Research Article

Improving Energy Utilization and Digestibility in Sheep through Calcium Hydroxide-Protected Lemuru Fish Oil

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Abstract | This study evaluated the effects of calcium hydroxide-protected Lemuru fish oil on the *in vitro* rumen and post-rumen digestibility of sheep feed. Lemuru fish oil, rich in omega-3 fatty acids such as EPA and DHA, was supplemented at varying inclusion levels (0%, 5%, 7.5%, 10%, and 12.5%) with and without calcium hydroxide protection. Results showed that fish oil protection significantly ($P < 0.01$) improved dry matter, organic matter, crude fiber, and crude protein digestibility, particularly in the post-ruminal phase. However, increasing the inclusion level of fish oil reduced digestibility across most nutrients, especially at 12.5%. Crude fat digestibility was higher in the unprotected treatment in the rumen but improved post-ruminally in the protected treatment due to better enzymatic utilization. These findings suggest that calcium hydroxide protection enhances the nutritional benefits of Lemuru fish oil while minimizing its negative effects on rumen fermentation, making it a promising strategy for improving ruminant feed efficiency.

Keywords | Lemuru fish oil, Protection, *In vitro*, Sheep

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INTRODUCTION

The success of increasing sheep populations largely depends on the quality of feed, particularly its nutritional value, which is critical for maintaining animal health, growth, and reproduction (Patience *et al*., 2015). One of the major challenges in sheep nutrition is the limited availability and high cost of energy-rich feed ingredients. To address this issue, dietary energy can be enhanced by supplementing with fats, while protein requirements may be met through the inclusion of protected proteins that resist degradation in the rumen (Pramono *et al*., 2013).

Lemuru fish oil, derived from the *Sardinella longiceps* species, presents a promising and cost-effective alternative energy source due to its high content of unsaturated fatty acids (Ibrahim *et al*., 2018). This oil, a by-product of the canning industry in Banyuwangi Regency, contains 38.15% saturated fatty acids (SFA), 32.18% monounsaturated fatty acids (MUFA), and 28.44% polyunsaturated fatty acids (PUFA), including substantial amounts of 14.36% eicosapentaenoic acid (EPA) and 4.60% docosahexaenoic acid (DHA) (Suseno *et al*., 2014). Supplementing ruminant diets with PUFA-rich fish oil can improve energy utilization, support protein synthesis, and promote better overall performance, as essential fatty acids cannot be synthesized endogenously by ruminants (Hatami *et al*., 2022; Hernandez-Casner *et al*., 2017).

However, incorporating high levels of unsaturated fat into ruminant diets must be approached cautiously, as it can disrupt rumen fermentation, inhibit fiber digestion, and impair microbial activity (Dewi *et al*., 2021). To overcome these challenges, protective treatments are necessary to stabilize the fat and preserve rumen function. One effective strategy is the use of calcium hydroxide ($Ca(OH)_2$), which helps prevent lipid oxidation, maintains fatty acid integrity, and supports normal rumen activity (Dewi *et al*., 2022). The protective mechanism involves the reaction between $Ca(OH)_2$ and free fatty acids to form calcium soaps, which are insoluble in the rumen's aqueous environment. These calcium soaps are less susceptible to microbial lipolysis and biohydrogenation, allowing unsaturated fatty acids to bypass rumen fermentation and reach the small intestine for absorption.

Calcium hydroxide treatment, through a saponification process, enables safe and effective inclusion of fish oil in ruminant rations without compromising rumen microbial ecosystems (Pramono *et al*., 2018; Pena *et al*., 2023). Studies have demonstrated that protected fish oil improves digestibility of organic and dry matter, enhances animal health, and contributes to better meat quality (Sudibya *et al*., 2016; Dewi *et al*., 2022). Therefore, this study aims to evaluate the impact of calcium hydroxide-protected Lemuru fish oil on *in vitro* digestibility in sheep.

MATERIALS AND METHODS

Study location and research design

This research was conducted at the Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Universitas Brawijaya, for the *in vitro* analysis. Rumen fluid was obtained from fistulated sheep at the Field Laboratory of Animal Nutrition and Feed, Faculty of Animal Husbandry, Universitas Brawijaya. The experimental design used in this study was a laboratory-based trial employing a 2 × 5 factorial randomized block design (RBD) with three replicates per treatment, as shown in Figure 1. The first factor was the type of fish oil treatment, comprising unprotected Lemuru fish oil (MUP) and Lemuru fish oil protected with calcium hydroxide (MP). The second factor was the level of Lemuru fish oil inclusion in the diet, which included 0% (no supplementation), 5%, 7.5%, 10%, and 12.5%. The feed used in this study was formulated to meet the standard nutritional requirements of sheep, with its composition and nutrient profile shown in Table 1.

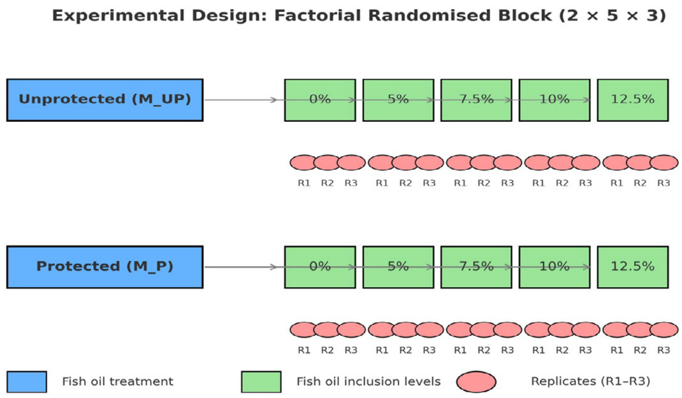

Figure 1: The experimental design of this study.

Preparation of calcium-protected lemuru fish oil

Lemuru fish oil was obtained as a byproduct from the sardine canning industry at PT Sumber Yalasamudra, Muncar, Banyuwangi, East Java, and calcium hydroxide ($Ca(OH)_2$) was used as the reacting agent. The formation of the calcium–oil complex is based on the hydrolysis of triglycerides by a basic compound, producing glycerol and fatty acid salts. In this reaction, the carboxyl (-COOH) groups of the fatty acids bind to calcium cations. The stoichiometric ratio involves two molecules of triglycerides reacting with three molecules of calcium hydroxide.

Table 1: Composition and nutrient content of experimental diets.

Feed ingredients	Treatment									
	M_{UP}					M_P				
	0	**5**	**7.5**	**10**	**12.5**	**0**	**5**	**7.5**	**10**	**12.5**
Odot grass (%)	50	50	50	50	50	50	50	50	50	50
Concentrates (%)	50	50	50	50	50	50	50	50	50	50
Fish oil (%)	0	5	7.5	10	12.5	0	0	0	0	0
Ca-fish oil (%)	0	0	0	0	0	0	5	7,5	10	12,5
Nutrient content (%)										
CP	11.15	11.19	11.43	11.52	11.61	11.15	11.29	11.36	11.43	11.50
CFa	4.09	7.60	9.36	11.12	12.87	4.09	6.73	8.04	9.36	10.68
CFi	20.91	20.95	20.97	20.99	21.00	20.91	20.94	20.91	20.97	20.98
Ash	11.15	11.15	11.46	11.56	11.67	11.15	11.42	11.56	11.70	11.84
NFE	59.07	59.87	60.27	60.67	61.07	59.07	59.67	59.97	60.27	60.57

Note: MUP = unprotected Lemuru fish oil; MP = Lemuru fish oil protected with calcium hydroxide; CP = crude protein; CFa = crude fat; CFi = crude fiber; NFE = Nitrogen-Free Extract

Based on the molecular weights of the fatty acid components and calcium hydroxide, the optimal ratio of fish oil to calcium hydroxide was determined to be 4:1. The preparation of calcium-protected Lemuru fish oil was carried out by first placing 1,000 g of Lemuru fish oil into a 1,000 mL Erlenmeyer flask. Calcium hydroxide was dissolved in distilled water at a 1:1 weight-to-volume (w/v) ratio and added to the fish oil, followed by thorough mixing until a homogeneous mixture was formed. The resulting calcium–fish oil complex was then aerated at room temperature to allow crystallization. Once hardened, the complex was ground using a blender or mortar and subsequently analyzed. A diagram of the saponification process in preparation of calcium-protected fish oil is shown in Figure 2.

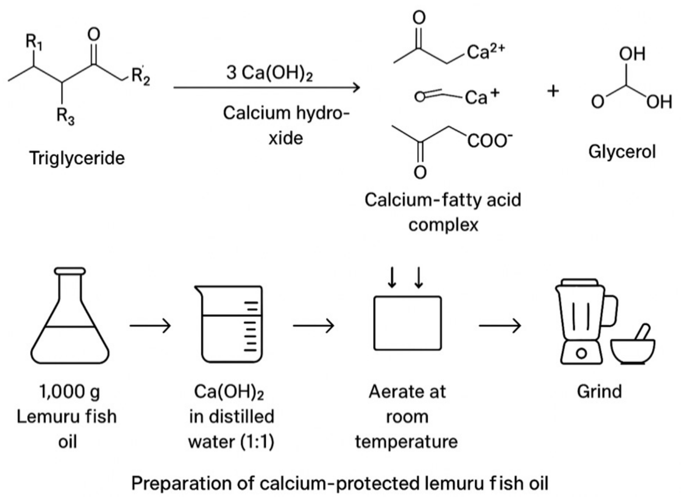

Figure 2: Preparation of calcium-protected Lemuru fish oil.

Preparation of rumen fluid and *in vitro* fermentation

Rumen fluid was collected directly from sheep using a stomach tube connected to a vacuum pump. The fluid was immediately filtered through layers of gauze, and the resulting filtrate was transferred into a pre-warmed crucible maintained at 40 °C, while being continuously flushed with CO_2 to preserve anaerobic conditions. To maintain microbial viability, the rumen fluid was kept at 40°C, and the time between collection and use did not exceed two hours.

A 500 mg feed sample was placed into a fermentation tube, followed by the addition of 40 mL of McDougall's buffer solution and 10 mL of rumen fluid. To establish anaerobic conditions, the mixture was flushed with CO_2 gas for 30 seconds before the tube was sealed with a ventilated rubber stopper. The tubes were then incubated at 39°C in a shaking water bath for 48 hours, with pH monitored at the 4-hour mark. After 48 hours, the rubber stopper was removed, and 1 mL of 5% $HgCl_2$ was added to halt microbial activity. The fermentation mixture was centrifuged for 15 minutes, after which 50 mL of a pepsin-HCl solution was added. The tubes were subsequently incubated for another 48 hours at 39°C in a water bath shaker.

At the end of the incubation period, the mixture was filtered to separate the supernatant and the sediment. The sediment, representing the undigested residue, was then analyzed to determine nutrient digestibility.

Statistical analysis

The variables observed in this study included the digestibility of dry matter, organic matter, crude fat, crude fiber, and crude protein in both the rumen and post-rumen regions. The obtained data were analyzed using Analysis of Variance (ANOVA) following a factorial pattern in a Randomized Group Design. When significant differences between treatments were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT) with the Genstat version 12.0 software. The mathematical model used for analysis is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} is observations of fish oil protection, μ is the mean, α_i is the effect of fish oil protection, β_j is the effect of the Lemuru fish oil's level, $(\alpha\beta)_{ij}$ is the interaction between fish oil protection and level of Lemuru fish oil factors, and ε_{ijk} is the random error.

RESULTS AND DISCUSSION

Fish oil, rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), supports immune function, reduces oxidative stress, optimizes rumen fermentation, and enhances livestock performance (Nurfatahilah *et al.*, 2022). The findings of this study revealed that the different methods (unprotected and protected) of feeding have different effects in rumen and post-rumen digestion (Figure 3). Our present study showed that increasing Lemuru fish oil inclusion significantly ($P < 0.01$) reduced the digestibility of dry matter (DM), organic matter (OM), crude fat (CFa), crude fiber (CFi), and crude protein (CP) in both rumen and post-rumen phases (Figure 4). The 0% inclusion consistently yielded the highest digestibility, whereas the 12.5% treatment resulted in the lowest values for DM (63.33% and 68.16%), OM (65.73% and 66.37%), CFa (62.00% and 59.59%), CFi (55.95% and 57.92%), and CP (63.11% and 68.04%). The reduction may be attributed to oil coating feed particles, limiting microbial access, and altering rumen pH and volatile fatty acid (VFA) profiles, which impair fermentation and nutrient absorption (Abdillah *et al.*, 2024; Vargas *et al.*, 2017). High fat inclusion can also suppress cellulolytic and proteolytic bacteria (Roy *et al*., 2017), consistent with previous findings of reduced digestibility and feed intake at elevated fish oil levels (Brelaz *et al*., 2024; Fadhilah *et al*., 2019).

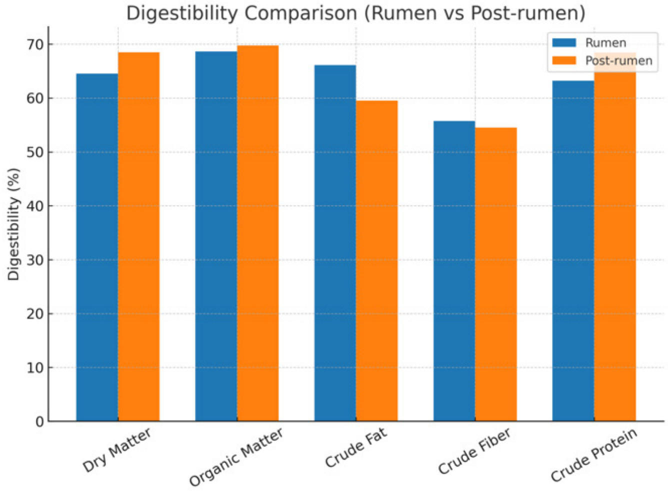

Figure 3: Rumen vs. post-rumen digestibility for each nutrient (DM, OM, CFa, CFi, CP).

Protection of fish oil with calcium hydroxide ($Ca(OH)_2$) effectively mitigated these negative effects by preventing microbial biohydrogenation and preserving fatty acid integrity, thereby supporting fiber-digesting microbes (Handojo *et al*., 2018; Pedroso *et al*., 2018). In the rumen, protected fish oil significantly ($P < 0.01$) improved DM (69.95%), OM (71.99%), CFi (61.86%), and CP (67.46%) digestibility compared to the unprotected form (Table 2).

Post-rumen digestibility was also significantly higher ($P < 0.01$) for protected oil diets, with averages of 71.05% for DM, 71.81% for OM, 65.41% for CFi, 63.39% for CFa, and 69.29% for CP, reflecting the release of bound fatty acids during enzymatic digestion (Hakim *et al*., 2022).

Crude fat digestibility, however, was significantly lower in the rumen for protected oil (57.92%) compared to unprotected oil (66.12%) (Table 2), due to reduced microbial access to protected fat (Putri *et al*., 2021). This limitation was offset post-ruminally, where calcium soaps dissociate under acidic conditions, enabling efficient emulsification and hydrolysis; as a result, post-rumen crude fat digestibility was higher in the protected group (65.41%) than in the unprotected group (59.52%) (Table 2).

A significant interaction ($P < 0.01$) between protection method and inclusion level was observed for rumen digestibility. The 12.5% unprotected oil treatment produced the lowest DM (58.29%), OM (62.72%), CFi (50.84%), and CP (58.78%) values, whereas crude fat digestibility was lowest (54.27%) in the 12.5% protected oil group. Excessive fat inclusion can form a lipid barrier that reduces microbial and enzymatic activity, increases digesta viscosity, and slows nutrient absorption (Ponnampalam *et al*., 2024). These effects were most pronounced at the 12.5% inclusion level, indicating that while protection alleviates some negative impacts, it cannot fully counteract the drawbacks of high-fat diets in ruminants.

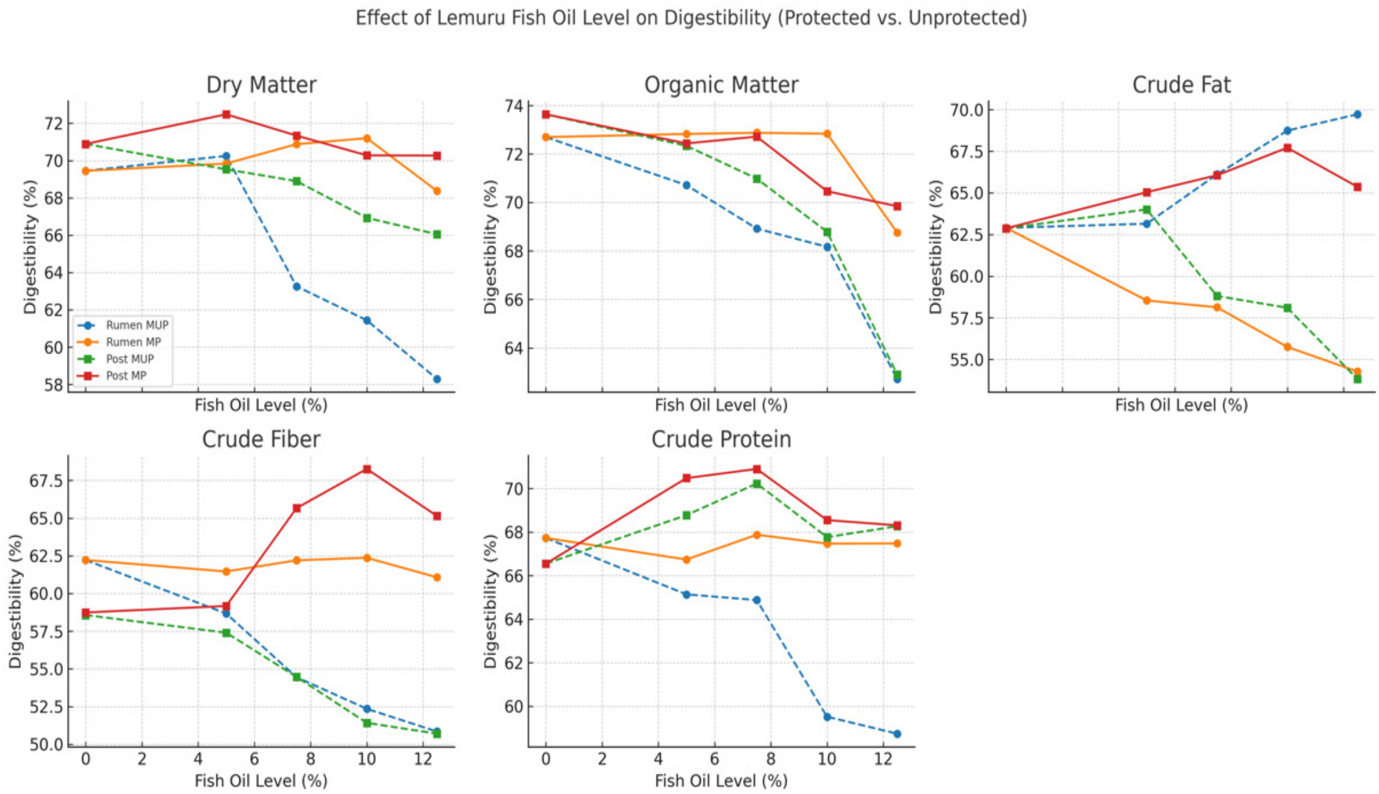

Figure 4: Trends in digestibility across inclusion levels (0% to 12.5%) for protected vs. unprotected oil.

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Table 2: *In vitro* digestibility evaluation of feed supplemented with Lemuru fish oil (Rumen)

Parameters	Method (M)	Level fish oil (L)						SEM	P-value		
		0	5	7.5	10	12.5	Mean		M	L	M*L
Lemuru fish oil (Rumen)											
Dry matter	M_{UP}	69.45^{de}	70.25^{e}	63.26^{c}	61.44^{b}	58.29^{a}	64.54^{A}	0.81	<0.01	<0.01	<0.01
	M_{P}	69.45^{de}	69.84^{de}	70.88^{e}	71.20^{e}	68.37^{d}	69.95^{B}				
	Mean	69.45^{C}	70.05^{C}	67.07^{B}	66.32^{A}	63.33^{A}					
Organic matter	M_{UP}	72.69^{d}	70.71^{c}	68.92^{b}	68.17^{b}	62.72^{a}	68.64^{A}	0.59	<0.01	<0.01	<0.01
	M_{P}	72.69^{d}	72.82^{d}	72.87^{d}	72.83^{d}	68.75^{b}	71.99^{B}				
	Mean	72.69^{D}	71.77^{CD}	70.89^{BC}	70.50^{B}	65.73^{A}					
Crude fat	M_{UP}	62.90^{d}	63.15^{d}	66.11^{e}	68.74^{f}	69.72^{f}	66.12^{A}	0.93	<0.01	<0.01	<0.01
	M_{P}	62.90^{d}	58.54^{c}	58.13^{c}	55.75^{b}	54.27^{a}	57.92^{B}				
	Mean	62.90^{B}	60.84^{A}	62.12^{B}	62.24^{B}	62.00^{B}					
Crude fiber	M_{UP}	62.22^{d}	58.67^{c}	54.45^{b}	52.35^{ab}	50.84^{a}	55.71^{A}	0.81	<0.01	<0.01	<0.01
	M_{P}	62.22^{d}	61.46^{d}	62.20^{d}	62.37^{d}	61.07^{d}	61.86^{B}				
	Mean	62.22^{D}	60.06^{C}	58.33^{B}	57.36^{AB}	55.95^{A}					
Crude Protein	M_{UP}	67.73^{d}	65.14^{bc}	64.88^{b}	59.52^{a}	58.74^{a}	63.20^{A}	0.62	<0.01	<0.01	<0.01
	M_{P}	67.73^{d}	66.74^{cd}	67.88^{d}	67.47^{d}	67.48^{d}	67.46^{B}				
	Mean	67.73^{C}	65.94^{B}	66.38^{B}	63.49^{A}	63.11^{A}					
Lemuru fish oil (After rumen)											
Dry matter	M_{UP}	70.89^{d}	69.53^{bc}	68.90^{b}	66.93^{a}	66.05^{a}	68.46^{A}	0.36	<0.01	<0.01	<0.01
	M_{P}	70.89^{d}	72.48^{e}	71.34^{d}	70.28^{cd}	70.27^{cd}	71.05^{B}				
	Mean	70.89^{C}	71.01^{C}	70.12^{B}	68.61^{A}	68.16^{A}					
Organic matter	M_{UP}	73.63^{e}	72.33^{d}	70.98^{c}	68.79^{b}	62.90^{a}	69.73^{A}	0.57	<0.01	<0.01	<0.01
	M_{P}	73.63^{e}	72.43^{de}	72.71^{de}	70.46^{c}	69.84^{bc}	71.81^{B}				
	Mean	73.63^{D}	72.38^{C}	71.84^{C}	69.63^{B}	66.37^{A}					
Crude Fat	M_{UP}	62.87^{b}	64.01^{cd}	58.81^{b}	58.10^{b}	53.82^{a}	59.52^{A}	0.76	<0.01	<0.01	<0.01
	M_{P}	62.87^{b}	65.04^{de}	66.06^{e}	67.71^{f}	65.36^{e}	65.41^{B}				
	Mean	62.87^{B}	64.52^{C}	62.43^{B}	62.91^{B}	59.59^{A}					
Crude fiber	M_{UP}	58.57^{d}	57.40^{c}	54.47^{b}	51.42^{a}	50.70^{a}	54.51^{A}	1.05	<0.01	<0.01	<0.01
	M_{P}	58.74^{d}	59.17^{d}	65.66^{e}	68.26^{f}	65.14^{e}	63.39^{B}				
	Mean	58.65^{a}	58.29^{a}	60.07^{b}	59.84^{b}	57.92^{a}					
Crude Protein	M_{UP}	66.55	68.78	70.23	67.77	68.27	68.44^{A}	0.31	0.029	<0.01	0.658
	M_{P}	66.55	70.48	70.90	68.55	68.31	69.26^{B}				
	Mean	66.55^{A}	69.63^{BC}	70.57^{C}	68.16^{B}	68.04^{BC}					

Note: SEM= standard error of mean; M= methode; L= level fish oil; M*L = interaction methode with level fish oil. ^{A,B}: Superscripts within in the same row or column show significant differences between treatment ($P<0.01$); ^{a,b,c,d}: Superscripts showed significant differences between the interaction of treatments ($P<0.01$).

Protein digestibility was similarly influenced by both factors. Protection improved CP digestibility by reducing ruminal degradation and increasing bypass protein available for post-rumen enzymatic digestion (Hakim *et al*., 2022). However, higher inclusion levels still reduced CP digestibility due to fermentation disruption, lower protease activity, and delayed protein release from the feed matrix (Mohammed *et al*., 2014).

Overall, Lemuru fish oil can enhance ruminant diets through its polyunsaturated fatty acids, but excessive inclusion, particularly above 7.5%, adversely affects nutrient utilization. Calcium hydroxide protection improves digestibility by safeguarding fatty acids from rumen microbial degradation and enhancing post-rumen absorption. These findings emphasize the need to optimize both inclusion level and protection method to maximize feed efficiency and animal productivity.

CONCLUSION

The supplementation of Lemuru fish oil in sheep feed significantly influences nutrient digestibility, with calcium hydroxide protection proving effective in mitigating the adverse effects of unsaturated fatty acids on rumen microbial activity. While higher inclusion levels of fish oil can impair fermentation and reduce nutrient availability, the use of protected oil enhances post-rumen digestibility by facilitating enzymatic breakdown and absorption. Overall, calcium hydroxide-protected Lemuru fish oil presents a viable approach to improve feed utilization and energy efficiency in ruminants, provided its inclusion level is optimized to avoid excessive fat interference with digestion.

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NOVELTY STATEMENT

This study is the first to evaluate the use of calcium hydroxide as a protective agent for Lemuru fish oil in ruminant feed, focusing on its impact on both rumen and post-rumen *in vitro* digestibility. The findings demonstrate that calcium hydroxide protection effectively enhances nutrient utilization while minimizing the negative effects of high unsaturated fat levels on rumen fermentation.

AUTHOR'S CONTRIBUTION

TIWK, MM, and HH designed and coordinated the study. HH and MM supervised the experiment. TIWK and RBL performed the experiment, analysed the data, and drafted the manuscript. LER and MM took part in preparing and critically checking this manuscript. All authors read and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that they did not use generative artificial intelligence (AI) and AI-assisted technologies in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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