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Blood Profiles, Microbial Population, Rumen Fermentation and Performance of Bali Calves Fed with Soybean Oil Calcium Soap

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Abstract: The purpose of this study was to assess the effects of Soybean Oil Calcium soap (SO-Ca) on blood profiles, rumen fermentation properties, microbial populations, and the performance of Bali's calves. Using a randomized block design, three treatment groups of six replications each contained 18 calves. Treatments included 0% SO-Ca (control/T0), 5% SO-Ca (T1), and 10% SO-Ca (T2). The diet of each treatment (Concentrate mix and Napier grass) used in the study was formulated based on the usual ration given to the cattle with energy levels approximately the same between treatments. Analysis of variance was used to assess the data and the Tukey Test was then used to determine whether there were any significant differences between the treatments. The findings demonstrated that the addition of SO-Ca up to 10% lowered ($p \leq 0.01$) the total protozoa population but had no effect on the overall bacterial population. The fraction of propionate increased ($p = 0.03$) and the concentration of NH_3 tends to rise ($p = 0.10$). However, the addition of SO-Ca up to 10% reduced the proportion of acetate ($p = 0.045$), butyrate ($p = 0.03$), C2/C3 ratio ($p = 0.02$) and tended to reduce valerate ($p = 0.1$) and methane estimation ($p = 0.06$). Blood metabolites, performance, and rumen pH were comparable between treatments. The addition of SO-Ca tends to lower the cholesterol level ($p = 0.10$). In conclusion, the addition of SO-Ca improves propionate proportions and reduces methane estimation as well as cholesterol levels, which are both positive impacts on rumen fermentation.

Keywords: Bali Calves, Blood Profiles, Fermentation, Rumen Microbial, Soybean Oil Calcium Soap

Introduction

The productivity of beef cattle in Indonesia is relatively low and does not meet domestic beef demand. Bali cattle (Bos sondaicus) has some superior characteristics compared to other beef cattle, including a high carcass percentage, the ability to better adapt to the tropical environment, and their ability to survive on poor nutrition during the dry season (Panjaitan *et al*., 2014). Furthermore, Bali Cattle's carcasses weren't at their best when the quality or amount of the feed wasn't adequate (Tahuk *et al*., 2018). Livestock productivity can be improved by improving the nutritional status of livestock, starting at the calving period. Well-maintained Bali cattle calves can produce high-quality cattle stock with optimum growth rates.

Livestock productivity can potentially be improved by the addition of fatty acids in feed rations as an energy source, but some of the unsaturated fatty acids can undergo biohydrogenation by rumen bacteria. Rumen bacteria such as *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* transform unsaturated fatty acids into saturated fatty acids during biohydrogenation (Dewanckele *et al*., 2020). The increased saturated fatty acid content in red meat is due to the subsequent absorption of the saturated fatty acids in the small intestine. Additionally, the fat can lessen the breakdown of fiber in the rumen by preventing bacteria from adhering to the fiber fraction.

When the rumen's unsaturated fatty acids are protected by calcium soap, biohydrogenation may be decreased.

Unsaturated fatty acids can be easily obtained from soybean oil, as it has a tremendous amount of linoleic acid (>50%), oleic acid (27.9%), and linolenic acid (6.7%) (Aguila, 2018). Calcium soap from soybean oil has been found to protect the fats in the feed ingredients, increase fiber digestibility and optimize the use of high-fat rations *in vitro* (Hidayah *et al*., 2014). Additionally, lactating buffaloes fed soybean oil at a level of 2.6% of dry matter intake without protection, according to Eldahshan *et al*. (2020), had an increase in the concentration of unsaturated fatty acids in their milk.

In an earlier *in vitro* experiment, we showed that the addition of calcium soap soybean oil (SO-Ca) at 5% of the total ration did not change rumen pH, the total number of bacteria and protozoa, as well as the digestibility of dry and organic matter, but reduced the Ammonia (NH_3) concentration and total Volatile Fatty Acid (VFA) production (Bain *et al*., 2016). The use of SO-Ca at 5% and cashew fruit flour at 10% in Bali male cattle at the fattening phase could increase the digestibility of organic matter, ether extract, and linoleic content in the meat (Bain *et al*., 2016). In contrast, the study regarding SO-Ca as a fat-protected source used in Bali cattle at the calving phase implemented in the traditional farmers is limited.

We hypothesize that the addition of SO-Ca can improve rumen fermentation to provide Volatile Fatty Acid (VFA) as energy reserves that are important for livestock productivity. This study evaluated the effect of SO-Ca addition in calf rations of Bali cattle calves on rumen fermentation, rumen microbial populations, blood metabolites, and overall livestock performances.

Materials and Methods

Preparation of Soybean Oil Calcium Soap (SO-Ca)

Production of SO-Ca was prepared according to Kumar *et al*. (2006) with slight modifications. Briefly, 5 mL soybean oil was mixed with 50 mL KOH solution, then placed into a neck flask to be refined and titrated, which resulted in the saponification number and the amount of NaOH used in this study. Oil and Butylated Hydroxytoluene (BHT) were heated at 80°C. Next, a 50% NaOH solution was slowly added to ensure that saponification occurred. A $CaCl_2$ solution and pollard were added to the mixture while stirring until it formed a solid that was dark in color. The resulting solid was placed on a plastic sheeted pan until it froze and hardened completely. Before it was added to the concentrate mix, the SO-Ca block was ground to become the meal.

Feeding Trial

A total of 18 male Bali calves at 11 months of age, with initial body weights of 124±25.0 kg were used. Each calf was kept in an individual stall for 80 days, including the preliminary period, and equipped with a feed bunk and water through. All of the clinical trials were performed following the safety procedures given by the faculty of animal science at Bogor agricultural university, Indonesia, and followed the principles given in the declaration of Helsinki.

Total rations consisted of Napier grass and concentrate, with a ratio of 70:30. The concentrate mix was commercially obtained from PT. Citra feed (East Java, Indonesia). The concentrate mix consists of pollard, palm kernel meal, copra meal, coffee husk, cassava waste, soy sauce dregs, peanut shell meal, rice bran, salt, and probiotics. The concentrate mix was a commercial product and there was no information on the percentage of each feedstuff. The SO-Ca was mixed with the concentrate mix at level 5 and 10% of the total ratio. The total ratio given was up to 2.5% of the calves' body weight. The feeding trial used a randomized block design with three different treatments and six replication blocks. Calves were grouped based on their body weights after the preliminary period. The treatments used included 0% SO-Ca (control/T0), 5% SO-Ca (T1), and 10% SO-Ca (T2). The nutrient content of the feedstuffs and mixed rations used in the experiment are shown in Tables 1-2. The diets of each treatment (concentrate mix and Napier grass) used in the study were the usual ration given to the cattle. Variables measured included feed intake, body weight gain, rumen fermentation characteristics including pH value, Ammonia (NH_3), Volatile Fatty Acid (VFA), bacterial and protozoa population, blood profiles, and metabolites.

During the 13-day preliminary period, the cattle were offered Napier grass and concentrate mix with SO-Ca addition in increased levels gradually from 0-5% and 10% depending on their treatment. After a 13-day preliminary period, the calves were weighed to determine the amount of feed to be given and to assign treatment groups. Calves were then fed their assigned feed twice daily at 08.00 AM (50% of the total ration) and 03.00 PM (50% of the total ration). Feed bunks were cleaned every morning and any residual feed was collected daily. The dry matter content of feed offered and residual rations were analyzed weekly before the morning feeding. Drinking water was provided ad libitum.

Feed intake was measured daily by weighing the ration given minus the remaining ration after 24 h of being given (dry matter basis). Body weights were recorded on days 0, 29, and 64 using a digital scale with an accuracy of two grams. Feed efficiency was calculated by dividing the body weight gain by total dry matter intake during the 64-day feeding period. No mortalities occurred during the study period.

Sampling and Measurement

Rumen fluid was collected using a stomach tube 3 h after feeding at day 64 of the trial period. The source of rumen fluid was taken by using the stomach tube and it was then filtered by using four layers of cheesecloth. The supernatant obtained was analyzed for NH_3, VFA, protozoa, and bacterial population.

Table 1: Nutrient composition of the concentrate mix, Napier grass, and Soybean Oil Calcium soap (SO-Ca) which were used in the experiment (% dry matter basis)

	DM	Ash	CP	EE	CF	NFE	TDN
Concentrate mix*	89.55	9.150	14.17	3.820	18.790	54.07	69.82
Napier grass	22.20	17.650	10.14	2.850	32.310	37.05	44.19
SO-Ca	81.53	26.780	0.520	13.600	0.550	58.56	72.36

Note: DM = Dry Matter, CP = Crude Protein, EE = Ether Extract, CF = Crude Fiber, NFE = Nitrogen Free Extract, TDN = Total Digestible Nutrient. Ash content represents the incombustible component remaining after a sample of the furnace oil is completely burned. *commercial product from company "Citra Feed Indonesia"

Table 2: Ration composition and nutrient content of each treatment (DM basis)

	Treatments		
	Control (C)	C+5% SO-Ca	C+10% SO-Ca
Napier grass (%)	70	70	70
Concentrate (%)	30	25	20
SO-Ca (%)	0	5	10
Total	100	100	100
Nutrient composition (%)			
Ash	15.10	15.98	16.86
Crude protein	11.35	10.67	9.98
The ether extract	3.14	3.63	4.12
Crude fiber	28.25	27.34	26.43
NFE	42.16	42.38	42.61
TDN	51.88	52.01	52.13

Note: SO-Ca = Soybean Oil Calcium Soap, NFE = Nitrogen Free Extract, TDN = Total Digestible Nutrient

The nutrient content of forage, concentrate mix, and SO-Ca was analyzed according to AOAC, (1990) for Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE), and Nitrogen Free Extract (NFE). Crude protein was calculated from nitrogen multiplied by 6.25. Ash content was measured by incinerating the sample for 8 h at 450°C in a furnace. Total Digestible Nutrient (TDN) was estimated using the following equation: TDN = -37.3039 +0.3618 (CF%) +2.1302 (EE%) +1.3630 (NFE%) +1.3048 (CP%) (Wardeh, 1981).

The NH_3 concentrations were analyzed according to the Conway micro-diffusion method (Waughman, 1981). Briefly, 1 mL of supernatant was placed on the left side of the Conway cup and 1 mL of saturated Na_2CO_3 solution was placed on the right side of the bulkhead. The small cup in the middle was filled with 1 mL of methyl red boric acid and green cresol bromine. The Conway cup was tightly closed with a Vaseline lid, then shaken so that the supernatant was mixed with the Na_2CO_3. The cup was then allowed to stand for 24 h at room temperature. The NH_3 bound by boric acid was titrated with H_2SO_4 0.0059 N until the color turned reddish.

The total VFA concentration and its molar proportion were analyzed using gas chromatography (Chrompack CP9002, Netherlands; flame ionized detector, oven temperature set to 60°C for conditioning, then running at 115°C, capillary column type WCOT Fused Silica 25 m × 0.32 mm and nitrogen as a gas carrier). A total of 1.5 mL rumen aliquot was mixed with 30 mg sulfosalicylic acid ($C_7H_6O_6S.2H_2O$), then centrifuged with a refrigerated centrifuge (7°C) at 12,000 rpm for 10 min. The sample of supernatant (0.5 µL) was injected into the GC and the chromatogram was calculated to obtain the total VFA, acetate, propionate, butyrate, and valerate concentration (Suharti *et al*., 2011). Methane estimation was calculated from the molar proportion of VFA according to the following equation: 0.45(C2) -0.275 (C3) +0.4(C4), where C2 = acetate, C3 = propionate and C4 = butyrate (Moss *et al*., 2000).

The protozoa population was counted using the Fuchs Rosenthal Counting Chamber (0.0625 mm^2 and 0.2 mm of depth). Rumen fluid (0.5 mL) was mixed with 0.5 mL of Trypan Blue Formalin Saline (TBFS) solution which consists of the following component: 100 mL of 35% formaldehyde, 900 mL of distilled water, 2 g of trypan blue, and 8 g of NaCl and diluted five times. The population of protozoa was enumerated directly by using a counting chamber under a microscope (40×) (Ogimoto and Imai, 1981).

Enumeration of the total bacterial population was done using anaerobic methods with roller tube cultivation (Ogimoto and Imai, 1981). Rumen-fluid-

Glucose-Cellobiose Agar (RGCA) modification was the media utilized for bacterial cultivation. The RGCA media had the following components: 15 mL of mineral mix solution I, 15 mL of mineral mix solution II, 0.1 mL of resazurin 0.1% solution, 40 mL of distilled water, 2 g of bacto agar, 30 mL of rumen fluid, 0.2 g of glucose, 0.2 g of cellobiose and 0.1 g of cysteine. HCl. H_2O, 1 mL Na_2CO_3 8% solution, 1 g of bacto casiton, 0.3 g of yeast extract, 0.2 g of starch-soluble yeast extract, 0.4 g of $NaHCO_3$, and 1 mL sodium lactate. The Hungate tube was filled with approximately 45 mL of the anaerobic dilution solution and 0.5 mL of the rumen sample, which was subsequently diluted 10 times. The 0.5 mL sample from dilutions 6-10 was added to petri dishes containing RGCA medium and rotated. Samples were cultured for 48 h at a temperature of 37-40°C. The following equation was used to determine the number of bacteria present: $C\times10^n\times2$, where C = the colony-forming unit's number and n is the number of dilutions.

At the end of the treatment (day 80), blood samples were taken. Blood was taken using jugular venipuncture with a 5 mL sterile syringe and put into an EDTA anti-coagulant tube that was stored in a cool box. Before analysis using a kit, the blood samples were centrifuged at 3,000 rpm for 10 min at room temperature. A vortex was used to blend a 10-l standard solution or sample with 1000 L of reagent and the mixture was then incubated for 10 min at 20-25°C. Blood hematology, cholesterol, glucose, triglycerides, total protein, and albumin were measured from the samples using the semi-autoanalyzer machine (photometer 5010, Reile, Berlin, Germany).

Data Analysis

A randomized block design with three treatment groups was used in this investigation with six animals per treatment. Calves were divided into weight blocks 1-6 to reduce the impact of initial body weight. Analysis of Variance (ANOVA) was performed using SPSS version 16 (IBM). The Duncan multiple range tests were used to examine the substantial difference between the treatments in more detail. The $p<0.05$ was found to be the significance level for the significant treatment and $0.05<p\leq0.10$ was found to be the relevance level for the tendency.

Results

The total protozoa population was dramatically reduced ($p = 0.0001$) by the addition of SO-Ca at levels of 5 and 10% in the rations compared to the control treatment, while the total bacterial population was unaffected (Table 3).

The addition of SO-Ca at the level of 10% in the rations also significantly increased the propionate proportion ($p = 0.03$) and NH_3 concentration was observed to rise ($p = 0.10$) in comparison to the control treatment. In contrast, It tended to lower valerate concentrations ($p = 0.1$) and methane estimation ($p = 0.06$) and reduced the proportion of acetate ($p = 0.045$), butyrate ($p = 0.03$ and C2/C3 ratios ($p = 0.02$), as well as the butyrate, butyrate, and C2/C3 ratios. Both total VFA production ($p = 0.20$) and rumen pH ($p = 0.23$) were unaffected (Table 4).

The addition of SO-Ca at the levels 5 and 10% increased the concentrate intake ($p = 0.01$) but had no impact on the amount of forage consumed ($p = 0.22$), total ration intake ($p = 0.17$), body weight gain ($p = 0.88$), feed efficiency ($p = 0.91$) or average daily gain ($p = 0.90$) (Table 5).

The following blood parameters did not change as a result of the treatments: Erythrocyte ($p = 0.78$), hemoglobin ($p = 0.93$), hematocrit ($p = 0.91$), mean corpuscular volume/MCV ($p = 0.93$), mean corpuscular hemoglobin/MCH ($p = 0.81$), mean corpuscular hemoglobin concentration/MCHC ($p = 0.96$), leukocyte ($p = 0.52$), neutrophil ($p = 0.58$), lymphocyte ($p = 0.88$), monocyte ($p = 0.44$), eosinophil ($p = 0.18$), basophil ($p = 0.88$) and neutrophil/lymphocyte (N/L) Ratio ($p = 0.72$). Blood metabolites such as albumin ($p = 0.94$), total protein ($p = 0.52$), and glucose ($p = 0.96$) were also not affected by the treatments. In contrast, the inclusion of SO-Ca at level 10% in the ratio resulted in a tendency for the level of cholesterol to drop ($p = 0.10$) and an increase in triglycerides ($p = 0.03$) (Table 6).

Table 3: Total protozoa and bacterial population of Bali calves fed diets containing or not calcium soaps of soybean oil (SO-Ca) at 5 or 10% of the diet

	Treatments			
Variable	Control (C)	C+5% SO-Ca	C+10% SO-Ca	p-value
Protozoa (log cell ml^{-1})	4.61 ± 0.07^a	4.40 ± 0.09^b	4.04 ± 0.10^c	0.0001
Total bacteria (log cfu ml^{-1})	6.35 ± 0.71	6.73 ± 0.88	6.45 ± 1.06	0.8500

Note: The different superscripts in the same row indicate significant differences

Table 4: Fermentation characteristics in the rumen of Bali calves fed diets containing or not calcium soaps of soybean oil (SO-Ca) at 5 or 10% of the diet

	Treatments			
Variable	Control (C)	C+5% SO-Ca	C+10% SO-Ca	p-value
Rumen pH	7.06±0.3400	6.70±0.290	6.90±0.270	0.230
NH_3 concentration	7.50±1.78^{b}	11.40±3.32^{a}	9.11±1.39^{ab}	0.100
VFA total (mm)	96.80±17.38	103.81±11.70	112.83±19.73	0.200
VFA proportion (%)	70.67±1.36^{ab}	72.13±3.56^{a}	68.32±1.46^{b}	0.045
Acetate (C_2)				
Propionate (C_3)	16.62±1.39^{b}	17.10±2.01^{b}	20.19±1.32^{a}	0.030
Isobutyrate + Butyrate (C_4)	10.68±0.78^{a}	9.28±1.45^{b}	9.70±0.63^{ab}	0.030
Isovalerate + Valerate (C_5)	2.04±0.54^{a}	1.49±0.35^{b}	1.80±0.20^{ab}	0.100
C_2: C_3	4.28±0.44^{a}	4.28±0.72^{a}	3.40±0.30^{b}	0.020
Methane estimation (mm)	31.50±0.83^{a}	31.47±1.64^{a}	29.07±0.95^{b}	0.060

Note: The different superscripts in the same row indicate significant differences, VFA = Volatile Fatty Acid

Table 5: Feed intake and performances of Bali calves fed diets containing or not calcium soaps of soybean oil (SO-Ca) at 5 or 10% of the diet (dry matter basis)

	Treatments			
Variables	Control (C)	C+5% SO-Ca	C+10% SO-Ca	p-value
Total ration (kg h^{-1} d^{-1})	3.31±0.75	3.47±0.60	3.50±0.72	0.17
Native grass (kg h^{-1} d^{-1})	2.33±0.54	2.42±0.42	2.46±0.50	0.22
Concentrate (kg h^{-1} d^{-1})	0.98±0.2^{a}	1.05±0.19^{b}	1.05±0.23^{b}	0.01
Initial BW (kg)	128±26.3	132±25.6	135±30.4	0.91
Final BW (kg)	147±31.6	150±28.5	155±33.7	0.92
Body weight gain (kg)	19.00±5.7	18.67±5.37	19.75±3.86	0.88
ADG (kg d^{-1})	0.30±0.09	0.29±0.08	0.31±0.06	0.90
Feed efficiency (%)	9.0±0.01	8.0±0.002	9.0±0.01	0.91

Note: The different superscripts in the same row indicate significant differences ($p<0.05$), DM = Dry Matter, BW = Body Weight, ADG = Average Daily Gain

Table 6: Blood profiles of Bali calves fed diets containing or not calcium soaps of soybean oil (SO-Ca) at 5 or 10% of the diet

	Treatments				
Variable	Control (C)	C+5% SO-Ca	C+10% SO-Ca	Standard	p-value
Erythrocyte (10^6 μl^{-1})	5.95±0.91	5.69±0.25	5.75±0.56	4.9-7.5	0.78
Hemoglobin (g dl^{-1})	9.28±1.35	9.07±0.71	9.08±1.20	8.4-12.0	0.93
Hematocrit (%)	25.18±3.22	24.55±2.06	24.72±3.58	21-30	0.91
MCV (fl)	42.53±3.04	43.13±2.79	42.92±3.23	36-50	0.93
MCH (pg)	15.62±0.81	15.93±0.80	15.77±0.81	14-19	0.81
MCHC (g dl^{-1})	36.82±1.97	36.95±1.03	36.78±1.13	38-43	0.96
Leukocyte (10^3 μl^{-1})	8.56±0.997	9.67±1.96	9.05±2.09	5.1-13.3	0.52
Neutrophil/N (10^3 μl^{-1})	0.35±0.23	0.38±0.21	0.24±0.14	0.0-0.2	0.58
Lymphocyte/L (10^3 μl^{-1})	7.39±0.89	7.70±1.38	7.78±2.05	1.8-8.1	0.88
Monocyte (10^3 μl^{-1})	0.65±0.29	0.95±0.37	0.62±0.51	0.1-0.7	0.44
Eosinophil (10^3 μl^{-1})	0.05±0.03	0.09±0.096	0.03±0.02	0.1-1.2	0.18
Basophil (10^3 μl^{-1})	0.07±0.06	0.08±0.08	0.08±0.07	0.0-0.2	0.88
N/L ratio	0.048±0.03	0.052±0.0295	0.037±0.025	≤1.15	0.72
Albumin (g dL^{-1})	2.52±0.40	2.55±0.35	2.54±0.3	2.1-3.6	0.94
Total protein (g dL^{-1})	7.67±0.30	7.83±0.48	8.02±0.35	5.7-8.1	0.52
Glucose (mg dL^{-1})	69.50±5.86	67.50±7.05	70.80±10.23	67.5-70.8	0.96
Cholesterol (mg dL^{-1})	138.00±33.69^{a}	127.75±15.13^{ab}	114.80±23.49^{b}	80-170	0.10
Triglyceride (mg dL^{-1})	91.17±2.14^{b}	87.25±4.57^{b}	109.00±17.55^{a}	0-14	0.03

MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, N/L: Neutrophil/lymphocyte

Discussion

The reduction in the total protozoa population with the addition of SO-Ca in the concentrate ratio indicates that SO-Ca causes defaunation activity in the rumen. The presence of free unsaturated fatty acids in SO-Ca may be the cause of this defaunation activity that might become dissociated in the rumen. It is possible that unsaturated fatty acids are not entirely resistant to microbial biohydrogenation in the rumen, despite the protection that calcium soap offers. Additionally, some free unsaturated fatty acids may be released into the rumen since the rumen is not completely inert to the unsaturated fatty acid salts in calcium and some of these bonds will break there (Sukhija and Palmquist, 1990).

Previous research by Klusmeyer and Clark (1991) has also reported the partial dissociation of soybean oil calcium salts, which suggests that calcium salts can protect up to 50% of the soybean oil against ruminal biohydrogenation. According to (Lundy Iii *et al*., 2004), the biohydrogenation activity of oleic acid (18:1) and linoleic acid (18:2) was up to 77.9% and 92.2%, respectively. In a previous study, we demonstrated that biohydrogenation activity in the rumen occurs up to 83% and 78%, respectively, with the use of 6% canola oil calcium soap or 6% flaxseed oil calcium soap (Suharti *et al*., 2017). The existence of biohydrogenation activity suggests that some free unsaturated fatty acids were liberated from the calcium soap made from canola and flaxseed oil.

It has been demonstrated that the overall protozoa population decreases as the number of double bonds in long-chain unsaturated fatty acids increases (Hristov *et al*. 2004). Our previous results also indicate that the use of oils containing unsaturated fatty acids reduced the protozoa population (Hidayah *et al*. 2014). Supplementation of oils that contain high amounts of unsaturated fatty acids without any protection decreased the protozoa population more than protected oils did during *in vitro* fermentation (Suharti *et al*., 2017). A disruption to the membrane integrity of protozoa may be the direct mechanism that causes a decrease in the protozoa population. By preventing chemotaxis and substrate acquisition, unsaturated fats may also be indirectly hazardous to protozoa, notably entodiniomorphids (Diaz *et al*., 2014).

The use of SO-Ca did not alter the bacterial population or growth in the rumen. Moreover, the calcium in the SO-Ca that may be dissociated from the rumen could also stimulate the growth of bacteria (Hidayah *et al*., 2014). The calcium soap, therefore, allows ruminants to optimize their rumen bacterial growth (Jenkins and Palmquist, 1984). Undesired bactericidal effects of unsaturated fatty acids can be controlled by certain alkaline minerals, such as calcium. Furthermore, calcium soap could also improve fiber digestibility by bacteria (Ferlay *et al*., 1993).

The use of SO-Ca did not change the rumen environment, as evidenced by the fact that the rumen pH was comparable across all treatments and remained within the normal range. The absence of pH change may be due to the pH level of SO-Ca, which is also in the normal range (6-7). Rumen pH has an essential role in optimal rumen fermentation, rumen development, and calf health. Calcium soap, which is inert, is not toxic to nor harms the rumen fermentation activity or the rumen ecosystem (Ferlay *et al*., 1993). The application of various oil preservation techniques, such as calcium soap or microencapsulation, had no effect on the rumen's pH (Hidayah *et al*., 2014). In the *in vitro* fermentation, the addition of canola and flaxseed oils protected by calcium soap at a level of 6% had no effect on the rumen's pH, dry matter digestibility, rumen protozoa, or total bacteria (Suharti *et al*., 2018).

The increase of NH_3 concentrations with the use of 5% SO-Ca demonstrated that the feed protein degradation was enhanced. This result indicates that the activity of rumen microbes was not altered in the presence of SO-Ca. One explanation for this would be the possibility of eliminating the detrimental effects of oil on rumen bacterial growth. The fatty acids present in the oil may have interfered with ruminal fermentation, including feed protein degradation, by inhibiting the direct adherence of microbes to plant membranes (Jenkins, 1993). The rumen's calcium soap did not impair the rumen bacteria's ability to break down the feed or ferment it.

In contrast, the use of SO-Ca at a higher level (10%) tended to decrease the NH_3 concentrations, which indicates that there was a reduction in feed protein degradation. This result also indicates that the oils that are released from SO-Ca to the rumen and used as energy sources, particularly glycerol, cause a reduction in the amount of protein degradation. Previous research showed that the use of calcium soap rapeseed fatty acids and soybean meal in the diet of Polish Friesian bulls also resulted in a slight decrease in ammonia concentrations (Kowalski *et al*., 1997).

The use of SO-Ca did not affect the total VFA production or methane estimations but did increase the amount of Propionate (C3) and reduced the quantity of Acetate (C2), Butyrate (C4), Valerate (C5), and C2:C3 ratio. The increase of the proportion of propionate without an increase in the overall production of VFA indicates that SO-Ca utilization might be able to modify the pattern of individual VFA, which decreased the acetate, butyrate, and valerate proportions and shifted to increase propionate production. These outcomes may be attributable to unsaturated fatty acids' capacity to promote the development of certain rumen microbes. Some butyrate-producing bacteria, such as the producers of stearate *Clostridium proteoclasticum, Butyrivibrio hungatei, and Eubacterium ruminantium,* are inhibited by

the presence of any polyunsaturated fatty acids (Maia *et al*., 2010). Unsaturated fatty acids' double bonds change the way they are shaped so that molecules with kinked double bonds cause lipid bilayers in bacterial membranes to become disrupted (Keweloh and Heipieper, 1996). The increasing propionate proportion with the use of SO-Ca might also be caused by the glycerol content in the SO-Ca which dissociated in the rumen. In the rumen, glycerol quickly ferments to produce propionate and butyrate (Kholif, 2019).

For ruminants, propionate serves as their main source of energy, especially beef cattle because propionate is the only major VFA that is glucogenic and rapidly converted to glucose (Yost *et al*., 1977). The increase of propionate production with the use of SO-Ca implies that SO-Ca enhanced the availability of energy sources for ruminants. Due to the shifting of propionate production, the acetate-to-propionate ratio (C2:C3) decreased with the inclusion of SO-Ca. Moreover, this ratio reduced methane estimates.

The increased concentrate intake indicates that concentrates containing SO-Ca are more palatable for calves than the control ration. This might be due to calcium soap's ability to reduce the rancidity activity of oils, thereby improving the palatability of the ration. The supplementation of corn or soybean oil containing unsaturated fatty had no impact on the intake of feed or nutrient digestibility of cattle fed long-stemmed alfalfa or orchard grass (Kouakou *et al*., 1994). On the other hand, Mahdavi *et al*. (2019), meta-analysis and meta-regression, found that lactating dairy cows fed with soybean oil produced more milk and had higher levels of unsaturated fatty acids in their milk when dry matter intake was reduced.

The similar growth, average daily gain, and efficiency of feed of the Bali cattle calves indicate that the addition of SO-Ca in this study could not improve the body weight gain of the calves. This result might be due to the growing phase of the calves, as more nutrients during this stage of development are used for organs, bone growth, and cell regeneration. Although the addition of SO-Ca 10% increased the propionate proportion, it does not yet promote the body weight growth caused by the short time observation in this study only 64 days. The Bali cattle calves's average daily gain used in this research remained in the normal range. The previous study reported that when compared to cohorts that weren't administered CSFA, the addition of Calcium Salt Fatty Acid (CSFA) to 0.3% of BW supplements boosted ADG (Cappellozza *et al*., 2020).

The similar blood metabolite levels of albumin, total protein, glucose, and triglyceride among the treatment groups may be due to the similar nutrient composition of each treatment. The level of albumin, glucose, and triglyceride was in the normal range, which indicates that the addition of SO-Ca did not alter the absorption or utilization of those nutrients.

The presence of unsaturated fatty acids in soybean oil may be the cause of the reduction in cholesterol that occurs when SO-Ca is added. This outcome suggests that there are some unsaturated fatty acids from the SO-Ca that are resistant to rumen microbial biohydrogenation and were absorbed in the intestines. Unsaturated fatty acids could reduce plasma cholesterol by decreasing lipogenesis and very low-density lipoprotein secretion and enhancing the return of cholesterol transfer (Fernandez and West, 2005). Previous research has shown that the use of olive oil, which is high in fats that aren't saturated, protected fish oil, and curcumin decreased the total cholesterol in the blood serum of Bali cattle (Kadarsih, 2011).

The increase of blood triglyceride concentrations with the addition of SO-Ca at the level of 10% might be due to an increase in fatty acid content in the rations. The major components of triglycerides are fatty acids and glycerol backbones (Chen, 2006).

The ratio of N/L of each treatment was lower than 1.5, which indicates that the cattle did not suffer stress with the supplementation of SO-Ca. Kannan and Jain (2000) suggested that cattle could be considered stressed when the N/L ratio was over 1.5. Thus the addition of SO-Ca up to 10% in their rations did not induce stress in this experiment.

Conclusion

The addition of SO-Ca up to 10% increased NH_3 concentrations and propionate proportions, decreased protozoa populations and the proportion of acetate, butyrate, valerate, and C2/C3 ratio, but did not affect bacterial populations, rumen pH, total VFA production or performance of Bali's calves. Furthermore, up to 10% SO-Ca use reduced cholesterol levels while having no effect on other blood metabolites such as albumin, total protein, and glucose.

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Author's Contributions

Sri Suharti: Study conception and design, data collection, analysis and interpretation of the result, drafted manuscript preparation, reviewed the final of the manuscript.

Sidik Yono and Dewi Kurniasari: Data collection, analysis, and interpretation of the result.

Lilis Khotijah, Dewi Ayu Warmadewi and I Gusti Lanang Oka Cakra: Data collection drafted manuscript preparation.

Komang Gede Wiryawan: Study conception and design, analysis, and interpretation of the result, drafted manuscript preparation, reviewed the final of the manuscript.

Ethics

There have been no disclosed potential conflicts of interest pertaining to this article.

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