, so the digestibility of fibre in the ration can increase [15]. These findings were in line according to the in vitro study of prilled fat addition by Riestanti et al. [26] showed that DMD in dairy ration has suitable quality feedstuffs and leads to a relatively higher feed digestibility. The use of PUFA-high oil as a source of energy is predicted to enhance the PUFA nutritional content in the ration. This finding was also supported by Riestanti et al. [27], that the depression of feed digestibility can be overcome by lowering infusion levels.

In this study, it demonstrates that the supplementation of prill fat and Ca-soap cannot prevented the feed digestibility depression as there was a little depression of DMD and OMD when fat was supplemented in the ration. However, this study shows that Ca-soap has higher DMD value and less adverse effect in ruminal microbial as we can see in Table 4. Decreasing dairy ration digestibility after infusion of protected-vegetable oil was assumed also due to methodological constraints. Riestanti et al. [26] showed that protected-vegetable oil supposed to be digested in abomasal, cannot be affected and well-imitated in the in vitro study due to the pepsin digestive enzyme in protein digestion.

5 CONCLUSION

From this study, it can be concluded that Ca-soap and prilled-vegetables oil addition in dairy ration can be used up to 3%, to reach the optimum fermentation activity, feed digestibility and less adverse effect on rumen microbial.

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