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Supplementation of Zinc Palm Oil Soap Improves Feed Fermentability and Unsaturated Fatty Acid Profile in Rumen Liquid

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ABSTRACT

This study aimed to evaluate the effects of energy and organic zinc supplements, specifically zinc palm oil soap (ZPOS), on digestibility and unsaturated fatty acid profiles *in vitro*. The study used a completely randomized design with 4 treatments and 5 replications. The treatments were: T0= basal diet without supplementation, T1= basal diet + 5% palm oil (PO), T2= basal diet + 5% partial ZPOS (3.75% ZPOS + 1.25% PO), and T3= basal diet + 5% ZPOS. The inoculum source was rumen liquid from three fistulated female dairy goats and was homogenized. The goats were fed ration consisting of corn straw, soybean hulls, and concentrate containing total digestible nutrients (TDN) 63%, crude protein (CP) 14%, and neutral detergent fiber (NDF) 35%. Results showed that both 5% partial ZPOS and 5% ZPOS supplementation (T2 and T3) resulted in the increase of total volatile fatty acids (VFA), acetate, propionate, butyrate, unsaturated fatty acids (USFA) and a decrease in the ratio of acetate/propionate (A/P) compared to the control and supplementation of 5% PO ($p<0.05$). Supplementation of 5% partial ZPOS (T2) is better than 5% ZPOS because increased the digestibility of ether extract (EE), crude fiber (CF), NDF, and acids detergent fiber (ADF) ($p<0.05$) and decreased of methane compared to the control ($p<0.05$). In conclusion, adding 5% partial ZPOS (3.5% ZPOS and 1.5% PO) increases fiber digestibility, VFA, LCFA, and USFA concentration, and decreases methane production in the rumen liquid.

Keywords: fermentability; rumen; zinc palm oil soap; unsaturated fatty acids

INTRODUCTION

Early lactation dairy cows experience negative energy balance due to the decreased dry matter intake (DMI). Lack or energy imbalance during that period may reduce production and stimulate metabolic disorders (Khotijah *et al.*, 2017). This opinion was supported by Tribout *et al.* (2023), who stated that energy deficiency has detrimental effects, including low production and weight loss in livestock. To address this challenge, especially during early lactation, providing additional energy sources through supplements is crucial.

Energy requirements of dairy livestock in tropical regions differ from those in temperate areas due to varying environmental conditions and feed resources. Assessment of the energy balance of tropical and temperate crossbred dairy cows revealed significant differences in serum metabolic profiles, indicating variations in energy utilization and metabolism between the two regions (Ranaweera *et al.*, 2020). Meta-analysis research by Oliveira (2015) found that tropical dairy cows (*Bos taurus* x *Bos indicus*) have lower MEm requirements and net energy efficiency for milk production is also lower than temperate dairy cattle (*Bos taurus*). Therefore, understanding these differences is very important to optimize feeding strategies, especially energy source supplementation in dairy livestock.

Palm oil is one of the most promising vegetable oils for energy sources, with a gross energy (GE) content of 9219 kcal/kg (Khaskheli & Chou, 2020). Palm oil is also easily obtainable, resistant to rancidity, and affordable. The global price of palm oil is $574 per metric ton (mt), which is cheaper compared to soybean oil at $684/mt, coconut oil at $830/mt, and peanut oil at $1,584/mt (World Bank, 2020). Palm oil contains saturated fatty acids in the form of palmitic acid 44% and unsaturated fatty acids, specifically oleic acid 39.2% and linoleic acid 10.1% (Mancini *et al.*, 2015). High levels of palmitate are very beneficial for cellulolytic bacteria. Cellulolytic bacteria can incorporate palmitic acid into their cell membranes, thereby increasing energy availability for metabolic processes. Recent findings by Sears *et al.* (2024) showed an increase in *Prevotella* and *Fibrobacter* populations in response to palmitic acid supplementation. Unsaturated fatty acids in palm oil (oleic acids and linoleic acids) contribute to energy efficiency by reducing protozoa (Muktiani *et al.*, 2020; Vargas-bello-p *et al.*, 2020), reducing methane (CH4) production and the acetate/propionate (A/P) ratio by increasing propionate production (Gao *et al.*, 2016).

However, oil supplementation also has negative effects. Fat particles tend to coat feed particles, which hinders the adhesion of rumen microbes, especially fibrolytic microbes, thus reducing fiber digestibility (Sears *et al.*, 2024). Rumen microbes defend against lipid toxicity by performing biohydrogenation, which converts unsaturated fatty acids into saturated fatty acids in the rumen (Shingfield & Wallace, 2014).

Palm oil contains 49.3% unsaturated fatty acids, and when used in animal feed, it must be protected to prevent biohydrogenation and to avoid disrupting rumen bacterial fermentation. It is essential to safeguard unsaturated fatty acids to preserve their biological roles, such as being structural components of bio membranes that uphold membrane integrity. Polyunsaturated fatty acids in biomembranes ensure membrane fluidity, which supports the activity of membrane-bound enzymes, facilitates intracellular metabolism and enhances nutrient utilization efficiency in livestock (Pereira *et al.*, 2022).

One effective method is saponification, which involves binding the free carboxyl groups of fatty acids with minerals to form fatty acid salts or soaps. Calcium (Ca) is commonly used for this purpose (Satir *et al.*, 2023; Vargas-bello-p *et al.*, 2020). In addition to Ca, NaOH can also be used to protect the oil (Wulandari *et al.*, 2020). However, the use of zinc (Zn) minerals for this purpose has not been widely explored.

Zinc (Zn) is an essential trace mineral that functions as a cofactor for over 300 enzymes and acts as a structural component in gene expression and signal transduction (Franco *et al.*, 2024). Zn is also a major component of metalloenzymes, lactate dehydrogenase, carboxypeptidase, and DNA and RNA polymerase, which play a role in protein synthesis (Sloup *et al.*, 2017). Studies *in vitro* observed that supplementing Zn in ruminal fluid increased cellulose digestibility (Martínez & Church, 1970) and concentrations of total volatile fatty acids (VFA) and acetate (Chen *et al.*, 2019). Research by Wang *et al.* (2021) found that supplementation of 20-30 mg/kg DM Zn sulfate led to increasing nutrient digestibility and ruminal fermentation.

In livestock, Zn is involved in multiple biochemical functions such as bone metabolism, the metabolism of carbohydrates, fats, and proteins, sexual development and spermatogenesis, immune function, and appetite regulation via its effects on the central nervous system (Duffy *et al.*, 2023). The most important of Zn are cellular respiration, cellular use of oxygen, DNA and RNA expression, maintenance of cell membrane integrity, sequestration of free radicals, and protection against lipid peroxidation (Sloup *et al.*, 2017). The use of Zn as an oil protector has a double benefit, namely protecting unsaturated fatty acids from the biohydrogenation process of rumen microbes while providing Zn needed for rumen microbes and livestock, whose needs increase in the early stages of lactation.

In vitro Zn soap supplementation research was conducted by Faizah *et al.* (2019), which compared the supplementation of 10% Zn soap from palm oil with 10% corn. Supplementation with 10% palm oil Zn soap resulted in no different digestibility of dry matter, organic matter, and crude fiber, but the total production of VFA, acetate, and A/P ratio was higher compared to corn oil Zn soap. High acetate levels strongly support the synthesis of milk fatty acids in dairy cows, especially short-chain fatty acids. However, no research has explored the contribution of long-chain fatty acids, MUFA, and PUFA due to Zn soap supplementation as a precursor for the synthesis of long fatty acids and unsaturated fatty acids in milk. This study's results provided information regarding the concentration of long-chain fatty acids, MUFA and PUFA in the rumen due to Zn soap supplementation, which is expected to be used as a consideration in providing unsaturated fatty acids to increase the production and health of dairy livestock.

This study aimed to evaluate the effects of energy and organic Zn supplements, specifically Zn palm oil soap (ZPOS), on fermentability, digestibility, and unsaturated fatty acid profiles *in vitro*. The benefit of this research is identifying the most efficient form of palm oil supplementation to increase energy supply and improve the profile of unsaturated fatty acids in the rumen without disrupting feed fermentability *in vitro*. Discover the most effective use of palm oil in providing an alternative solution to energy and Zn deficiencies in dairy cattle.

MATERIALS AND METHODS

Zinc Soap and Feed Preparation

The preparation of Zn soap was carried out according to Cabatit (1979). The palm oil used to make Zn soap was a commercial palm oil that was generally sold on the market. The zinc soap of palm oil was made based on saponification number. Palm oil is measured for its saponification number and the KOH added is proportional to the saponification number. In order to protect palm oil, zinc chloride ($ZnCl_2$) is added in an amount equal to the KOH required to soak the oil, which is determined by the outcomes of reaction stoichiometric calculations. Palm oil was put into a glass beaker and incubated in a water bath at 90 ^{o}C. KOH that has been dissolved in ethanol is added to hot oil and stirred until the oil is completely hydrolyzed. Next, the $ZnCl_2$ solution was added and mixed continuously until a paste formed. The final step is to remove the remaining KOH by adding water and washing using a centrifuge. This process produces cream soap called zinc palm oil soap (ZPOS). The nutrient content of ZPOS is 24.36% ether extract, 4.2% crude fiber, 4.94% Zn, and gross energy 5257 kcal/kg.

The basal feed consists of forage and concentrates containing CP 14% and TDN 63%, formulated for feeding lactating dairy cows with a body weight 400 kg, milk yield 15 kg, and fat content 3.5% (National Research Council, 1988). The feed was composed of corn straw, soybean hulls, rice bran, pollard, cassava waste meal, coconut meal, soybean meal, and molasses. The composition of ingredients and nutrient content of the basal feed are shown in Table 1.

Table 1. Ingredients and chemical composition of experimental diet (dry matter basis)

Items	Composition
Ingredient:	
Corn straw	33.38
Soybean hull	12.25
Rice bran	7.42
Pollard	9.32
Cassava waste meal	9.93
Coconut meal	17.01
Soybean meal	6.98
Molasses	3.00
Vitamin and mineral mixture	0.80
Nutrient composition:	
Dry matter (DM), %	84.68
Ash, %DM	8.63
Crude protein, %DM	14.32
Ether extract, %DM	4.43
Crude fiber, %DM	20.02
Calcium, %DM	0.33
Phosphor, %DM	0.28
Zinc, mg/kg DM	16.93
Neutral detergent fiber, %DM	35.51
Acid detergent fiber, %DM	14.77
Total digestible nutrient (TDN) [1], %	63.15

Note: [1]Total digestible nutrients (TDN) were calculated using TDN (%DM). TDN = -17.2649 + 1.2120(PK) + 0.8352(BETN) + 2.4637(LK) + 0.4475(SK), according to Wardeh (1981).

In Vitro Experiment

The experiment was designed using a completely randomized design with four treatments and five replications. The treatments tested were T0= basal diet without supplementation, T1= basal diet + 5% palm oil (PO), T2= basal diet + 5% partial ZPOS (3.75% ZPOS + 1.25% PO), and T3= basal diet + 5% ZPOS. Rumen liquid as a source of inoculum was taken from three female goats with fistulas that belong to the Faculty of Animal and Agricultural Sciences Diponegoro University, and were homogenized. The goats were given a dietary trial following the ration for *in vitro* substrate for one week before rumen liquid was collected. Rumen liquid was collected before morning feeding from a fistulated rumen. The rumen liquid was filtered using cheesecloth and placed into a 39 °C flask under anaerobic conditions.

In vitro experiments were carried out according to the method of Tilley & Terry (1963). In the fermenter tube, 0.55 g of feed samples from each treatment were added, followed by 40 mL of McDaugall's solution and 10 mL of rumen liquid. The fermenter cylinder was flowed by CO_2 gas and closed to anaerobic conditions. The fermenter tube was incubated at a 39 °C water temperature.

Nutrient Digestibility

The digestibility of nutrients, including dry matter (DMD), organic matter (OMD), crude protein (CPD), ether extract (EED), and crude fiber (CFD) were measured through two stages of incubation, namely fermentative and enzymatic. In the first stage, the fermenter tube was incubated at 39 °C water temperature for 2x24 hours, and the process was stopped by immersing the fermenter tube in ice water for 20 minutes. Then, the tube was centrifuged for 15 minutes at a speed of 3,000 rpm, and the supernatant was discarded. In the second stage, the HCl pepsin solution was added 50 mL into the tube and incubated again for 2x24 hours in a 39 °C water temperature under aerobic conditions. The sample was filtered with Whatman No.41 filter paper using a vacuum pump. The filter results were analyzed for DM, Ash, CP, EE and C, NDF, and ADF (AOAC, 2012) to calculate nutrient digestibility values. Fermentation is also carried out without feed samples, which are called blanks. Nutrient digestibility samples are calculated by the formula:

Nutrient digestibility (%)=
{[Nutrient sample, g - (Nutrient residue, g - Nutrient blank, g)] / Nutrient sample, g} x 100

pH Value, VFA, NH_3, and Methane Production

The process of measuring pH, VFA, and NH_3 followed the same procedure as that for nutrient digestibility, but the fermentation stopped at the 4-hour mark. After centrifuging the fermenter tube at 3,000 rpm for 15 minutes, the supernatant was taken to measure the rumen pH, NH_3, and total and partial VFA concentrations. A digital pH meter from the brand "ACT" was used to measure the rumen liquid pH, and each sample was tested three times. NH3 levels were determined using a spectrophotometer, employing a spectrophotometric method based on the catalyzed endophenol reaction to form a stable blue compound (Chaney & Marbach, 1962). Cottyn & Boucque (1968) reported that gas chromatography was used to quantify the generation of partial VFAs (acetic acid, propionate, and butyrate). A milliliter of 95%–97% H_2SO_4 was combined with a 10 mL incubation sample. A milliliter of the sample combination was mixed with 0.0003 g of dehydrated sulfosalicylate. After that, the mixture was centrifuged for 10 minutes at 7 °C at 12,000 rpm. The samples were ready for gas chromatography identification.

The methane gas concentration and energy conversion efficiency were determined by calculating the VFA stoichiometry, which was the estimation using the formula of Orskov & Ryle (1990). The formula used for methane concentration was Methane (mM)= 0.5a - 0.25p + 0.5b, where a, p, and b are moles of acetic, propionic, and butyric acids, respectively. The efficiency of conversion of hexose energy to VFA was calculated based on the stoichiometry of the carbohydrate fermentation reaction from hexose to VFA (acetate, propionate, and butyrate). The calculation formula used was:

E (%)= [(0.622 pA + 1.091 pP + 1.558 pB) / (pA + pP + 2pB)] x 100

Where E is energy, and pA, pP, and pB are the proportions of acetic, propionic, and butyric acids.

Protozoa, Microbial Protein, and Fatty Acids Rumen Liquid

To calculate protozoa, microbial protein and fatty acid concentrations in the rumen liquid, the fermentation process was carried out for 24 hours. The populations of protozoa were calculated following the procedures of Ogimoto & Imai (1981). The solution used was trypan blue formalin saline (TBFS), made from a mixture of 100 mL 35% formaldehyde, 2 g trypan blue, 8 g NaCl, and 900 mL distilled water. The protozoa population was carried out on each treatment rumen fluid mixed with Trypan Blue Formalin Saline (TBFS) solution with a ratio of 5:1. The counting chamber with a thickness of 0.1 mm was dripped with 2 drops of the mixture. The area of the smallest box was 0.0625 mm, totaling 16 boxes with a box area of 5 boxes read. The number of protozoa was calculated by using a microscope at magnification 100 times. The formula used for total population of protozoa was: [1 / (0.1 x 0.0625 x 16 x 5)] x 1000 x DF x C, where C is protozoa population in the counting chamber and DF is diluent factor the specimen.

Measurement of rumen liquid protein microbes using the method of Makkar *et al.* (1982) on the principle of gradual centrifugation. The fatty acid profile of rumen liquid was analyzed using a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan). Samples were stored at -20 °C until analysis. Following the method of Cocks & Rede (1966), the fatty acid composition was determined by converting oil to fatty acid methyl esters. This involved adding 950 µL of n-hexane, 50 mg of oil, and 50 µL of sodium methoxide. The peaks of the fatty acid methyl esters were identified by comparing their retention times with those of authentic standards. The relative percentage of each fatty acid was calculated based on its peak area relative to the total peak area of all fatty acids in the sample. Fatty acids were categorized into short-chain fatty acids (SCFA) as having fewer than six carbon atoms, medium chain fatty acids (MCFA) ranging from C6 to C12, and long-chain fatty acids (LCFA), which are greater than C12 (Wang *et al.*, 2020).

Statistical Analysis

The data were analyzed using a completely randomized design in SPSS 16. Duncan's Multiple Range Test with significance was set at $p<0.05$ to compare treatments when a significant effect was observed.

RESULTS

Feed Fermentability in The Rumen

Detailed rumen feed fermentation parameters can be seen in Table 2. The pH levels in all treatments were not significantly different and were in the normal range of 6.75-6.82. Supplementation of 5% PO (T1) had an effect on reducing the number of protozoa and microbial proteins compared to the control and other treatments ($p<0.05$). On the other hand, 5% partial ZPOS (T2) and 5% ZPOS (T3) supplementation caused a decrease in NH3 concentration and an increase in VFA production compared to the control and 5% PO supplementation ($p<0.05$).

Nutrient Digestibility

Table 3 presents the nutrient digestibility due to 5% PO, 5% partial ZPOS, and 5% ZPOS supplementation. All treatments did not show significant differences in dry matter (DMD), organic matter (OMD), and crude protein (CPD) digestibility, but there were significant differences ($p<0.05$) in ether extract digestibility (EED), crude fiber digestibility (CFD), neutral detergent fiber digestibility (NDFD), and acid detergent fiber digestibility (ADFD). Both supplementation of 5% PO (T1), 5% partial ZPOS (T2), and 5% ZPOS (T3) increased EED, but fiber digestibility (CFD, NDFD, and ADFD) was only increased by 5% partial ZPOS supplementation (T2), while 5% PO and 5% ZPOS supplementation did not differ from the control.

Relative Proportion of VFA

Table 4 shows the partial VFA and methane production in rumen liquid. Supplementation of 5% PO, 5% partial ZPOS, and 5% ZPOS) significantly influenced ($p<0.05$) acetate, propionate, butyrate, the A/P ratio, and methane but did not impact the efficiency of hexose energy conversion into VFA. The relative proportion of VFAs in this study was 59%–62% acetic acid, 24%–29% propionic acid, and 12%–13% butyric acid. Both 5% partial ZPOS (T2) and 5% ZPOS supplementation increased acetate, propionate, and butyrate compared to 5% PO supplementation (T1) and the control (T0). Conversely, A/P ratio and methane production decreased with 5% PO and 5% partial ZPOS supplementation. The efficiency of hexose energy conversion into VFA between treatments was not significantly different.

Table 2. *In vitro* feed fermentability in rumen liquid with supplementation of zinc palm oil soap (ZPOS)

Variables	Treatments				SEM	p-Value
	T0	T1	T2	T3		
pH	6.76	6.75	6.73	6.82	0.018	0.224
Protozoa (10^3 cell/mL)	98.14^{a}	32.57^{c}	82.83^{ab}	69.31^{b}	0.044	0.000
Microbial protein (mg/mL)	13.01^{a}	9.37^{b}	13.37^{a}	11.41^{ab}	0.469	0.002
NH^3 (mM)	14.06^{a}	14.45^{a}	9.64^{b}	10.36^{b}	0.543	0.000
VFA (mM)	87.52^{b}	101.78^{b}	164.38^{a}	157.89^{a}	8.134	0.000

Note: ^{a,b} Means in the same row with different superscripts differ significantly ($p<0.05$). T0= basal diet without supplementation; T1= basal diet + 5% palm oil (PO); T2= basal diet + partial ZPOS (3.75% ZPOS + 1.25% PO); T3= basal diet + 5% ZPOS.

Table 3. *In vitro* nutrient digestibility in rumen liquid with supplementation of zinc palm oil soap (ZPOS)

Variables	Treatments				SEM	p-Value
	T0	T1	T2	T3		
Dry matter (%)	64.06	63.82	67.20	69.24	0.85	0.054
Organic matter (%)	69.16	65.89	69.65	69.98	0.632	0.068
Crude protein (%)	68.69	67.99	66.39	65.11	0.569	0.100
Ether extract (%)	88.45^{b}	94.34^{a}	93.22^{a}	92.35^{a}	0.587	0.000
Crude fiber (%)	54.46^{b}	51.46^{b}	73.63^{a}	66.11^{ab}	3.057	0.020
Neutral detergent fiber (%)	56.20^{b}	46.33^{b}	70.03^{a}	64.62^{ab}	2.830	0.006
Acid detergent fiber (%)	45.12^{b}	44.56^{b}	65.81^{a}	53.76^{ab}	2.704	0.006

Note: ^{a,b} Means in the same row with different superscripts differ significantly ($p<0.05$). T0= basal diet without supplementation; T1= basal diet + 5% palm oil (PO); T2= basal diet + partial ZPOS (3.75% ZPOS + 1.25% PO); T3= basal diet + 5% ZPOS.

Table 4. *In vitro* VFA and methane production in rumen liquid with supplementation of zinc palm oil soap (ZPOS)

Variables	Treatments				SEM	p-Value
	T0	T1	T2	T3		
Acetate (mM)	41.17^{b}	51.10^{a}	59.31^{a}	65.80^{a}	2.579	0.000
Propionate (mM)	15.91^{c}	25.41^{b}	28.73^{a}	29.89^{a}	1.348	0.000
Butyrate (mM)	8.73^{b}	10.28^{b}	12.55^{a}	14.20^{a}	0.546	0.000
A/P	2.59^{a}	2.01^{b}	2.06^{b}	2.20^{b}	0.061	0.000
Methan (mM)	31.87^{a}	28.04^{b}	28.58^{b}	29.57^{ab}	0.489	0.014
Efficiency of energy conversion (%)	73.05	73.19	74.74	74.47	0.357	0.226

Note: ^{a,b} Means in the same row with different superscripts differ significantly ($p<0.05$). T0= basal diet without supplementation; T1= basal diet + 5% palm oil (PO); T2= basal diet + partial ZPOS (3.75% ZPOS + 1.25% PO); T3= basal diet + 5% ZPOS.

Fatty Acids in Rumen Liquid

Table 5 outlines the fatty acid composition in rumen liquid. Supplementation of 5% PO (T1), 5% partial ZPOS (T2), and 5% ZPOS (T3) supplementation decreased significantly ($p<0.05$) short-chain fatty acids (SCFA). The long chain fatty acids (LCFA), especially palmitate (C16:0), increased, but stearate (C18:0) decreased by all treatments ($p<0.05$). Unsaturated fatty acids (USFA), particularly trans 11 C18:1, cis 9 C18:1, C18:2, C20:5 (EPA), and C22:6 (DHA), increased by 5% partial ZPOS (T2) and 5% ZPOS (T3) supplementation.

These fatty acids are classified into various categories, including short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), long-chain fatty acids (LCFAs), saturated fatty acids (SFAs), unsaturated fatty acids (USFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). According to Table 6, the diets supplemented with 5% PO, 5% partial ZPOS, and 5% ZPOS decreased SCFAs compared to the control diet ($p<0.05$) but increased LCFA. SFAs showed an increase in control and 5% PO supplementation, whereas 5% partial ZPOS and 5% ZPOS supplementation demonstrated an ability to elevate USFAs. Notably, the 5% partial 5% ZPOS and 5% ZPOS supplementation increased MUFAs and PUFAs than the other treatments.

DISCUSSION

Feed Fermentability

The pH is one of the crucial factors in assessing rumen health. The findings indicated that adding palm oil and protected palm oil Zn soap did not disrupt the rumen fermentation process. Palm oil contains high palmitic acid, which actually supports the growth of rumen bacteria (Sears *et al*., 2024). Apart from being high in palmitate content, palm oil is also high in unsaturated fatty acids (oleic acids), palm oil protection causes unsaturated fatty acids to escape rumen microbial biohydrogenation so that negative effects on the rumen environment can be eliminated. Similar results have been reported in other studies, such as those conducted by Adeyemi *et al.* (2015), Amanullah *et al.* (2022), and Roy *et al.* (2017).

The reduction in protozoa is attributed to the high content of saturated fatty acids in palm oil, particularly palmitic acids (C16:0). According to Rahman *et al.* (2022), palm oil contains about 44% palmitic acid. It has been reported that supplementation of LCFA in the form of palmitate (C16:0) and stearate (C18:0) resulted in a decrease in the protozoa population, although the effect of the decrease was not as strong as MCFA, especially capric acids (C10:0) and lauric acid (C12:0). (Matsumoto *et al*., 1991). The decrease is caused by the inability of protozoa to digest fat because they do not have lipolytic enzymes; as a result, the addition of oil can interfere with the metabolic activity of protozoa and subsequently cause the number of protozoa to decrease (Hartanto *et al*., 2017).

Ibrahim *et al.* (2021) discovered that supplementing with palm oil altered the rumen microbial population in ruminants, potentially affecting their overall protein synthesis capability. Sears *et al.* (2024) reported that oleic acid significantly reduces Fibrobacteraceae. The study further indicated that high levels of unsaturated fatty acids could adversely affect cellulolytic bacteria, which are crucial for fiber digestion, resulting in decreased

Table 5. *In vitro* proportion of fatty acids in rumen liquid with supplementation of zinc palm oil soap (ZPOS) (% fat)

Fatty acids	Treatments				SEM	p-Value
	T0	T1	T2	T3		
Short-chain fatty acids (SCFA)						
C4	7.34[a]	5.34[b]	5.27[b]	2.36[c]	0.435	0.000
Medium chain fatty acids (SCFA)						
C6	<0.1	<0.1	<0.1	<0.1	-	-
C8, caprylic	<0.1	<0.1	<0.1	<0.1	-	-
C10, capric	<0.1	<0.1	<0.1	<0.1	-	-
C12, lauric	1.38	1.15	1.22	1.20	0.044	0.299
Long-chain fatty acids (LCFA)						
C13	0.3[a]	0.17[a]	<0.1[b]	<0.1[b]	0.030	0.000
C14, myristic	2.19	1.85	2.30	1.68	0.063	0.054
C14:1	1.00	0.50	0.57	0.44	0.072	0.053
C15,	0.51	0.75	0.36	0.33	0.056	0.055
C15:1	0.34	0.23	0.16	0.21	0.025	0.051
C16, palmitic	22.03[b]	29.34[a]	29.23[a]	31.14[a]	0.854	0.000
C16:1, n7	1.12[b]	1.04[b]	1.36[ab]	1.5[a]	0.058	0.005
C17	0.39	0.23	0.23	0.28	0.025	0.052
C17:1	<0.1	<0.1	<0.1	<0.1	-	-
C18, stearic	40.13[a]	30.74[b]	23.17[b]	21.5[b]	2.198	0.002
trans 11 C18:1	12.55[c]	17.84[b]	26.16[a]	28.05[a]	1.476	0.834
cis 9 C18:1, oleic	1.09[b]	1.43[b]	1.99[a]	1.83[a]	0.099	0.000
C18:2	1.04[c]	1.31[bc]	1.6[ab]	1.86[a]	0.084	0.000
C18:3	<0.1	<0.1	<0.1	<0.1	-	-
C18:3, omega 6	0.22[b]	0.26[b]	0.45[a]	0.53[a]	0.032	0.000
C18:3, gamma linolenic	0.79[a]	<0.1[c]	0.38[b]	0.41[b]	0.064	0.000
C20	1.31	0.83	0.64	0.67	0.050	0.058
cis 11 C20:1	0.16[b]	0.59[a]	0.31[b]	0.23[b]	0.041	0.000
cis 11-14 C20:2	<0.1	<0.1	<0.1	<0.1	-	-
C20:3	<0.1	<0.1	<0.1	<0.1	-	-
C20:4, arachidonic	0.48[a]	<0.1[b]	<0.1[b]	<0.1[b]	0.048	0.000
C20:5, EPA	0.07[c]	0.55[b]	0.59[b]	0.76[ab]	0.069	0.000
C21	1.38	0.31	0.20	0.26	0.017	0.000
C22, behenic	0.94[a]	0.43[ab]	0.23[b]	0.25[b]	0.042	0.050
C22:1	<0.1	<0.1	<0.1	<0.1	-	-
C22:6, DHA	3.19[b]	4.81[a]	4.57[a]	4.42[a]	0.159	0.000
C24	<0.1	<0.1	<0.1	<0.1	-	-
C24:1, nervonic omega 9	<0.1	<0.1	<0.1	<0.1	-	-

Note: [a,b] Means in the same row with different superscripts differ significantly (p<0.05). T0= basal diet without supplementation; T1= basal diet + 5% palm oil (PO); T2= basal diet + partial ZPOS (3.75% ZPOS + 1.25% PO); T3= basal diet + 5% ZPOS.

Table 6. *In vitro* proportion of short-chain, long-chain, saturated, and unsaturated fatty acids in rumen liquid with supplementation of zinc palm oil soap (ZPOS) (% fat)

Fatty acids	Treatments				SEM	p-Value
	T0	T1	T2	T3		
SCFA	7.34[a]	5.34[b]	5.27[b]	2.36[c]	0.453	0.000
MCFA	1.38[a]	1.15[b]	1.22[b]	1.20[b]	0.028	0.009
LCFA	91.23[c]	93.41[b]	93.50[b]	96.35[a]	0.443	0.000
SFA	77.9[a]	71.14[b]	61.85[c]	59.67[c]	1.766	0.000
USFA	22.05[c]	28.76[b]	38.14[a]	40.24[a]	1.722	0.000
MUFA	16.26[c]	21.63[b]	30.55[a]	32.26[a]	1.531	0.000
PUFA	5.79[c]	7.13[b]	7.59[a]	7.98[a]	0.201	0.000

Note: [a,b] Means in the same row with different superscripts differ significantly (p<0.05). SCFA= short-chain fatty acids, MCFA= medium-chain fatty acids, LCFA= long-chain fatty acids, SFA= saturated fatty acids, USFA= unsaturated fatty acids, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids. T0= basal diet without supplementation; T1= basal diet + 5% palm oil (PO); T2= basal diet + partial ZPOS (3.75% ZPOS + 1.25% PO); T3= basal diet + 5% ZPOS.

protein synthesis. The use of ZPOS protection, both 5% partial ZPOS (T2) and 5% ZPOS (T3) can mitigate the harmful effects of unsaturated fatty acids, enabling microbes to develop as effectively as in the control group.

Yanza *et al*. (2021) found that ruminants given protected medium chain fatty acids (MCFA) experienced a decrease in total VFA production compared to those given unprotected oil. Similarly, T2 and T3 treatments resulted in high VFA levels due to the increased propionate production. Propionate is formed from the breakdown of glycerol during fat hydrolysis. The NH_3 concentration in the T2 treatment was the lowest among all treatments, while microbial protein production was the highest. This indicates that NH_3 is utilized for microbial protein synthesis (Getabalew & Negash, 2020).

Nutrient Digestibility

The digestibility values for DMD, OMD, CPD, NDFD, and ADFD were unaffected by 5% PO supplementation, indicating that this level of supplementation is safe for rumen microbial growth. These results are consistent with previous research, which found that 6% supplementation with vegetable oils (olive oil, sunflower oil, and linseed oil) yielded OMD, NDFD, and ADFD values comparable to the control, although linseed oil resulted in higher digestibility than sunflower oil (Vargas-bello-p *et al.*, 2020). The effect of oil supplementation on digestibility is highly dependent on the nutrient composition of the feed and the fatty acid profile of the oil. The experimental feed in this study, characterized by high fiber content and low ADF (13.77%), supports the proliferation of cellulolytic microbes. A high-fiber diet may mitigate the potential negative impact of oil on fiber digestion by rumen microbes (Benchaar *et al.*, 2015). Both non-protected (T1) and protected (T2 and T3) palm oil increased EED, supporting the conclusion that palm oil can be used as an energy supplement without compromising rumen feed fermentability.

A notable finding from this research is that both partial and total Zn soap supplementation with palm oil (T2 and T3) significantly enhances CFD, NDFD, and ADFD. This effect is likely due to the fat and Zn content in palm oil. Palm oil contains high levels of palmitic acid (39.8%) and oleic acid (46.14%) (Koushki *et al.*, 2015). Research by Sears *et al.* (2024) indicates an increase in Prevotella and Fibrobacter populations in response to palmitic acid supplementation. This suggests that cellulolytic bacteria can incorporate palmitic acid into their cell membranes, thereby increasing energy availability for metabolic processes. Furthermore, Zn is crucial for various metabolic functions in rumen microbes.

Zinc (Zn) plays a pivotal role in numerous biological processes, encompassing enzyme activity, cell membrane stability, gene expression, and cell signaling (Swain *et al.*, 2016; Uniyal *et al.*, 2017). It is indispensable for preserving the structural and functional integrity of over 2000 transcription factors and 300 enzymes. It can be contended that virtually all metabolic pathways are reliant, to varying degrees, on at least one Zn-dependent protein (Ranasinghe *et al.*, 2015). Recent research by Fellner *et al.* (2021) elucidated that Zn supplementation precipitated increased acetate production and a heightened acetate/propionate ratio. This delineates an augmented fiber digestion process facilitated by cellulolytic bacteria, consequent to heightened microbial protein synthesis. This conclusion aligns with findings indicating elevated levels of microbial protein compared to PO supplementation (T1), specifically 13.37 (T2) and 11.41 (T3) versus 9.37 mg/mL in the T1 treatment (Table 3).

Relative Proportion of VFA

The relative proportion of acetate observed is slightly lower than that reported by Anzhany *et al.* (2024), which ranged from 69% to 72%, while the proportions of propionate and butyrate are comparatively higher. According to Amanullah *et al.* (2022), palm oil supplementation modifies the rumen environment, thereby altering the ratio of cellulolytic to amylolytic microbes. Both 5% partial ZPOS and 5% ZPOS supplementation resulted in significantly higher acetate production compared to non-protected palm oil ($p<0.05$). Acetate is the yield of digestion of fibrous carbohydrates by cellulolytic bacteria, which means that the increase in acetate is in line with the increase in the number of cellulolytic bacteria in the rumen. This is likely related to the decreasing protozoa population resulting from oil supplementation. Protozoa are predators of bacteria (Dayyani *et al*., 2013), so a decrease in the number of protozoa will increase the number of bacteria because it reduces competition for nutrients. These findings are consistent with the increased microbial protein levels observed with 5% partial ZPOS and 5% ZPOS supplementation, suggesting enhanced bacterial growth. Zinc (Zn) is an essential trace mineral cofactor for over 300 enzymes, including DNA and RNA polymerase, which play a role in protein synthesis (Franco *et al.*, 2024; Sloup *et al.*, 2017).

The increase in propionate in the ZPOS supplementation treatment resulted from the hydrolysis of fat into fatty acids and glycerol, with glycerol subsequently converted into propionate. Protozoa in the rumen contribute significantly to the flow of unsaturated fatty acids leaving the rumen, potentially protecting chloroplast unsaturated fatty acids from rumen bio-hydrogenation and increasing the duodenal flows of mono and polyunsaturated fatty acids (Newbold *et al.*, 2015). These findings are consistent with studies showing that ZPOS supplementation leads to an increase in protozoan populations (Table 4). Zn supplementation has been associated with shifts in rumen volatile fatty acid profiles, notably an increase in propionate and a reduction in the acetate-to-propionate ratio (Hilal *et al.*, 2016). Moreover, the lack of an increase in methane production and a lower A/P ratio is advantageous, as higher propionate levels provide a carbon framework and synthetic energy for livestock.

Fatty Acids in Rumen Liquid

Previous research showed that oil supplementation caused a decrease in the proportion of short-chain fatty acids in rumen fluid while increasing the presence of long-chain fatty acids. Mancini *et al.* (2015) argue that this transition is caused by the large number of long-chain fatty acids (LSFA) in palm oil, namely 98.3%, and the remaining 1.7% consists of short-chain fatty acids (SCFA). Among long-chain fatty acids, which account for 98.3% of the total, almost half (49.9%) are unsaturated fatty acids (USFA), consisting of 39.2% monounsaturated fatty acids (MUFA) and 10.5% polyunsaturated fatty acids (PUFA). The prevalence of saturated fatty acids (SFA) in palm oil contributes to the range of rumen saturated fatty acid levels, where PO supplementation treatment (T1) has higher SFA levels compared to ZPOS supplementation, namely T1 71.14%, T2 61.85%, and T3 59.67% (Table 6).

Palm oil contains MUFA in the form of oleic acid (18:0) in large quantities, namely 39.2%. In the rumen, feed fat will undergo lipolysis to produce fatty acids and glycerol. Unsaturated fatty acids, including oleic acid (18:1), undergo a biohydrogenation process, being converted into saturated fatty acids, stearic acid (18:0), in the rumen by bacteria (Buccioni *et al.,* 2012). Harvatine *et al.* (2009), who tracked the biohydrogenation process of oleic acid, found that oleate in the rumen can change into trans and cis forms because rumen microbes produce cis/trans isomerase enzymes, and there is an isomerase that changes the 18:2 and 18:3 cis bonds (linoleate and linolenate) to trans 18:1. The dominant biohydrogenation process that occurs in the rumen is bacteria hydrogenate polyunsaturated fatty acids (PUFA) into trans-11 18:1 and cis-9, trans-11 fatty acids, then trans 18:1 fatty acids into stearic acid (18:0).

Consistent with our study's findings, supplementation with ZPOS (T2 and T3) resulted in elevated levels of trans18:1 and cis 18:1, alongside reduced stearate (18:0) concentrations compared to non-protected oil supplementation (T1). Amanullah *et al.* (2022) conducted a comparative analysis involving corn oil, linseed oil, and Ca salt (protected C18:2n-6), revealing that protected fatty acids (Ca Salt) led to diminished stearate levels but higher proportions of MUFA and PUFA. These findings are consistent with the research by Satir *et al.* (2023), which demonstrated that 3% protected palm oil supplementation can increase the content of palmitic acid (SFA) and oleic acid (MUFA). Stearate is the final product of the biohydrogenation of oleic, linoleic, and linolenic acids. This indicates a complete inhibition of the biohydrogenation process in the ZPOS treatment. The protection of polyunsaturated fatty acids through saponification involves bonding the carboxyl group with Zn, resulting in the inhibition of isomerization, which is a prerequisite for the hydrogenation of unsaturated fatty acids into saturated fatty acids. This is reflected in the higher proportion of MUFA in the ZPOS treatment compared to PO and the control (Table 6). The substantial presence of PUFA, notably EPA and DHA, underscores Zn's role in the elongation and desaturation process, facilitated by the formation of arachidonic acid (20:4) through delta-6-desaturase (D6D) and delta-5-desaturase (D5D) enzymes (Ridgway, 2016). Zn's indispensability is further highlighted as a pivotal mineral cofactor for D6D and D5D enzymes (Noland & Drisko, 2020).

Trans 18:1 acts as a precursor for saturated fatty acid formation in the rumen, particularly stearate, while on a tissue level, it serves as a precursor for conjugated linoleic acids (CLA), known for their antioxidant and anticarcinogenic properties in livestock products. Hence, the augmentation of trans 18:1 fatty acids attributed to ZPOS supplementation holds the potential for enhancing the quality of meat and milk fat.

CONCLUSION

Based on the results of this research, it can be concluded that zinc soap from palm oil (ZPOS) can be developed as a source of energy and organic Zn. Supplementation of 5% partial ZPOS (3.75% ZPOS + 1.25% PO) is better than 5% ZPOS. The beneficial effects of this supplementation are increased fiber digestibility, VFA, LCFA, and USFA, as well as decreased methane production in the rumen. Further investigation is required to test the ZPOS on dairy cows *in vivo*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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