Effectiveness of Calcium Hydroxide-Protected Lemuru Fish Oil in Optimizing Essential Fatty Acids under In Vitro Rumen Fermentation

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Abstract. nsaturated fatty acids (UFAs), particularly polyunsaturated fatty acids (PUFAs) such as oleic, linoleic, linolenic, arachidonic acids, EPA, and DHA, play a vital role in livestock productivity and in improving the nutritional quality of animal products. However, PUFAs are highly susceptible to biohydrogenation in the rumen, reducing their biological availability. The novelty of this study lies in evaluating lemuru fish oil protected with calcium hydroxide [$Ca(OH)_2$], which has not been specifically assessed in previous fat-protection research. This study aimed to determine the effect of unprotected and $Ca(OH)_2$-protected lemuru fish oil on the in vitro fatty acid profile. A nested randomized block design was applied under laboratory conditions, with treatments consisting of unprotected fish oil (P1) and $Ca(OH)_2$-protected fish oil (P2) at levels of L0 (0%), L5 (5%), L7.5 (7.5%), L10 (10%), and L12.5 (12.5%), each replicated three times. Variables observed included oleic, linoleic, linolenic, arachidonic acids, EPA, and DHA. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test. Results indicated that both treatment type and supplementation level had highly significant effects ($P<0.01$) on fatty acid profiles. The highest lipid concentrations were achieved with 10% supplementation of protected fish oil, while the best outcome for unprotected oil occurred at the 5% level. These findings provide practical implications for livestock feeding, showing that unprotected lemuru fish oil can be effectively utilized up to 5% inclusion, whereas $Ca(OH)_2$-protected lemuru fish oil can be safely applied up to 10%, offering a sustainable strategy to improve ruminant productivity and product quality.

Keyword: Protected fish oil; Unsaturated fatty acids; Calcium soap; In vitro fermentation

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1 Introduction

Improving the quality of ruminant livestock products, particularly meat and milk, has become a key focus in modern animal production since it is directly associated with nutritional value and consumer health. One of the widely studied approaches is the modification of dietary fatty acid profiles to enhance the concentration of polyunsaturated fatty acids (PUFA) in animal-derived products [1].

Fish oil, including lemuru fish oil, serves as a natural source of long-chain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play important roles in metabolic health, reproductive function, and immune system performance in livestock [2, 3]. However, the major challenge in PUFA supplementation in ruminants lies in the high rate of biohydrogenation by rumen microbes, which convert unsaturated fatty acids into saturated forms, thereby reducing their availability for absorption in the intestine [4].

One strategy to address this limitation is lipid protection through the formation of calcium soaps using calcium hydroxide. This technology has been shown to decrease PUFA degradation in the rumen and improve their transfer to the small intestine [5]. Consequently, the application of protected fish oil has the potential to improve fatty acid profiles in animal tissues and products, while simultaneously enhancing the functional value of animal-based foods for consumers [6].

Although several fat-protection methods such as encapsulation and calcium salts of palm oil have been widely investigated, no specific evaluation has been conducted on lemuru fish oil protected with $Ca(OH)_2$. This research gap highlights the need for a focused study on this locally available marine resource. Moreover, emphasizing lemuru fish oil is highly relevant for the Indonesian livestock industry, where sustainable and cost-effective feed additives are required to support both productivity and product quality. Based on this background, research on the effects of protected and unprotected lemuru fish oil on fatty acid profiles under in vitro conditions is crucial to evaluate the effectiveness of calcium hydroxide protection in preserving PUFA and its implications for livestock product quality.

2 Materials and methods

The primary material used in this study was lemuru fish oil, both in unprotected form and protected with calcium hydroxide ($Ca(OH)_2$). The treatments consisted of five supplementation levels of lemuru fish oil in the in vitro fermentation medium, namely 0%, 5%, 7.5%, 10%, and 12.5%. Protection was carried out through a saponification process with calcium hydroxide to produce calcium soaps that are relatively inert under ruminal conditions.

The calcium-soap complex was prepared by weighing 1,000 g of lemuru fish oil into a 1 L Erlenmeyer flask and heating it on a hot plate. Subsequently, 125 g of calcium hydroxide ($Ca(OH)_2$) was added to the heated oil and mixed using a magnetic stirrer until a homogeneous mixture was obtained and the solution reached boiling, indicating the formation of the Ca-oil complex. The mixture was then continuously stirred while being cooled to room temperature. Once cooled, the Ca-oil complex was ready for use as a feed supplement.

The experiment was conducted using the in vitro fermentation technique to simulate rumen conditions. A nested randomized block design was applied with two treatment factors: unprotected lemuru fish oil (P1) and calcium hydroxide-protected lemuru fish oil (P2), each tested at five supplementation levels. Samples were incubated with rumen fluid collected from healthy donor cattle. Incubation was performed at 39°C to represent physiological rumen conditions. After incubation, samples were analysed to determine fatty acid profiles,

including oleic acid, linoleic acid, linolenic acid, arachidonic acid, EPA, and DHA, using Gas Chromatography (GC).

The experimental data were analysed using analysis of variance (ANOVA) according to the experimental design. If significant differences were detected ($P<0.05$), Duncan's Multiple Range Test (DMRT) was conducted to compare treatments

3 Results and discussion

The fatty acid profile in livestock feed refers to the composition and proportion of various types of fatty acids present in the feed ingredients provided to animals, either in their natural form or through supplementation. Fatty acids are the main components of lipids (fats) and function as a dense source of energy, precursors of bioactive compounds, and essential elements in cell membrane structure. Characterization of the fatty acid profile is crucial, as the type and concentration of fatty acids in the diet strongly influence animal health, feed efficiency, and the quality of animal products such as meat, milk, and eggs. The in vitro fatty acid profiles resulting from the effects of unprotected and protected lemuru fish oil are presented in Table 1.

Table 1. In vitro fatty acid profile influenced by unprotected and calcium hydroxide-protected lemuru fish oil.

Variable	Treatment	Treatment mean	Lemuru Fish Oil Levels				
			0%	5%	7,5%	10%	12,5%
Oleic acid	P1	2,282±0,02^{a}	2,253±0,01	2,271±0,01	2,278±0,01	2,294±0,01	2,315±0,01
	P2	4,144±0,98^{b}	2,254±0,01^{a}	4,564±0,02^{b}	4,579±0,03^{b}	4,607±0,02^{b}	4,719±0,11^{c}
Linoleic acid	P1	0,628±0,02^{a}	0,598±0,01	0,617±0,01	0,624±0,01	0,639±0,01	0,661±0,01
	P2	1,023±0,22^{b}	0,592±0,01^{a}	1,104±0,01^{b}	1,114±0,01^{bc}	1,127±0,01^{bc}	1,175±0,07^{c}
Linolenic acid	P1	0,046±0,02^{a}	0,016±0,01^{a}	0,035±0,01^{a}	0,042±0,01^{a}	0,056±0,01^{a}	0,080±0,01^{b}
	P2	0,063±0,04^{b}	0,016±0,01^{a}	0,053±0,01^{a}	0,056±0,01^{a}	0,069±0,00^{b}	0,123±0,06^{b}
Arachidonic acid	P1	0,062±0,02^{a}	0,031±0,00^{a}	0,051±0,00^{a}	0,058±0,01^{a}	0,072±0,01^{a}	0,096±0,01^{b}
	P2	0,121±0,08^{b}	0,032±0,00^{a}	0,095±0,01^{b}	0,099±0,00^{b}	0,115±0,00^{b}	0,266±0,06^{b}
EPA	P1	0,108±0,02^{a}	0,078±0,00	0,097±0,00	0,104±0,01	0,119±0,01	0,142±0,01
	P2	0,190±0,08^{b}	0,081±0,00^{a}	0,169±0,01^{b}	0,174±0,01^{bc}	0,216±0,06^{c}	0,310±0,01^{d}
DHA	P1	0,058±0,02^{a}	0,028±0,00	0,046±0,00	0,055±0,01	0,069±0,01	0,089±0,00
	P2	0,131±0,09^{b}	0,027±0,00^{a}	0,096±0,01^{b}	0,116±0,03^{b}	0,146±0,06^{b}	0,267±0,06^{c}

Note:

P1: unprotected lemuru fish oil

P2: calcium hydroxide-protected lemuru fish oil

a–b: different superscripts in the same column indicate that unprotected and calcium hydroxide-protected lemuru fish oil had a highly significant effect ($P<0.01$) on oleic, linoleic, linolenic, arachidonic, EPA, and DHA concentrations.

a–c: different superscripts in the same row indicate that supplementation levels of calcium hydroxide-protected lemuru fish oil had a highly significant effect ($P<0.01$) on oleic, linoleic, and DHA concentrations.

a–b: different superscripts in the same row indicate that supplementation levels of unprotected and calcium hydroxide-protected lemuru fish oil had a highly significant effect ($P<0.01$) on linolenic and arachidonic acid concentrations.

a–d: different superscripts in the same row indicate that supplementation levels of calcium hydroxide-protected lemuru fish oil had a highly significant effect ($P<0.01$) on DHA concentrations.

The in vitro fatty acid profiles influenced by unprotected (P1) and calcium hydroxide-protected (P2) lemuru fish oil are presented in Table 1. Overall, supplementation with protected lemuru fish oil resulted in higher concentrations of oleic, linoleic, linolenic, arachidonic acids, EPA, and DHA compared to the unprotected form. The most pronounced improvements were observed at the 10% supplementation level of protected oil, whereas the

optimal level for unprotected oil was found at 5%. The concentration profile of fatty acids under in vitro conditions is presented in Figure 1.

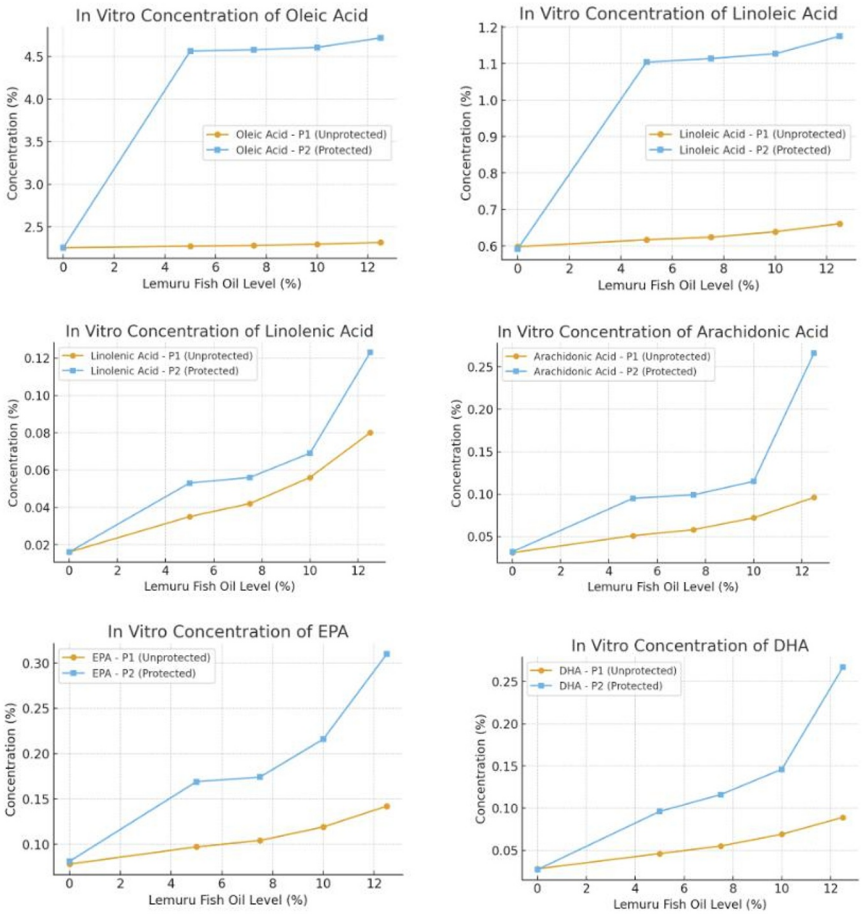

Fig. 1. In vitro concentration of lipid acid profile

The visualization results demonstrated that calcium hydroxide protection consistently increased the concentrations of unsaturated fatty acids across all supplementation levels. In P2, oleic acid (C18:1) concentration increased sharply from the 5% to the 12.5% level, while P1 remained relatively stable. A similar trend was observed for linoleic acid (C18:2 n-6), where P2 showed a substantially greater increase than P1, confirming that fat protection effectively prevents PUFA biohydrogenation in the rumen. In the case of linolenic acid (C18:3 n-3), both treatments showed increases with higher supplementation levels, but P2 consistently yielded higher concentrations, particularly at 10–12.5%. Arachidonic acid (C20:4 n-6) also showed more pronounced increases in P2 compared with P1, emphasizing the protective role of calcium hydroxide. The most significant improvements were found in EPA (C20:5 n-3) and DHA (C22:6 n-3), where P2 achieved concentrations more than twice those of P1.

Oleic and linoleic acids are highly susceptible to ruminal biohydrogenation but can be preserved through fat protection strategies [6]. Calcium soap technology increases PUFA flow through the rumen without impairing the digestibility of other nutrients [4,7]. Arachidonic acid plays an important role in reproductive function through eicosanoid

synthesis [8]. Moreover, enrichment of EPA and DHA has been shown to improve metabolism, immune balance, and the nutritional quality of ruminant-derived products [1,2]. Therefore, calcium hydroxide protection of lemuru fish oil not only enhances PUFA retention but also provides important biological implications for livestock productivity and the functional value of animal products.

The protective effect of $Ca(OH)_2$ can be attributed to the formation of calcium soaps, which are insoluble at ruminal pH, thereby reducing microbial biohydrogenation and enabling a greater proportion of unsaturated fatty acids to bypass the rumen and reach the small intestine. Biologically, this mechanism implies that increased oleic acid supports improved lipid metabolism, while linoleic and arachidonic acids are crucial for reproductive and immune functions. Furthermore, higher concentrations of EPA and DHA contribute to stronger anti-inflammatory responses, metabolic balance, and improved nutritional quality of meat and milk.

The ANOVA results, which showed highly significant differences ($P<0.01$), further confirm the effectiveness of calcium hydroxide protection. From a practical perspective, unprotected lemuru fish oil can be safely used up to 5% in ruminant diets, while protected oil can be applied up to 10%. This strategy provides a sustainable approach to improving livestock productivity and enhancing the functional value of animal-derived products for consumers. Nevertheless, this study was conducted under in vitro conditions, which may not fully reflect the complex dynamics of in vivo digestion and metabolism. Factors such as feed intake behaviour, ruminal passage rate, and systemic absorption could influence the actual outcomes in animals. Therefore, further in vivo studies are required to validate these findings and to determine the long-term effects of protected lemuru fish oil on livestock performance and product quality.

4 Conclusion

Calcium hydroxide protection of lemuru fish oil effectively preserved unsaturated fatty acids (oleic, linoleic, linolenic, arachidonic, EPA, and DHA) under in vitro rumen fermentation. Protected oil performed best at the 10% inclusion level, while unprotected oil was effective only up to 5%. This strategy enhances lipid metabolism, reproductive and immune functions, and improves the nutritional quality of animal products.

Unprotected lemuru fish oil can be safely used up to 5%, while calcium hydroxide-protected oil is recommended up to 10% in ruminant diets. Future in vivo trials on dairy and beef cattle are necessary to validate long-term effects on animal performance and product quality.

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