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Utilization of Calcium Sources in the Saponification Process of Lemuru Fish Oil for Protected Fat Supplements

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ABSTRACT

This study evaluated the effectiveness of several calcium compounds in the saponification of lemuru fish (*Sardinella lemuru*) oil to produce protected fat supplements for ruminants. Four calcium sources—Ca $(OH)_2$, $CaCO_3$, $CaCO_3 \cdot MgCO_3$ (dolomite), and $CaSO_4$ (gypsum)—were tested using a completely randomized design with five replications. The parameters observed included product texture, proximate composition (moisture, ash, fat), fatty acid profile, and solubility under rumen-like (neutral) and gastric (acidic) conditions. Results indicated that Ca $(OH)_2$, $CaCO_3 \cdot MgCO_3$, and $CaSO_4$ were effective in forming calcium or mixed fatty acid salts, while $CaCO_3$ failed to bind free fatty acids. Among the effective compounds, Ca $(OH)_2$ produced the fastest hardening product, whereas dolomite and gypsum provided more balanced mineral contributions (Ca, Mg, and S). All protected fat products were less soluble in the rumen environment but readily decomposed under acidic gastric conditions, ensuring nutrient bypass. These findings suggest that low-cost and locally available calcium sources can be utilized to develop protected fat supplements that are both nutritionally safe and practical for ruminant feeding. Further research is needed to optimize the water-to-oil ratio and enzyme application for improved efficiency.

Keywords: calcium, fat, oil, lemuru, supplement

INTRODUCTION

Fat plays a crucial role in ruminant nutrition as a concentrated source of energy and essential fatty acids, which can enhance the quality of milk and meat (Toral et al., 2018). However, supplementation with unprotected fats above 5–7% of the diet may negatively affect fiber digestibility in the rumen, as excess lipids can inhibit microbial activity and reduce cellulose degradation (Faradilla et al., 2019; Enjalbert et al., 2017). Moreover, unprotected unsaturated fatty acids are highly susceptible to biohydrogenation, leading to the loss of their beneficial effects (Gadeyne et al., 2015; Gadeyne et al., 2016; Huda et al., 2023). To overcome this limitation, fats are usually provided in a protected form that bypasses ruminal degradation but is digested in the abomasum or small intestine, ensuring effective absorption and utilization.

Protected fat can be produced through the saponification of fatty acids using alkaline compounds, commonly NaOH or KOH, to form sodium or potassium fatty acid salts (Proaño et al., 2015). These salts are stable in the neutral rumen environment but dissociate in the acidic abomasum, allowing enzymatic digestion. Although this method has been widely applied, reliance on Na- or K-based salts has practical disadvantages: they are relatively expensive, not always accessible for smallholder farmers, and may cause mineral imbalances in ruminant diets. Excess Na or K intake can increase water consumption, reduce feed intake, and potentially contribute to environmental waste through mineral excretion (Palmquist, 1994; Suprayitno et al., 2020).

Calcium-based compounds represent an attractive alternative because calcium is required in larger amounts by ruminants and is safer compared with Na or K salts. Previous studies have primarily focused on $Ca(OH)_2$, but this compound is costly and not widely available in rural areas (Gallardo et al., 2014; Mierlita, 2018). Other calcium sources, such as $CaCO_3$ (lime), $CaCO_3 \cdot MgCO_3$ (dolomite), and $CaSO_4$ (gypsum), are inexpensive, widely accessible in local markets, and may provide additional macrominerals such as magnesium (Mg) and sulfur (S), which support microbial growth and metabolic functions in the rumen (Mayasari et al., 2015). However, limited information is available on the potential of these alternative calcium compounds in producing protected fat supplements from fish oil.

Therefore, this study aimed to evaluate the use of several calcium compounds—Ca $(OH)_2$, $CaCO_3$, $CaCO_3 \cdot MgCO_3$, and $CaSO_4$—in the saponification of lemuru fish (Sardinella lemuru) oil to produce protected fat. The evaluation focused on product texture, proximate composition, fatty acid profile, and solubility under rumen and gastric conditions to identify suitable and cost-effective calcium sources for developing protected fat supplements in ruminant nutrition.

MATERIALS AND METHODS

Materials

This study used calcium sources including $Ca(OH)_2$, $CaCO_3$ (lime), $CaCO_3 \cdot MgCO_3$ (dolomite), and $CaSO_4$ (gypsum). The fat source was oil from the lemuru fish (Sardinella lemuru), obtained from the fish processing industry in Muncar, Banyuwangi. The enzyme used for hydrolysis was lipase (Lypozyme®, Habio Bioengineering Co., Ltd., China), a commercial product known for its high hydrolytic activity on fish oil triglycerides.

Methods

This research used a Completely Randomized Design (CRD) method with four treatments and five replications, with treatment details as follows:

T1 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lipase + 20 grams of $Ca(OH)_2$

T2 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lipase + 20 grams of $CaCO_3$

T3 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lipase + 20 grams of $CaCO_3.MgCO_3$

T4 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lipase + 20 grams of $CaSO_4$

The process of making protected lemuru fish oil is carried out using the hot method according to the following procedure:

a. Each ingredient was weighed according to the specified formula for each treatment.
b. 100 grams of lemuru fish oil, 20 grams of water, and 1 gram of Lypozyme Lipase Habio enzyme were mixed and stirred until homogeneous, then allowed to stand for approximately 5 minutes.
c. The calcium source, based on the treatment, was then added to the mixture of lemuru fish oil, water, and lipase enzyme, and stirred until entirely homogeneous.
d. The mixture was left to rest for several days to produce a protected oil product or solid fatty acid salts.

Subsequently, the product was analyzed in the laboratory for texture tests (including color and hardness), moisture content analysis (by drying in an oven at 60°C for 24 hours, followed by a second oven drying at 105°C for 3 hours), and ash content (mineral content), which was measured by heating in an oven at 550°C for 3 hours. Additionally, the fatty acid content was assessed using chromatography. Finally, the solubility of the product was tested in both a rumen-like environment (pH above 6) and an acidic environment (pepsin-HCl solution/stomach pH).

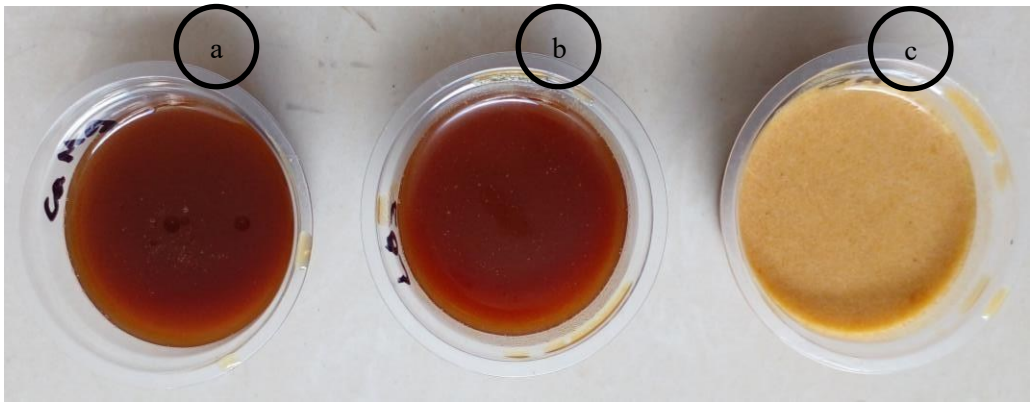
Statistical Analysis

The experiment was arranged in a Completely Randomized Design (CRD) with four treatments and five replications. Data collected on product texture, proximate composition (moisture, ash, fat), and solubility were analyzed using Analysis of Variance (ANOVA) to determine the effect of different calcium sources. When significant differences ($P < 0.05$) were detected, means were further compared using Duncan's Multiple Range Test (DMRT) to identify differences among treatments. All statistical analyses were performed using Microsoft Excel 2019.

RESULTS AND DISCUSSION

The process of producing protected fat or calcium fatty acid salts from lemuru fish oil begins with the mixing of lemuru fish oil, water, and lipase enzyme to break down the fish oil into glycerol and fatty acids (Rosmalina et al., 2021). When 100 grams of lemuru fish oil was mixed with 20 grams of water and stirred, the result showed that the two did not mix uniformly. Water bubbles were observed in the oil, and the colour of the mixture remained brown, similar to the original colour of lemuru fish oil. However, after adding 0.5 grams of lipase enzyme to the oil and water mixture and stirring, a homogeneous, cream-coloured mixture was produced with no visible water bubbles, as shown in Figure 1. When the homogeneous mixture of lemuru fish oil, water, and lipase enzyme was left to stand for approximately 5 minutes, it separated into two distinct layers: a brown liquid layer at the bottom and a cream-coloured solid layer on top, as shown

in Figure 2. This indicates that a hydrolysis process occurred, breaking down the lemuru fish oil using hydrolyzing agents such as water and lipase enzyme, resulting in fatty acids and glycerol (Sholeha and Agustini, 2021). The lipase enzyme has an essential role in fat metabolism, this enzyme hydrolyzes fat into free fatty acids, glycerin, and monoglycerides for use by livestock.



Note:
a = Lemuru fish oil
b = Lemuru fish oil + water
c = Lemuru fish oil + water + lipase enzyme

Figure 1. Conditions for the sample preparation of protected fat products

Figure 2. Conditions for the sample hydrolysis of lemuru fish oil into fatty acids and glycerol

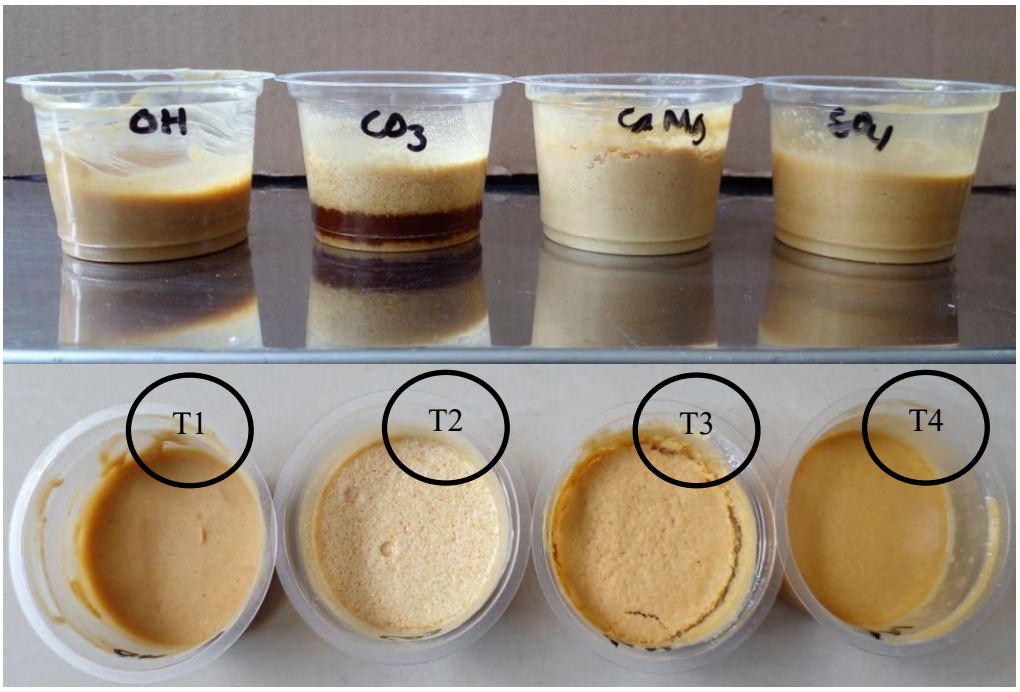
Texture (colour and hardness level)

Protected fat or calcium-fatty acid salts from lemuru fish oil produced with four different calcium sources are shown in Figure 3, illustrating apparent differences in texture. When $CaCO_3$ was added to the mixture of lemuru fish oil, water, and lipase enzyme, separation occurred, producing a cream-white solid layer on top and a brown liquid at the bottom. This unstable condition persisted for up to seven days, indicating that $CaCO_3$ is not suitable for producing protected fat, which ideally should form a homogeneous and solid mixture that can be further processed into granules.

In contrast, the use of $Ca(OH)_2$, $CaCO_3 \cdot MgCO_3$ (dolomite), or $CaSO_4$ (gypsum) produced uniform mixtures with cream-colored pastes. $Ca(OH)_2$ and dolomite generated thicker pastes compared to $CaSO_4$. Both $CaCO_3$ and dolomite caused an increase in product volume due to the release of CO_2 from carbonate decomposition. Dolomite produced more gas because it contains two carbonate groups. The $Ca(OH)_2$-based product hardened within 24 hours, dolomite required about three days, while gypsum hardened more slowly, reaching a firm texture after seven days.

Overall, $Ca(OH)_2$, dolomite, and gypsum can be used to produce protected fat from lemuru fish oil, while $CaCO_3$ is ineffective. $Ca(OH)_2$ produces the fastest hardening product but may result in an excessively high calcium content, making it challenging to balance dietary minerals, particularly phosphorus (P) and magnesium (Mg). Excessive calcium intake also poses health risks such as urolithiasis in ruminants (Benu et al., 2023). These limitations can be mitigated by using dolomite or gypsum, which not only lowers calcium levels but also provides additional

macrominerals. Dolomite contributes magnesium, while gypsum provides sulfur—both essential for rumen microbial growth and amino acid synthesis, particularly methionine and cysteine.



Note:
T1 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lypozime Lipase Habio enzyme + 20 grams of $Ca(OH)_2$
T2 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lypozime Lipase Habio enzyme + 20 grams of $CaCO_3$
T3 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lypozime Lipase Habio enzyme + 20 grams of $CaCO_3.MgCO_3$
T4 = 100 grams of lemuru fish oil + 20 grams of water + 1 gram of Lypozime Lipase Habio enzyme + 20 grams of $CaSO_4$

Figure 3. Saponification of lemuru fish oil with the addition of a calcium source

The moisture, ash, and fat content

The moisture, ash, and fat content of the protected fat product from lemuru fish oil made using three different calcium sources are presented in Table 1. The data in Table 1 show that the use of different calcium sources did not have a significant effect ($P > 0.05$) on moisture content. However, it has a considerable impact ($P < 0.05$) on ash or mineral content and organic matter in the protected fat product. The low moisture content across all treatments is due to the water in the materials being used in the reaction, which breaks down fat or triglycerides into glycerol and free fatty acids through hydrolysis. The hydrolysis reaction of lemuru fish oil into fatty acids and glycerol breaks down triglycerides in the oil using water.

Table 1. The moisture, ash, and fat content of the protected fat product

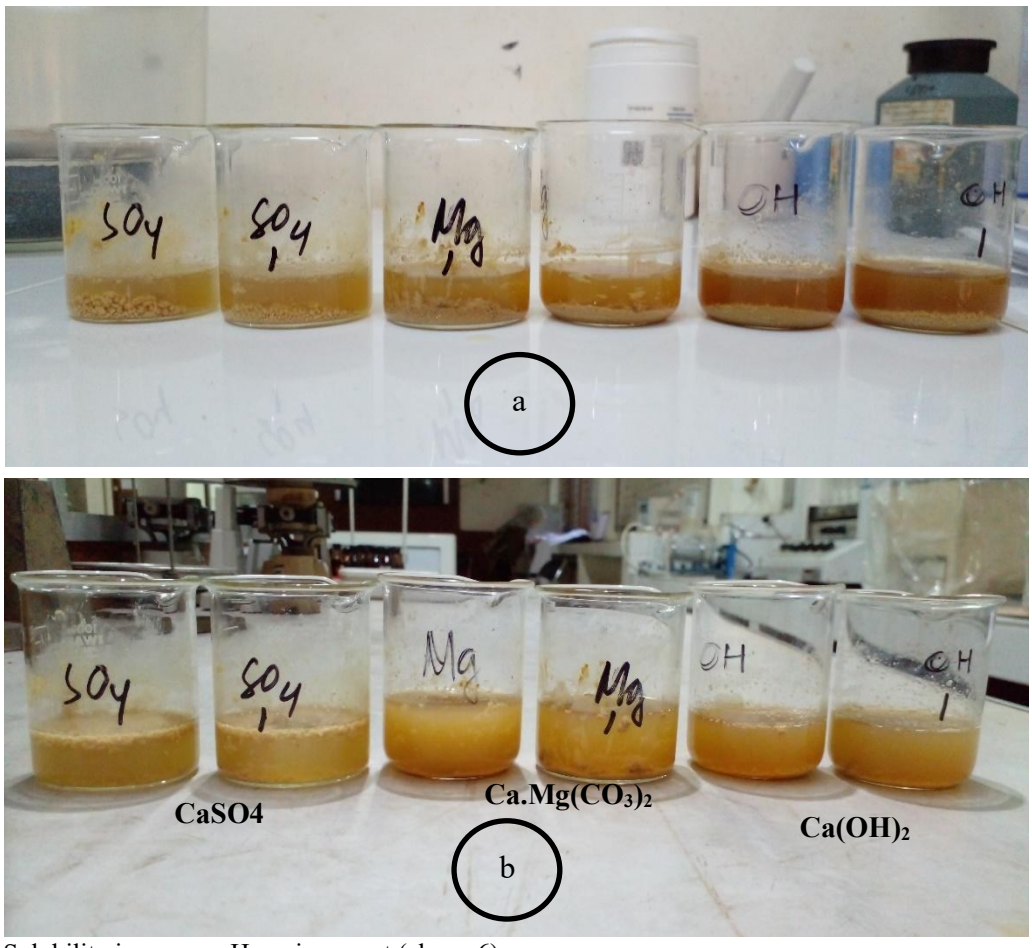
Content	Calcium source treatment		
	$Ca(OH)_2$	$Ca.Mg(CO_3)_2$	$CaSO_4$
Water (%)	3.78 ± 136	4.13 ± 0.94	3.95 ± 0.75
Ash (%)	$5.82^a \pm 0.78$	$9.02^b \pm 0.45$	$8.84^b \pm 0.94$
Fat (%)	$94.13^b \pm 3.55$	$90.67^b \pm 2.67$	$90.15^a \pm 4.37$

Note: $^{a, b, c}$ The superscripts a, b, and c in the columns for $Ca(OH)_2$, $Ca.Mg(CO_3)_2$, and $CaSO_4$ indicate significant differences ($P < 0.015$).

Triglycerides consist of one glycerol molecule bound to three fatty acid molecules through ester bonds. When lemuru fish oil undergoes hydrolysis, water breaks this ester bond and produces glycerol and free fatty acids (Sarungallo et al., 2014). The remaining moisture content, although very low, may be attributed to the residual water in the used lemuru fish oil. The significant differences in ash content are primarily influenced by the type, amount, percentage, and atomic weight of each mineral present in the respective calcium sources used, which remain in the protected oil product. When Ca $(OH)_2$ is used as the calcium source, the only mineral present is calcium, which constitutes 10.8% of the Ca $(OH)_2$ molecule. In contrast, the use of Ca. Mg $(CO_3)_2$ introduces both calcium and magnesium, totalling 6.97%, and $CaSO_4$ adds calcium and sulfur, totalling 10.6%—the lower ash content when using Ca. Mg $(CO_3)_2$ is due to calcium being substituted by magnesium, which has a lower atomic weight (40.1 compared to 24.3). Likewise, the use of $CaSO_4$, where calcium is substituted by sulfur, which has a lower atomic weight (40.1 compared to 32.1). The organic matter content in the product is inversely proportional to the ash content, as the organic matter is calculated as 100 minus the percentage of ash. The organic matter in the product may include unhydrolyzed fat, free fatty acids not bound to Ca and Mg from the calcium sources, as well as fatty acids bound to Ca and Mg, and glycerol.

Solubility in rumen pH environment (above 6) and in pepsin-HCl/gastric pH solution (acidic)

The results of the solubility test of protected fat products in rumen pH environment (above 6) and in pepsin-HCl/gastric pH solution (acidic) are presented in Figure 4.



Note: a. Solubility in rumen pH environment (above 6)
b. Solubility in pepsin-HCl solution/gastric pH (acid)

Figure 4. Solubility in rumen pH environment (above 6) and in pepsin-HCl/gastric pH solution (acidic)

Based on Figure 4, the residue or lumps of protected fat products made using $CaSO_4$, $Ca.Mg(CO_3)_2$, or $Ca(OH)_2$, in the rumen pH environment (Figure 3) is more than the residue in the pepsin-HCl/gastric pH solution (Figure 3). This indicates that the protected fat products are more difficult to dissolve in the rumen environment than in the pepsin-HCl/gastric pH solution (which is acidic). Normal pH in the rumen (digestive system of ruminant animals such as cows, goats and sheep) ranges from 5.7 to 7.0 (Purbowati et al., 2014; Debevere et al., 2020; Nurhaita et al., 2020). The rumen has a more neutral pH, ranging from around 5.7 to 7, and contains various microorganisms that can digest fibrous materials and carbohydrates. However, protected fat products are usually coated with materials that are resistant to rumen microbial attacks, such as fatty acids protected by proteins or calcium compounds such as $Ca(OH)_2$, $CaSO_4$, $Ca.Mg(CO_3)_2$, so that protected fat is more difficult to dissolve and digest in the rumen because the pH is not low enough and because of protection from microbes. While in the true stomach (abomasum) or small intestine, protected fat can be digested more effectively which has a more acidic pH of 2-4 where in this acidic environment, the protective material that coats the fat will be more easily dissolved or degraded, allowing fat to be digested and absorbed more efficiently by ruminant livestock (Febrianti et al., 2016; Suryani et al., 2023).

CONCLUSION

This study demonstrated that $Ca(OH)_2$, $CaCO_3 \cdot MgCO_3$ (dolomite), and $CaSO_4$ (gypsum) are effective calcium sources for producing protected fat from lemuru fish oil. At the same time, $CaCO_3$ is unsuitable due to its inability to bind free fatty acids. Among the effective compounds, $Ca(OH)_2$ produced the fastest hardening product, whereas dolomite and gypsum provided more balanced mineral contributions, including magnesium and sulfur. All protected fat products were resistant to solubility in the rumen environment but readily degraded under acidic gastric conditions, confirming their function as bypass fat. These findings highlight the potential use of inexpensive and locally available calcium compounds as alternatives to conventional Na- or K-based salts, thereby supporting the development of cost-effective and nutritionally safe fat supplements for ruminants. Further in vivo research is recommended to evaluate the feeding value, optimal inclusion levels, and long-term effects of these protected fat products on ruminant performance.

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