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The Influence of Lipase Enzyme Level and $Ca(OH)_2$ Usage in the Production of Protected Lemuru Fish Oil Supplement on the Texture, Nutrient Content, and Fatty Acid Value of the Product

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ABSTRACT: The scarcity of feed energy source to ruminant can be anticipated by adding oil to the feed. However, the effect of adding oil to the feed more than 7% will reduce the activity of fiber-degrading microbes because fat is toxic. In addition, fat in the feed will easily undergo biohydrogenation by rumen microbes so that it will reduce its function. Making fat protection feed using lemuru fish oil with a combination of lipase enzymes and $Ca(OH)_2$ can be a solution because it aims to protect fat from biohydrogenation in the rumen. The experiment used a completely randomized design method with a 3x2 factorial pattern with factors 1) lipase enzyme (0%, 0.5%, and 1%) and factor 2) $Ca(OH)_2$ (20% and 25%). The product results obtained showed that the product quality was in good condition with the product hardness criteria at number 4 (hard). Analysis of variance showed no significant effect on interaction,enzyme factor and $Ca(OH)_2$ factor ($P>0.05$) on DM, OM, CF and acid number where the combination of L_1K_{20} treatment (1% enzyme and 20% $Ca(OH)_2$) produced the highest DM content of 90.25±1.12%; OM 81.98±1.35%; CF 14.62±0.53%; and the lowest acid number of 0.54±.0.06 mg KOH/g sample. The conclusion that can be given is that the use of a combination of 1% lipase enzyme and 20% $Ca(OH)_2$ in fat protection products produces good quality products in terms of product hardness, high nutrient content and low acid number according to the standard fatty acid number below 1 mg KOH/g sample.

KEYWORDS: acid number, calcium soap, $Ca(OH)_2$, lipase enzyme, nutrient content.

INTRODUCTION

Energy in feed is an important nutrient and is needed by livestock to support health, productivity and livestock reproduction. Increasing energy intake in ruminant livestock can be attempted by supplementing fat (oil) because fat contributes 2.25 times more energy than energy sources from carbohydrates and proteins (Pantoja, Firkins, Estridge, and Hull, 1994). Oil as a feed ingredient has several advantages as a source of energy, a source of essential fatty acids, a carrier of vitamins, and increasing feed efficiency (Fernandez, 1999). One of the oils that has the potential to be used as an energy source can be obtained from lemuru fish oil. Lemuru fish oil is a by-product of making fish meal and canning lemuru fish which can produce around 5% of fish oil yield from the fish canning process and 10% from the fish flour process (Lamid, Pursetyo, Tri, and Istiqomah, 2017). Research by Maulana, Sukraso, and Damayanti (2014) stated that lemuru fish oil has a fatty acid content of 38.15% SFA, 32.18% MUFA, and 28.58% PUFA.

Oil supplementation in ruminant livestock feed has a weakness because it will inhibit the activity and growth of rumen microbes (Adawiah, Sutardi, Toharmat, Manalu, and Tanuwiria, 2006). Fat is known to have toxic properties because it is not easily soluble in rumen fluid and tends to associate with feed particle components so that microbes will find it difficult to degrade feed optimally (Palmquist and Jenkins, 1980). Oil is generally antimicrobial against protozoa so that it is necessary to adjust the use of fat concentration supplemented in feed (Suryahadi, Toharmat, Sudarman, and Amrullah, 2004). In addition to being toxic, fat given in feed will undergo biohydrogenation in the rumen so that it will change unsaturated fat into saturated fat (Agnihotri, Mishra, Goda, and Arora, 2012). Research by Sudibya, Riyanto, and Sari (2015) stated that the use of fish oil in feed for meat goats, dairy goats, sheep, and beef cattle is optimally 4%. Efforts to eliminate the negative effects of fatty acids that are toxic to rumen microbes can be anticipated by fatty acid protection treatment. The saponification method can be used to protect fat using a mixture of strong bases such as CaO, KOH, and $CaCl_2$ (Purwati, 2016), NaOH (Sudibya, Akbar, Sabar, and Riyanto, 2017) and quite a few use $Ca(OH)_2$. Fat protection in this study will go through the hydrolysis stage using the lipase enzyme to convert fat (triglycerides) into

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fatty acids and glycerol which will then be continued to the saponification stage using a strong base, namely $Ca(OH)_2$ into Ca-protected lemuru fish oil. Through the combination of lipase enzymes and $Ca(OH)_2$ in the manufacture of Ca-protected lemuru fish oil, it is expected to reduce the negative impact of fat that is toxic to rumen microbes. The bond between calcium is stable at neutral pH such as the rumen and is reducible (easily released again) at acidic pH or post-rumen (Pramono, Kustono, Widayati, Putro, Handayanta, and Hartadi, 2013). This study aims to evaluate the protection of Ca-protected lemuru fish oil as a supplementary feed product using different ratios of lipase and $Ca(OH)_2$ enzymes based on the level of product hardness, nutrient content, and acid number.

MATERIALS AND METHODS

This study was conducted at the Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Brawijaya University, Malang. The materials used in this study used fat from lemuru fish oil obtained from fish oil processing in Bayuwangi, East Java, Indonesia. The lipase enzyme is the Habio Enzyme brand and the $Ca(OH)_2$ is the Pudak brand. The procedure for making Ca-protected lemuru fish oil is as follows:

1. Weigh 100g of fish oil and put it in a 250 ml Erlenmeyer flask.
2. First add the lipase enzyme as much as the treatment level given then stir until homogeneous.
3. Add $Ca(OH)_2$ based on the treatment level and homogenize until it clumps.
4. The Ca-protected lemuru fish oil complex that has been made is then air-dried at room temperature (air dry) until it hardens/crystallizes, then mashed using a blender/mortar.

Ca-protected lemuru fish oil in this study was made through 2 reaction stages, namely through a hydrolysis reaction first using the lipase enzyme to convert the triglyceride fat from lemuru fish oil into fatty acids and glycerol. Then the fatty acids produced from the hydrolysis process will be continued by adding $Ca(OH)_2$ to go through a saponification/soaping reaction to produce a Ca-fatty acid bond. The experimental design used was the Factorial RAL pattern with 6 treatments and 3 replications consisting of 2 treatment factors 1) lipase enzyme (L) usage factor (0%, 0.5%, and 1%) and 2) $Ca(OH)_2$ (K) usage factor (20% and 25%). The design for adding lipase enzyme and $Ca(OH)_2$ concentrations in making protected fat is as follows.

- L_0K_{20} = use of 0% lipase enzyme + 20% $Ca(OH)_2$
- $L_{0.5}K_{20}$ = use of 0.5% lipase enzyme + 20% $Ca(OH)_2$
- L_1K_{20} = use of 1% lipase enzyme + 20% $Ca(OH)_2$
- L_0K_{25} = use of 0% lipase enzyme + 25% $Ca(OH)_2$
- $L_{0.5}K_{25}$ = use of 0.5% lipase enzyme + 25% $Ca(OH)_2$
- L_1K_{25} = use of 1% lipase enzyme + 25% $Ca(OH)_2$

The variables observed included the hardness texture of the product with a rating scale of 1) watery; 2) soft; 3) semi-hard; 4) hardness, nutrient content (DM, OM, and CF), and acid number analysis. The data obtained was then analyzed using analysis of variance and if there was an influence on the treatment, it was continued with the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results obtained from the use of various enzyme lipase concentration combinations (0%, 0.5%, and 1%) and $Ca(OH)_2$ concentrations (20% and 25%) in the production of fat-protected feed (Ca-protected lemuru fish oil) showed no significant difference ($P>0.05$) in terms of texture, interaction, enzyme factor, and $Ca(OH)_2$ factor on texture, nutrient content (crude protein, crude oil, and crude fiber), and acid value.

Texture of Ca-protected Lemuru Fish Oil Product

Observations of the Ca-protected lemuru fish oil product under all treatments showed a good product quality level, as indicated by a score of 4 with a "hard" criterion. This suggests that all lipase enzyme concentrations and $Ca(OH)_2$ levels used resulted in products of similar quality across each treatment. The texture score of 4 with a "hard" criterion for all treated lemuru fish oil samples relates to the success achieved during the product manufacturing process, which was carried out under good and optimal conditions. Lipase enzymes, which hydrolyze fats, convert triglycerides into fatty acids and glycerol. Then, with the addition of $Ca(OH)_2$, saponification occurs, where fatty acids react with Ca (a strong base) to form solid calcium salts (Indarto et al., 2020).

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The combination of lipase enzyme and $Ca(OH)_2$ addition enables a more optimal hydrolysis and saponification process of fats. The lipase enzyme plays a role in hydrolyzing fats, converting triglycerides into fatty acids and glycerol, which is then followed by the addition of $Ca(OH)_2$ for the saponification process, where fatty acids react with the calcium compound (a strong base) to form solid calcium salts. This is in line with the statement of Sholeha and Agustini (2021) that lipase, a complex protein, functions similarly to other enzymes, acting as a biocatalyst to accelerate the hydrolysis reaction of fats into fatty acids and glycerol under normal temperature and conditions. The role of $Ca(OH)_2$ as a strong base in the saponification process of fatty acids aligns with the statement of Indarto et al. (2020), which explains that fatty acids can react with calcium sources through a modified fusion reaction to form solid crystals known as protected Ca lemuru fish oil.

Nutrient Content of the Product: Dry Matter (DM), Organic Matter (CO), and Crude Fat (CF)

The nutrient content (DM, OM, and CF) of the Ca-protected lemuru fish oil product made with different levels of lipase enzyme and Ca(OH)2 is presented in Tables 1, 2, and 3, respectively. The partial average of the dry matter content of the Ca-protected lemuru fish oil is shown in Table 1 below.

Table 1. Dry Matter Content (%) of the Fat-Protected Product from Lemuru Fish Oil, Lipase Enzyme, and $Ca(OH)_2$

Enzyme (L)	Calcium (K)		Average
	20	25	
0	89.08±1.61	88.28±1.96	88.68±1.66
0.5	89.45±1.79	89.55±2.11	89.50±1.75
1	90.25±1.12	88.92±1.23	89.58±1.28
Average	89.59±1.43	88.92±1.66	

Based on Table 1, it is shown that the treatment levels of lipase enzyme, $Ca(OH)_2$, and their interaction did not have a significant effect ($P>0.05$) on the dry matter (DM) content of the Ca-protected lemuru fish oil product. This result is indicated by a P value of >0.05, meaning there were no significant differences between the treatments of lipase enzyme, $Ca(OH)_2$, or their interaction. The use of 0%, 0.5%, and 1% lipase enzyme levels, as well as 20% and 25% $Ca(OH)_2$, resulted in average DM content that did not differ much. Based on Table 1, it is shown that the DM content of the Ca-protected lemuru fish oil across all treatments ranged from 88.28±1.96% to 90.25±1.12%. Although there was no significant effect, there was a tendency for the highest DM content to be found in the L_1K_{20} treatment (1% lipase enzyme and 20% $Ca(OH)_2$), which was 90.25±1.12%. Sudibya et al. (2017) in their study on fat-protected products from lemuru fish oil reported a DM content of 90.11%, which is slightly lower than the results of this study. According to Proano, Stuart, Chongo, Flores, Herrera, Medina, and Sarduy (2015), the standard dry matter content of calcium soap or Ca-protected lemuru fish oil made from animal fat is at least 65%. This indicates that although the average DM content of the Ca-protected lemuru fish oil product did not show significant differences, it still meets the standard CP content for a fat-protected product.

The organic matter (OM) content in the effect of using different levels of lipase enzyme and $Ca(OH)_2$ in the Ca-protected lemuru fish oil product showed that the treatments of lipase enzyme, $Ca(OH)_2$, and their interaction did not significantly differ ($P>0.05$) in terms of OM content. The partial average of the organic matter content of the Ca-protected lemuru fish oil is presented in Table 2 below.

Table 2. Organic Matter Content (%) of the Fat-Protected Product from Lemuru Fish Oil, Lipase Enzyme, and $Ca(OH)_2$

Enzyme (L) (%)	Calcium (K) (%)		Average
	20	25	
0	79.91±1.11	80.79±0.89	80.35±1.02
0.5	81.23±1.65	81.25±0.87	81.24±1.18
1	81.98±1.35	80.88±1.76	81.43±1.52
Average	81.04±1.50	80.97±1.10	

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Based on Table 2, it shows no significant effect (P>0.05) from the lipase enzyme factor, $Ca(OH)_2$, or their interaction. This indicates that the use of 0%, 0.5%, and 1% lipase enzyme levels, as well as 20% and 25% $Ca(OH)_2$ levels in the production of Ca-protected lemuru fish oil resulted in average organic matter (OM) content that did not differ significantly. Based on Table 2, the average OM content of the Ca-protected lemuru fish oil across all treatments ranged from 79.91±1.11% to 81.98±1.35%. Although there was no significant difference, the average OM tended to increase in the L_1K_{20} treatment (1% lipase enzyme and 20% $Ca(OH)_2$) to 81.98±1.35%. Pramono et al. (2018) in their study on fat-protected products using saponification and microencapsulation methods reported an average OM content of 81.33%, which is slightly lower than the results of this study. This suggests that although the effect was not significant, the use of lipase enzyme and $Ca(OH)_2$ tends to increase the OM content. The lower OM content in the Ca-protected lemuru fish oil is due to the higher ash content in the lemuru fish oil material. Additionally, the $Ca(OH)_2$ compound added for the saponification reaction of fatty acids increases the mineral content in the product, as Ca (calcium) is a chemical compound that is a source of minerals. Therefore, there is a relationship between the ash content and the organic content of a material. The ash content of a material is an indicator of its micromineral content and serves a structural function (P, Ca, Mg, Fe, and others), which is important for membrane balance and tissue permeability (Murillo et al., 2013).

Based on the analysis of crude fat (CF) content from the lipase enzyme level, $Ca(OH)_2$, and their interactions in the Ca-protected lemuru fish oil product, no significant effect was observed (P>0.05). The partial average crude fat content of Ca-protected lemuru fish oil is presented in Table 3 below.

Table 3. Crude Fat Content (%) of the Fat-Protected Product from Lemuru Fish Oil, Lipase Enzyme, and $Ca(OH)_2$

Enzyme (L) (%)	Calcium (K) (%)		Average
	20	25	
0	13.16±0.08	13.63±0.45	13.40±0.38
0.5	13.72±0.57	13.49±0.15	13.60±0.39
1	14.62±0.53	13.82±0.94	14.22±0.81
Average	13.83±0.75	13.65±0.55	

Based on Table 3, the effect of lipase enzyme levels, $Ca(OH)_2$, and their interaction on the crude fat (CF) content showed no significant differences (P>0.05) across all treatments. This indicates that the use of lipase enzyme levels of 0%, 0.5%, and 1%, as well as $Ca(OH)_2$ levels of 20% and 25%, resulted in average CF contents that did not differ significantly. According to Table 3, the CF content obtained in all treatments ranged from 13.16±0.08% to 14.62±0.53%. However, there was a tendency for the highest average CF to be observed in the L_1K_{20} treatment, which was 14.62±0.53%. Based on research by Wibowo et al. (2012), pure lemuru fish oil without any protective treatment had a CF content of around 70.45%. Although there were no significant differences, the use of lipase enzyme levels and $Ca(OH)_2$ in protecting lemuru fish oil tends to reduce the CF content afterwards. The protected treatment of lemuru fish oil using a combination of lipase enzyme and $Ca(OH)_2$ appears to reduce the CF content in the Ca-protected lemuru fish oil product. The low fat content is known to be influenced by the physical treatment provided, in the form of heat generated by the hydrolysis process by lipase enzymes and saponification from $Ca(OH)_2$.

The low fat content is known to be influenced by the physical treatment given. According to Luque (2008), the saponification process also involves the breakdown of carbon bonds in fats, similar to the hydrolysis process, due to the changes in temperature and pH during saponification, which leads to a reduction in the crude fat content of the resulting fat-protected product. Furthermore, according to Berghuis and Maulana (2023), fatty acids that have undergone saponification by strong bases will form more stable soap esters, which are harder to dissolve by fat extraction solvents, such as ether compounds. Soap ester compounds have a hydrophilic (polar head) group that tends to dissolve in water, and a hydrophobic (non-polar tail) group that is soluble in ether compounds. Ether is a non-polar solvent, so when the Ca-protected lemuru fish oil product is extracted using ether, the compounds that will be extracted are the remaining non-polar compounds from the fats, such as sterols, fat-soluble pigments, or lipophilic components that have not undergone saponification.

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Acid Value

The acid value in the use of lipase enzyme levels, $Ca(OH)_2$, and their interaction showed no significant differences ($P>0.05$) across all treatments. The partial average acid value of the Ca-protected lemuru fish oil product is presented in Table 4 as follows.

Table 4. Acid Value (mg KOH/g sample) of the Fat-Protected Product from Lemuru Fish Oil, Lipase Enzyme, and $Ca(OH)_2$

Enzyme (L) (%)	Calcium (K) (%)		Average
	20	25	
0	0,63±0,11	0,59±0,14	0,61±0,11
0,5	0,54±0,13	0,62±0,14	0,58±0,13
1	0,54±0,06	0,54±0,12	0,53±0,09
Average	0,56±0,10	0,58±0,12	

Based on the data in Table 4, it is shown that the treatments of lipase enzyme, $Ca(OH)_2$, and their interaction had no significant effect ($P>0.05$) on the acid value of the Ca-protected lemuru fish oil product. However, there was a tendency for the lowest average to be found in the combination of L_1K_{20} (1% enzyme and 20% $Ca(OH)_2$), which was 0.54±0.06 mg KOH/g sample, compared to the other treatments. This indicates that the use of lipase enzyme levels of 0%, 0.5%, and 1%, along with $Ca(OH)_2$ levels of 20% and 25%, resulted in average acid values that did not differ significantly across all treatments. According to Table 4, the acid values obtained for all treatments ranged from 0.54±0.06 mg KOH/g sample to 0.63±0.14 mg KOH/g sample. Although there was no significant effect, there was a tendency for the lowest average to be observed in the L_1K_{20} treatment, at 0.54±0.06 mg KOH/g sample. This suggests that as the concentration of the lipase enzyme level increases, the acid value of the Ca-protected lemuru fish oil decreases. In contrast, the addition of $Ca(OH)_2$ was found to increase the acid value as the concentration level increased. Research by Handojo et al. (2020) produced a low acid value for calcium soap derived from the byproduct of crude palm oil distillation (Palm Fatty Acid Distillate, PFAD), with a minimum value of 0.37 mg KOH/g sample. This result is lower compared to this study, but overall, the Ca-protected lemuru fish oil product from this research has a low acid value of less than 1 mg KOH/g sample. Additionally, products with low acid values are known to be non-toxic to the digestive systems of ruminant livestock, particularly in the rumen (Handojo et al., 2020). The standard acid value that determines the quality of Ca-protected lemuru fish oil in good condition is below 1 mg KOH/g sample. Therefore, it can be concluded that all the Ca-protected lemuru fish oil products in this study are of good quality, as they are still below the standard.

CONCLUSION

The product of Ca-protected lemuru fish oil made using lemuru fish oil with varying levels of lipase enzyme and $Ca(OH)_2$ as sources of fat protection feed for ruminant livestock showed no significant effects across all treatment combinations. However, it was able to produce a high-quality product, as seen from the product's hardness level, which met the criteria of 4 (hard). The treatment combination of L_1K_{20} (1% lipase enzyme and 20% $Ca(OH)_2$) can be used as a reference in the production of fat protection products, as it resulted in high dry matter (DM), organic matter (OM), and crude fat (CF) contents, and a low acid value of less than 1 mg KOH/g sample.

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