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Research Article
Fermentability and Nutrient Digestibility of Ration Supplemented with Soybean Oil Calcium Soap and Cashew Fruit Flour

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Abstract

Objective: An *in vitro* study was conducted to evaluate the effect of soybean oil calcium soap (SOCS) and cashew fruit flour (CFF) supplementation on fermentability characteristics, microbial population and nutrient digestibility using rumen fluid of Bali cattle. **Materials and Methods:** The experiment was arranged in a complete randomized block design with 4 different ration treatments and 3 replicates. The ration treatments were R1: 40% native grass+60% concentrate, R2: 40% NG+60% C, containing 5% soybean oil and calcium soap, R3: 40% NG+60% concentration (C), containing 5% SOCS+10% CFF and R4: 40% NG+60% C, containing 5% SOCS+20% CFF. The measured variables were pH, NH_3-N, total volatile fatty acids and total gas production (fermentability characteristics), total bacteria and protozoa (microbial populations), dry matter and organic matter digestibility. The data were analyzed using one-way analysis of variance and differences between treatments were examined using the Duncan's multiple range test. **Results:** The results showed that the treatments significantly decreased ($p<0.05$) ammonia (NH_3-N) concentration and total volatile fatty acids production. The different feed treatments did not have any significant effect on pH, total bacteria, protozoa population, dry matter and organic matter digestibility. **Conclusion:** Supplementation of 5% SOCS in R2 and 5% SOCS+10% CFF in R3 treatments has better NH_3-N concentration, total VFA and total gas production compared to the other treatments.

Key words: Polyunsaturated fatty acid, biohydrogenation, calcium soap, cashew fruit, tannin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the past decades, increasing number of studies have shown that vegetable oils contain high polyunsaturated fatty acid that can be used to substitute animal fat in feed ingredients. Vegetable oils, such as soybean oil, contain highly unsaturated fatty acid and they have been widely used to supplement the cattle, poultry and pig's feeds[1]. Soybean oil contains polyunsaturated fatty acid (PUFA), such as linoleic acid (>50%), oleic acid (21-25%) and linolenic acid (5-6%)[2] are beneficial to animals. Supplementation of the vegetable oil in feed has been proven beneficial for ruminant. However, its effectiveness is limited due to the negative effect of PUFA on rumen digestion. Typically, PUFA undergoes many alterations inside the rumen[3] and can cause a negative impact on rumen fermentation, reduce crude fiber digestibility and lead to dry matter consumption[4]. The transformation of unsaturated fatty acids to saturated fatty acids in ruminants represents a major human health issue[5]. In this context, only small amount of PUFA will be able to reach small intestine of the animal due to this biohydrogenation and esterification process[6]. The biohydrogenation process of fatty acid in the rumen serves as a detoxification mechanism to avoid the bacteriostatic effect of the unsaturated fatty acid that could damage membrane integrity and reduce the microbial growth[7].

The negative impacts of PUFA in the rumen digestion process are the underlying reason to control the biohydrogenation of the PUFA to maximize vegetable oil supplemented for ruminant ration. However, this PUFA needs to be protected prior to supplementation to avoid biohydrogenation process by rumen microbes, to decrease rumen microbial growth and activity and improve feed digestibility. Some protection methods that have emerged over the years involve either encapsulation of unsaturated fatty acids inside a microbial-resistant shell, (such as formaldehyde-treated or lipid encapsulated) or alteration of fatty acid structure, (such as calcium salts or fatty amides) to resist the action of microbial enzymes[8]. Fat protection with calcium soap method may depress PUFA biohydrogenation process in the rumen, increase ether extract (EE) digestibility in the post-ruminal digestion[9] and increase PUFA in the body tissue of beef cattle[8].

In addition to chemical methods, plant polyphenolic compounds, such as tannin can be used to suppress the negative effects and PUFA biohydrogenation process inside the rumen[10]. Tannin compound can reduce the biohydrogenation process of vaccenic acid (t11 C18:1) to stearic acid (C18:0), increase the accumulation of vaccenic acid, decrease the concentration of stearic acid in the post-rumen digestive tract[11], lower the production of methane gas and escalate the flow of "by pass" protein[12]. In spite of this, the usage of tannin needs to be controlled because the nutrient digestibility can clearly be hampered by increasing level of condensed tannin as confirmed by both *in vitro* and *in vivo* studies[13].

One of the local feed ingredients that contain tannin is the cashew nuts (*Anacardium occidentale* L.) and dried cashew fruits that contain condensed tannin around 0.18 mg/100 g[14]. Although, the tannin compound is one of the limiting factors that prevents the use of cashew apples, the potency of its biomass and nutrient contents gives cashew fruits as potential to be used as a source of energy in ruminant rations[15]. Dried cashew fruit contains 12.68% crude protein, 10.05% crude fiber, 2.15% ash, 12.21% calcium, 0.26% phosphorus and 22.80% total sugar[16]. Bain *et al.*[17] reported that the supplementation of 5% soybean oil calcium soap and 10% cashew fruit flour in the ration improved the digestibility of organic matter, ether extract and linoleic fatty acids content in Bali cattle meat.

Despite its high nutritional content, the potential use of the cashew fruit as a ruminant feed ingredient still faces some issues related to its astringent and itchy taste when consumed[18]. The astringent taste of cashew fruit is associated with its tannin content and itchy nature triggered by its urushiol and phytic acid contents. Tannin, phytic acid and urushiol acid may prevent optimal consumption, digestibility and nutrient absorption, which may lead to poor cattle's productivity[15]. Inadequate information on the use of cashew fruits as a cattle feed necessitates an evaluation of its impact on fermentation process and nutrient digestibility in ruminant rations. In assessing nutritive value, the rate at which a feed or its chemical constituents are digested in the rumen is as important as its digestive process. The pattern of feed fermentation (kinetics of fermentation) is one of the factors that influences voluntary feed intake by ruminants[19].

Following the above-mentioned potentials and drawbacks, a rumen fluid-based *in vitro* research needs to be carried out to assess the supplementation effectiveness of soybean oil, calcium soap and cashew fruit flour on ruminant ration's fermentability, microbial population and nutrient digestibility using rumen fluid of Bali cattle.

Moreover, this study explores energy sources from a combination of soybean oil calcium soap (SOCS) and cashew fruit flour (CFF) as PUFA for ruminant livestock ration. The goal of the study is to assist researchers to uncover the critical roles of soybean oil calcium soap and tannin compound in cashew fruit flour, which assist in improving the efficiency of PUFA and energy supplementation for increasing the productivity and quality of cattle meat for human consumption.

MATERIALS AND METHODS

Treatment rations: The experimental rations consisted of 40% native grass (NG) and 60% concentrate (C). The concentrate's material consisted of tapioca waste, Pollard, coconut meal, cashew fruit flour, soybean oil, calcium soap, molasses, $CaCO_3$ and urea. The SOCS was produced following the method developed by Kumar *et al.*[20] using soybean oil (Mazola brand, produced by © ACH Food Companies, Inc), distilled water, NaOH (Supplied by Wuhai Xinye Chemical Industry Co., Ltd) and $CaCl_2$ (Keg King, 2/33-35 Smith Road Springvale, Vic 3171). The production of SOCS was initiated by using a specified soap saponification amount in determining the amount of required NaOH concentration[21]. The NaOH solution and soybean oil were mixed and heated on a hot plate up to a temperature of 180°C and stirred at 800 rpm rotation until achieving the NaOH solution and soybean oil dissolved completely. The $CaCl_2$ solution (made from 2.35 g of $CaCl_2$ and mixed into 4.7 mL of distilled water or in the scale of $CaCl_2$: water = 1: 2) was added slowly to the mixture and stirred up until the SOCS was formed. Then, SOCS was dried up for 24 h at 60°C and ready to be mixed with other concentrate materials.

The cashew fruits (*Anacardium Occidentale* L.) used for the experiment were red and yellow originating from a cashew farm in Muna District, Southeastern Sulawesi. The preparation of the cashew fruit flour (CFF) was done by slicing cashew fruits into small pieces and drying them in the sunlight to reduce the water content. The next step was removing the water content completely by drying the CFF in an oven (manufactured by Memmert GmbH+Co.KG, Äuβere Rittersbacher Startβe 38) set at 60°C. After that, the coffee grinder is used to convert them to cashew fruit flour (CFF).

The research was arranged in a completely randomized block design (rumen fluid collection period) to test 4 types of treatment rations with 3 replicates. The treatment rations consisted of, R1-40% native grass (NG)+60% concentrate (C), R2-40% NG+60% C, containing 5% soybean oil calcium soap (SOCS), R3-40% NG+60% C, containing 5% SOCS and 10% cashew fruit flour (CFF) and R4-40% NG+60% C, containing 5% SOCS and 20% of cashew fruit flour (CFF). The proportion of the raw materials of each treatment is presented in Table 1. Nutrient composition of the treatment ration was compiled with 68% TDN, 13% crude protein (CP) and 3.91-6.54% ether extract (EE) in accordance with the standard nutrient requirement of Bali cattle that weighed 250 kg[22]. The nutrient composition of the treatment rations (% dry matter) is presented in Table 2.

Table 1: Proportion of the raw materials of each treatment ration (% dry matter)

	Treatments			
Ration ingredients	R1	R2	R3	R4
Native grass	40.00	40.00	40.00	40.00
Concentrate	60.00	60.00	60.00	60.00
Tapioca waste	30.00	25.00	23.00	19.00
Pollard	31.50	31.50	26.00	25.00
Coconut meal	20.50	20.50	18.00	13.00
Cashew apple flour	0.00	0.00	10.00	20.00
Molasses	15.00	15.00	15.00	15.00
$CaCO_3$	1.50	1.50	1.50	1.50
Urea	1.50	1.50	1.50	1.50
Soybean oil calcium soap	0.00	5.00	5.00	5.00

Table 2: Nutrient composition of the treatment rations (% dry matter)

	Treatments			
Nutrient compositions	R1	R2	R3	R4
Dry matter	76.00	76.09	75.56	75.01
Ash	6.37	6.91	6.94	6.94
Crude protein	13.21	13.17	12.91	12.83
Ether extract	3.91	6.48	6.54	6.53
Crude fiber	16.71	16.38	16.58	16.58
NFE	59.80	57.06	57.03	57.12
TDN	71.19	73.76	73.60	73.57
Ca	0.52	0.51	0.52	0.53
P	0.25	0.25	0.21	0.19

R1: 40% native grass (NG)+60% concentrate (C), R2: 40% NG+60% C, containing 5% soybean oil calcium soap (SOCS), R3: 40% NG+60% C, containing 5% SOCS+10% cashew fruit flour (CFF), R4: 40% NG+60% C, containing 5% SOCS+20% cashew fruit flour

Research procedure: The study was conducted for 2 months, from May-July, 2014, in a number of laboratories in the Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, namely (a) *In vitro* testing, measurements of total gas production were held in the Laboratory of Dairy Animal Nutrition (b) Measurement of the microbial population was conducted in the Laboratory of Microbiology, Physiology and Nutritional Biochemistry (c) Proximate analysis of rations was performed in the chemical analytic laboratory of The Livestock Research Center, Ciawi, Bogor.

Research variables observed consisted of (1) Fermentation characteristics (pH, NH_3-N concentration, total VFA and total gas production), (2) Population of microbes (total bacteria and protozoa) and (3) Nutrient digestibility consisted of dry matter and organic matter. The number of samples measured for each variable was Duplo in each replication of the experiment.

In vitro fermentation was conducted following Tilley and Terry[23] method. Each treatment ration sample (500 mg) and 40 mL McDougall solution was poured into a 100 mL fermenter tube. The tube was then added with 10 mL of the

Bali's cattle rumen fluid, slowly stirred and flushed with CO_2 gas and then incubated in the shaker water bath (manufactured by Memmert GmbH+Co.KG, Äußere Rittersbacher Startβe 38) set at 39°C for 24 h. Rumen fluid of the Bali cattle, used in the treatment, was previously collected 4 h after morning feeding using a stomach tube connected to a vacuum pump. The rumen fluid sample collection procedure has been approved by Animal Care and Use Committee (ACUC), Bogor Agricultural University, No. 08-2015 IPB.

The pH, VFA, NH_3-N, total bacteria and protozoa population measurement of the fermented fluid samples in the fermenter tube were conducted 4 h after the incubation. The measurements of dry matter and organic matter digestibility were carried out by incubating a fermenter tube filled with the ration sample in the shaker bath set at 39°C for 48 h.

Collection and measurement of samples: Rumen fermentation characteristic variables were pH (measured with pH meter, manufactured by Hanna Instruments, 3820 Packard Road, Suite 120 Ann Arbor, Michigan 48108), the concentration of NH_3-N analyzed with Conway micro diffusion method of Conway[24] and total VFA concentration was analyzed using steam distillation method. Protozoa population was counted with the aid of a microscope of 40x magnification using Fuchs Rosenthal Counting Chamber, size 4×4×0.2 mm (manufactured by Hausser Scientific, Montgomery County, Pennsylvania, USA) on which the *in vitro* liquid (0.5 mL) was incubated for 4 h and mixed with 0.2 mL methyl green formaldehyde saline solution[25]. Bacteria in the Hungate tube were visually counted to determine the bacterial population. Before counting, the bacterial culture was diluted using BHI medium of Ogimoto and Imai[25] and homogenized in a roller tube.

The 48 h incubated fermentation liquids were measured for their dry matter and organic matter digestibility. The process started with *in vitro* liquid, where the fermenter tube was taken out from the shaker bath after 24 h incubation. At the end of the first incubation period, the 2-3 drops of the $HgCl_2$ solution were added to stop the microorganism fermentation process, then centrifuged at 3500 rpm to separate the supernatant and residue. After discarding the supernatant, 50 mL of freshly-made pepsin solution were added to the residue in each tube. The tubes were then incubated at 39°C for 48 h with occasional shaking. Anaerobic conditions were not necessary during this stage. At the end of the incubation, the supernatants were discarded and the insoluble residues were washed with water in the centrifuge. The substrate was then filtered using Whatman paper No. 41 (distributed by Voigt Global, P.O. Box 1130, Lawrence, Kansas 66044 USA), placed in a porcelain cup and then dried in an oven set at 105°C for 8 h at a constant weight. The dry weight of residue was calculated. Then, it was followed by incineration set at 550°C for 4 h to measure the ash weight.

The total gas production was measured with the gas test method of Menke *et al.*[26]. Using this method, the ration samples (230 mg each) were ground and filtered by using 1 mm filter and placed into syringes. The samples in the syringe tubes were flushed with CO_2 gas, then incubated in a water bath set at 39°C. Each rumen fluid sample (amount 30 mL), that had been filtered and saturated with CO_2 gas for 10 min, was poured into a new tube and closed with a tube piston lubricated with Vaseline. The piston was then pressed until there was no gap between the piston and the syringe. The first volume of each syringe was recorded prior to the incubation (0 h). Gas production was observed in the series of incubation times of 4, 8, 12 and 24 h. The total gas production was measured using the following equation (Menke *et al.*[26]):

$$\text{Gb (mL/230 mg DM, 24 h)} = (\text{Gb24-Gb0})\times 230\times\left[\frac{(\text{FH+FC})/2}{\text{DM material}}\right]$$

Where:

FH = Standard gas production divided by the actual production of the native grass

FC = Standard gas production divided by actual production of the concentrate

Data analysis: The data were analyzed using one-way ANOVA followed by Duncan's multiple range test using the Statistical Package for the Social Sciences (IBM®SPSS® version 21.0, Armonk, New York). Differences were considered significant at $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

Fermentation characteristics: The supplementation of 5% SOCS combined with CFF at the level of 10 and 20% in the concentrate ration had no significant effect on pH level and total gas production, but the treatments significantly decreased ($p<0.05$) NH_3-N concentration and total VFA production (Table 3).

Table 3: Effect of soybean oil calcium soap and cashew fruit flour addition on *in vitro* fermentation products

	Treatments				
Variables	R1	R2	R3	R4	p-value
pH	6.75±0.24	6.75±0.15	6.75±0.10	6.75±0.10	1.00
NH_3-N (mM)	10.23±1.86^{a}	10.43±1.98^{ab}	7.95±1.30^{c}	7.76±1.91^{cd}	0.03
Total VFA (mM)	139.35±16.79^{a}	142.72±21.95^{a}	110.77±54.52^{a}	49.08±9.43^{b}	0.03
Total gas production (mL/230 mg DM)	237.08±54.65	154.29±7.29	211.82±7.89	189.35±29.46	0.06

R1: 40% native grass (NG)+60% concentrate (C), R2: 40% NG+60% C, containing 5% soybean oil calcium soap (SOCS), R3: (40% NG+60% C, containing 5% SOCS+10% cashew fruit flour (CFF) and R4: 40% NG+60% C, containing 5% SOCS+20% cashew fruit flour (CFF). Different superscripts on the same row show significant differences ($p<0.05$)

Table 4: Effect of soybean oil calcium soap and cashew fruit flour addition on microbial population

	Treatments				
Variables	T1	R2	R3	R4	p-value
Total bacteria (log CFU mL^{-1})	8.30±1.10	7.82±1.12	6.90±0.34	6.91±0.79	0.42
Protozoa (log cell mL^{-1})	5.09±0.32	5.20±0.28	4.96±0.74	4.99±0.32	0.91

R1: 40% native grass (NG)+60% concentrate (C), R2: 40% NG+60% C, containing 5% soybean oil calcium soap (SOCS), R3: 40% NG+60% C, containing 5% SOCS+10% cashew fruit flour (CFF) and R4: 40% NG+60% C, containing 5% SOCS+20% cashew fruit flour (CFF)

The data showed that different ration treatments have relatively similar pH values and total gas production. The pH values for all treatments were relatively similar around 6.75, which was a normal pH level to support the fermentation process in the rumen[27]. The normal pH level indicated that the 5% addition of SOCS in R2 ration and 5% of SOCS combined with 10% of CFF and 20% of CFF had no negative effects on rumen ecosystem condition. The normal pH condition is very important to support the growth and microbe's activity in decomposing the ration. Franzolin and Dehority[28] stated that pH concentration had an important role in preserving rumen stability and microbial growth.

The supplementation of 5% SOCS in the ration resulting in normal pH level, which was contributed by calcium soap that protected unsaturated fatty acid in the soybean oil from the microbes poisoning. The undisrupted growth and activity of the microbes had resulted in the normal rumen ecosystem condition. This was confirmed with the similar values on all treatments of the microbial population (total bacteria and protozoa) and nutrient digestibility (dry matter and organic matter) (Table 4). This condition was expected as calcium soap reduces PUFA solubility, avoids disruption of microbial growth and increases ration digestibility in the rumen. In addition, the use of the SOCS and CFF in cattle rations did not harm the rumen protozoa population, which contributed to the pH normalization. Franzolin and Dehority[28] reported that protozoa had an important role in preserving the rumen pH due to its faster capability in digesting starch, slowing down the fermentation process by the rumen bacteria and producing organic acids (volatile fatty acids). This capability can be linked with protozoa's capability, which is able to grow in the substrate containing tannin. Protozoa are not as sensitive as bacteria in the presence of tannin in the ration[29].

Different treatment rations also resulted in different levels of NH_3-N. The levels of NH_3-N of R1 (control) and R2 rations were significantly ($p<0.05$) higher than R3 and R4 treatments. There were no significant differences in NH_3-N levels between R1 and R2 or between R3 and R4. Nevertheless, the averages of NH_3-N in all treatments were still in a normal range (7.76-10.43 mM). The ammonia production averages were still capable of supplying the nitrogen source for rumen microorganisms to keep growing and conducting the fermentative activities. The ammonia was the most important nitrogen source for microbial protein synthesis in the rumen[30]. McDonald *et al.*[27] stated that fermentation level inside the rumen was normal if the level of average NH_3-N was in the range of 6-21mM.

The average of the NH_3-N production that was decreasing in R3 and R4 treatments could be caused by the addition of the CFF. The possible explanation for this trend was the tannin content of 10 and 20% of CFF that bound the protein and causing a decrease of the ration protein degradation inside the rumen. Jayanegara and Palupi[13] reported that tannin can create a complex bond with protein compound and carbohydrates. The complex bond is stable in the normal rumen pH, but the bond is deteriorated if the pH falls below 3.5, such as pH 2.5-3.0 in the abomasum or if the pH increases above 8 (pH in the duodenum)[31]. The strong bond between tannin and protein might result in the decrease of the protein degradation by the microbial enzymes with further implication on the decrease of ammonia production inside the rumen. A study by Wina *et al.*[12] found that inclusion of 4% tannin concentration in the feed depressed ammonia production in the rumen of sheep before and after 3 h of morning feeding. Despite the tannin present in the ruminant ration, this still resulted in a decrease of the protein

digestibility and the complex bond of the tannin-protein was beneficial to the protein flow in the post rumen digestive system[32]. This was confirmed by Wina *et al.*[12], who concluded that even if tannin component reduced the crude protein digestibility, it still provided benefit in increasing protein bypass required for cattle's growth.

The supplementation effect of 5% SOCS+10% CFF in the R3 ration and 5% SOCS+20% CFF in R4 ration also resulted in some changes of total VFA production. Despite total VFA levels in the ration treatments, R1, R2 and R3 were still in the normal range of 70-150 mM[27]. Total VFA levels in the treatment rations of R1, R2 and R3 were not significantly different. The supplementation of 5% SOCS+20% CFF resulted in the lowest total VFA. The low total VFA production in R4 treatment was suspected to be caused by the effect of high tannin concentration in the CFF. This effect could be linked to the study of Khiaosa-Ard *et al.*[11], who reported that tannin compound in the rumen inhibited the lipolysis process due to its behavior of poisoning bacteria species responsible in fatty acid biohydrogenation process. The low total VFA production in the R3 and R4 treatments was possibly related to the side effect of tannin that could decrease the ammonia concentration. According to Jayanegara and Palupi[13], condensed tannin phenolic compound may modify the rumen fermentability by inhibiting the production of ammonia and methane gas (CH_4). The ammonia is the main substrate for microbes to grow and synthesizing the enzymes[33]. The non-optimal growth and activities of bacteria will subsequently affect the ration fermentation process in producing higher total VFA during the incubation period. Nevertheless, the total VFA productions are achieved when the treatment of R1, R2 and R3 is in a normal range. McDonald *et al.*[27] stated that the total VFA levels capable of supporting normal fermentation process are in the range of 70-150 mM.

The supplementation of SOCS and CFF in concentrate resulted in varying levels of total gas production. The low total gas production in R2 ration was possibly caused by linoleic acid contained in SOCS depressed hydrogen production (H_2). Hydrogen is used by methanogen microbes to produce CH_4 (methane). The consequence of the inhibited methanogen activities will reduce the CH_4 and total gas production[34]. It was confirmed by Li *et al.*[5], who reported that the addition of linoleic fatty acid (C18:2) and linolenic acid (C18:3) significantly reduced the population of *Methanobacterium formicicum* compared to oleic acid addition. The strong influence of soybean oil linoleic acid content (reaching 52.16%) in limiting the total gas production and methane gas in this research was caused by the dissociation of the linoleic fatty acid bond with the calcium ion from the soybean oil calcium soap. Jenkins and Bridges Jr.[8] confirmed this by stating that long chain of unsaturated fatty acids, such as linoleic acid and linolenic acid, were difficult to be protected from the biohydrogenation process of the rumen bacteria compared to oleic fatty acid to increase the unsaturated fatty acid content of milk and red meat. The alteration of the nutritional characteristics and the strength of the unsaturated fatty acid calcium soap were affected by rumen ecosystem dynamics and microbial biohydrogenation process inside the rumen[35]. The high total production of gas in the rumen may be related to the high content of total sugars in the ration. The raw materials and the carbohydrate content in the diet contribute to 40% of the total gas production[27]. Cashew fruit contains 52.82% of total sugars in fresh form[36]. Akinfemi *et al.*[37] reported that the fastest and the high levels of gas production were influenced by the soluble carbohydrate fraction readily available in the feed that could be used by microbes. The high content of easily fermentable starch, sugar and hemicellulose are the compounds for rumen microbes to produce gas[38]. Numerous microorganisms in the rumen use carbohydrates as an energy source.

Microbial population: The supplementation of 5% SOCS in R2 ration, 5% SOCS+10% CFF in R3 and 5% SOCS+20% CFF in R4 ration had no significant effect on the total population of bacteria and protozoa (Table 4). The data showed that the supplementation of 5% SOCS (R2), combination of 5% SOCS+10% CFF (R3) and 5% SOCS+20% (R4) had no significant difference in the total population of bacteria and protozoa. The inclusion of 5% SOCS and CFF in the level of 10 and 20% in the ration might play a role in this aspect, which did not cause the rumen ecosystem variable to decrease and the rumen microbes to grow and function normally. The phenomenon was confirmed with dry matter and organic matter digestibility of the ration which was relatively similar for all treatments (Table 5).

The SOCS and CFF supplementation in the rations were unsuccessfully promoting an optimized growth of bacteria and protozoa. This was possibly related to the unsaturated fatty acid content and tannin compound existed in each supplement components. The unsaturated fatty acids could be toxic to rumen due to its ability to inhibit cell integrity and microbial growth, therefore disrupt feed fermentation process, especially feed fiber fermentation[7]. The non-optimal microbe population in this study was caused by the effect of tannin suppressing the ammonia productions in the rumen. Wallace *et al.*[33] reported that ammonia was the main substrate in protein synthesis and supporting microbial growth.

Table 5: Effect of soybean oil calcium soap and cashew fruit flour addition on nutrient digestibility values

	Treatments				
Variables (%)	R1	R2	R3	R4	p-value
DMD	71.81±3.50	73.10±1.65	72.46±0.71	71.46±0.79	0.77
OMD	74.64±2.34	75.44±2.79	74.86±1.28	74.10±2.11	0.86

R1: 40% native grass (NG)+60% concentrate (C), R2: 40% NG+60% C, containing 5% soybean oil calcium soap (SOCS), R3: 40% NG+60% C, containing 5% SOCS+10% cashew fruit flour (CFF) and R4: 40% NG+60% C, containing 5% SOCS+20% cashew fruit flour (CFF)

The addition of SOCS (5%) and CFF (10 or 20%) in the feed concentrate did not significantly affect the protozoa population. Sultana *et al.*[39] reported a similar finding that protozoa population of cattle fed with a ration containing calcium soap made from soybean and linseed oils was not changed. In contrast, Meraj *et al.*[40] found that fatty acid calcium soap significantly decreased the number of rumen protozoa without affecting the rumen's pH and NH_3-N. The inconsistency of fatty acid calcium soap effect on rumen microbial growth could be related to the physical stability and composition of the calcium soap product[35]. The concentration and level of the unsaturated fatty acid, as well as feeding frequency, might also contribute in the inconsistency[35].

The SOCS supplementation at 10% in R3 ration and 20% CFF in the R4 ration had no significant effect on the protozoa population during the incubation process. This is theorized by Patra and Saxena[29] that protozoa are not as sensitive as bacteria in the presence of tannin in the ration.

Nutrient digestibility: The supplementation of 5% SOCS combined with CFF at the level of 10 and 20% had no significant effect on dry matter digestibility (DMD) and organic matter digestibility (OMD) (Table 5). The data showed that the supplementation of 5% SOCS and 10 and 20% CFF in the rations resulted in relatively similar values of DMD and OMD in all treatments. Hidayah *et al.*[41] reported similar findings where vegetable oil supplementation (sesame oil, canola oil and linseed oil) protected by calcium soap had no effect on dry matter and organic matter digestibility, as well as the total population of rumen bacteria.

The averages of DMD and OMD obtained from all treatments were relatively similar, ranged between 71.46-73.10 and 74.10-75.44%, respectively. This was possible due to the effect of SOCS and CFF supplementation, which had no negative impact on the microbial population, pH and NH_3-N concentration. Fiorentini *et al.*[42] reported that fatty acid calcium soap had no effect on ration nutrient digestibility (dry matter digestibility, crude protein digestibility and crude fat digestibility, propionate concentration and total fatty acid). pH level of 6.75 and NH_3-N concentration of 7.76-10.43 mM were both considered within the optimum ranges to support the fermentation process in the rumen. McDonald *et al.*[27] also found similar results where the normal variations of temperature, pH, NH_3-N during the fermentation process in the rumen were between 38-42°C, 6.0-7.0 and 6-21 mM, respectively.

The total VFA production in R4 treatment was below the normal average (49.08 mM) to support the fermentation process. Nevertheless, the low total VFA production had no negative effect on the growth and total population of the rumen microbes. This result was confirmed by the relatively similar values of microbial population, NH_3-N concentration and nutrient digestibility (DMD and OMD) in R4 treatment compared to the other treatments.

Rations contain 5% SOCS (R2) and 5% SOCS combined with 10% CFF (R3) that can be used as a source of PUFA and energy in the fattening of Bali cattle. To increase the effectiveness of SOCS utilization as an *in vivo* PUFA source, it is necessary to improve the method used in the manufacturing of soybean oil calcium soap to minimize the negative effects and PUFA biohydrogenation process in rumen digestion.

CONCLUSION

The addition of 5% SOCS and 10% CFF in ration resulted in better ammonia concentrations, total VFA production and total gas production compared to the other treatments without any negative effect on the microbial population.

SIGNIFICANCE STATEMENT

This study explores the possible use of soybean oil calcium soap (SOCS) and cashew fruit flour (CFF) as PUFA and energy sources in ruminant livestock ration. The study uncovers some critical roles of SOCS and CFF's tannin compound in cattle's feed. The SOCS improved the absorption efficiency of PUFA while CFF's tannin compound increased energy supplementation in cattle's feed. The combination of SOCS and CFF has prospects for application in the fattening of Bali cattle.

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