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The Effect of Lemuru Fish Oil Microencapsulation using *Uncaria Gambir Roxb*. as Coating Material on *In Vitro* Fermentation

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ABSTRACT

The primary objective of this study was to evaluate fish oil microencapsulation (FOM) using *Uncaria Gambir Roxb*. (UG) residues *in vitro* fermentation. At first stage, there were 4 treatments (T1=3% UG, T2=6% UG, T3=9% UG, T4=12% UG) and 4 replications. The results showed that unencapsulated oil (UO), encapsulated oil (EO), and efficiency encapsulation (EE) were significantly affected. The best treatment was at T4, with 11.33% UO, 17.29% EO, and 63.02% EE. The second stage evaluated FOM using 12% UG on in vitro fermentation. There were 5 treatments (T1=2% FO without protection, T2=2% FOM, T3=4% FOM, T=6% FOM, T5=8% FOM). The results showed that FOM using UG did not affect nutrient digestibility, pH, NH3, VFA branches, butyrate and protozoa population, yet acetate, propionate, and microbial protein synthesis were affected. It is concluded that UG can be used with 8% of FO.

Key words: Fish oil supplementation, Polyphenol compound, Rumen, In-vitro.

INTRODUCTION

Lemuru Fish oil contains high amounts of unsaturated fatty acids such as linoleic acid, linoleic acid, EPA and DHA (Ibrahim 2013), which can be potential energy sources for animals by increasing the feed's energy density. The content of fatty acids plays various important roles in human health, such as reducing cardiovascular diseases, cancer, and hypertension (Selim et al. 2021). Additionally, the oil in ruminants is not only a source of energy but also helps enhance physiological functions due to the essential fatty acids in fish oil, which are deposited in the meat, thereby improving its quality. Fish oil supplementation, especially in ruminants, has several challenging aspects, such as the biohydrogenation process, which alters unsaturated fatty acid to saturated fatty acid and coats other nutrient particles, which leads to inhibiting microbes in the rumen from digesting nutrients. Those mechanisms are lipolysis and biohydrogenation (Oematan 2023). Thus, protection treatment is needed to make fish oil supplementation more effective and gain real benefit from its presence.

The selection of a protection method depends on economic aspects, sensitivity of the core, size of the microcapsule desired, physical and chemical properties of both core and coating, application for the ration ingredient, and the release mechanism (Jackson and Lee 1991). The most effective and efficient method is microencapsulation, which is a method for preserving essential fatty acids in fish oil using coating materials and turning those substances into powder (Tolve et al. 2019). The advantage of the microencapsulation technique is its stability, ensuring a site-specific release of the intended wall or core material in the animals' gastrointestinal tracts and the rumen, abomasum, and small intestine (Amin et al. 2021). It serves as a widespread technique to safeguard the core, such as Lemuru fish oil.

Nowadays, microencapsulation technology has been applied broadly in the feed industry to microencapsulate sensitive ingredients and to reduce the rate at which physical, chemical and biological stimuli cause core components to degrade, shielding the entire bioactive composition from outside impacts by keeping the core material intact for a long time. By passing through cell membranes, microcapsules can also improve absorption and bioavailability at the nanoscale (Saadi 2023). Through previous studies, various levels of fish oil are used in microencapsulation, such as 5% (Riemas et al. 2021; Hasyaftala 2021) and 6% (Yilmaz and Kara 2022). Moreover, the level of fish oil used in this research is up to 8% to be evaluated.

The major factor influencing the effectiveness of microencapsulation is the coating material. Previous studies have shown that the encapsulation efficiency varies,

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depending on the coating material used. For example Gimenez et al. (2023) achieved an efficiency of 54.62% using whey protein isolate and tannin, while Selim et al. (2021) reported a higher efficiency of 64.71% with gum Arabic and maltodextrin. Currently, there is a growing interest in finding accessible and affordable coating materials with an effective impact on ruminants.

Montesqrit (2007) reported that feedstuffs such as meat bone meal and coconut meal were coating materials for poultry. However, additional sources must be added to the feedstuff in ruminants due to the microbe's presence in the rumen. Moreover, the effects of different secondary metabolites, such as tannin, have been investigated in a variety of alternative plant-based bioactive compounds. Additionally, other works showed that incorporating polyphenol compounds such as tannin from olive leaf extract (Ozyurt et al. 2022) and citric acid (Darmus 2023) could enhance the stability of fish oil microencapsulation products. Thus, it can be more effective in the ruminant digestive system. On top of that, the previous study showed that polyphenol compounds such as tannin were effective as coating material.

Uncaria Gambir Roxb. is one of the tannin sources (12.5% of tannic acid) and is potentially used as feed supplementation (Ningrat et al. 2017). From 21.412 hectares of *Uncaria Gambir Roxb*. field, there would be 14.220 tons of production (Nurdin and Fitrimawati 2018) and only 6% would be exported, and there would be 94% Gambir extraction waste (Kasim 2010). That potential made *Uncaria Gambir Roxb* widely used as ruminant feed but not as a coating material in microencapsulation, while it has the potential to protect the fish oil in microencapsulation products. Thus, a study to investigate the potential of *Uncaria Gambir Roxb*. as a coating material is needed. This research presents novel strategies of accessible and affordable coating material using *Uncaria Gambir Roxb*. in fish oil microencapsulation for ruminants.

MATERIALS AND METHODS

Ethical approval

All animal procedures were conducted under the authority of the Faculty of Animal Science, Andalas University (Scientific Procedures), approval by the local animal welfare and ethical review body, and local ethical clearance No. B/026/UN.16.06.3.8/TA.00.03-2024. This research was conducted in two steps. The first step examined the best level of Gambir leaf (*Uncaria Gambir Roxb*) residues (GLR) in coating materials and the second step evaluated the effect of Lemuru fish oil microencapsulation using the best level of GLR on the *in vitro* parameters.

Experimental design and microencapsulation process

The microencapsulation procedure was adapted from previously described methods (Montesqrit 2007). The ingredients of the experimental coating materials are presented in Table 1. All ingredients were dried and ground to 18 mash. A fish oil-to-coating ratio of 1:4 was employed with a carbohydrate-to-protein ratio of 1:3. The process commenced by weighing the coating materials and dissolving those in an aqueous solvent. Subsequently, the mixture was stirred for approximately 15min at 40-50°C until a homogeneous blend was achieved. Fish oil was weighed according to the experimental design, and lecithin was added as an emulsifier, constituting 2.5% of the fish oil's weight. The mixture was then stirred for approximately 15min at a temperature of 40-50°C. The coating material solution and fish oil were combined and homogenized for 10min. The final product was then dried using spray drying. The spray-drying process was conducted with an inlet temperature of 180°C and an outlet temperature of 90°C.

Table 1: Formulation of coating materials in Lemuru fish oil microencapsulation

Coating Materials	Treatment (%)			
	1	2	3	4
Meat Bone Meal	76	79	83	87
Coconut Meal	21	15	8	1
Uncaria Gambir Roxb	3	6	9	12
Total	100	100	100	100
Carbohydrate and Protein Content (%)				
Carbohydrate (C)	15.21	15.21	14.78	14.36
Protein (P)	49.99	49.06	48.19	47.33
C:P ratio	1:3	1:3	1:3	1:3

This research used a completely randomized design (CRD) consisting of four treatments and five replications. All data obtained were processed and analyzed for variance using ANOVA, followed by Duncan's Multiple Range Tests (DMRT).

Unencapsulated Oil Content (Wanasundara and Shahidi 1995)

1 gram microcapsule sample was weighed, wrapped in fat-free filter paper, and tied with a fat-free string. Then, it was washed three times with 20mL of hexane for 60s and collected in a pre-weighed fat flask. The hexane in the fat flask was distilled, and the sample in the filter paper was dried in an oven. The fat flask, after distillation, was dried in an oven at 105°C for 3 hours (until constant weight), then cooled in a desiccator and weighed.

$$Unencapsulated\ oil = \frac{final\ weight\ of\ fat\ flask - initial\ weight\ of\ fat\ flask}{samples\ weight}\ x\ 100\%$$

Encapsulated Oil Content (AOAC 1984)

The fat flask was dried in an oven at 105°C, cooled in a desiccator and weighed. The sample to be extracted was placed in a Soxhlet apparatus and extracted with hexane as a solvent for 6 hours. Thus, the solvent was distilled and the fat flask was dried in an oven for 3 hours, cooled in a desiccator and weighed.

$$Encapsulated\ oil = \frac{final\ weight\ of\ fat\ flask - initial\ weight\ of\ fat\ flask}{samples\ weight}\ x\ 100\%$$

Encapsulation Efficiency (Lin et al. 1995)

Encapsulation efficiency (%) is determined by comparing the amount of oil in the microcapsules or the amount of encapsulated oil with the oil content and fat content of the coating material used, using the formula:
Encapsulation efficiency (%) =[(amount of encapsulated oil / total solids) x 100%]

Total solids is the sum of the coating material, fish oil, and emulsifier used, and it is assumed that all of the total solids are converted into microcapsules or the microcapsule yield is 100%.

Dietary treatments and *in vitro* procedure

The dietary composition is similar to the previous publication of Montesqrit et al. (2024) and is shown in Table 2.

Table 2: Dietary composition in *in vitro* rumen fermentation (%)

Feedstuff	Treatments				
	1	2	3	4	5
Brancharia decumbens	60	60	60	60	60
Rice brand	15	6	6	6	6
Coconut meal	17	28	28	28	28
Mineral	1	5	5	5	5
Corn meal	2	1	1	1	1
Soybean meal	5				
LFO	2				
Protected LFO	-	2	4	6	8
Chemical composition in basal ration					
Dry matter	87.85	87.85	87.85	87.85	87.85
Crude protein	12.30	12.30	12.30	12.30	12.30
Fiber crude	19.32	19.32	19.32	19.32	19.32
Crude fat	4.13	4.13	4.13	4.13	4.13
BETN	56.19	56.19	56.19	56.19	56.19
TDN	68.32	68.32	68.32	68.32	68.32

The *in vitro* procedure was adapted from previously described methods (Tilley and Terry 1963). A day before fermentation, the buffer solution was prepared and placed in a shaking water bath at 39°C. To maintain anaerobic conditions, it was flushed with CO_2 gas for 30-60 seconds. The pH was adjusted to near neutral.

Rumen fluid was collected in the morning from goats slaughtered at the farm. The rumen fluid was placed in a thermos maintained at 39°C under anaerobic conditions. 50mL of rumen fluid and 200mL of buffer were added in a 2.5g sample, which was placed in a 250mL Erlenmeyer. The samples were incubated in a shaker at 39°C for 48 hours. Then, the samples were centrifuged at 4000rpm for 4min. The residue settled at the bottom, and the supernatant was collected.

This study used a randomized block design with five treatments, each repeated four times. All data obtained were processed and analyzed for variance using ANOVA, followed by Duncan's Multiple Range Tests (DMRT).

Nutrient digestibility

Dry Matter Digestibility (DMD)

The analysis of dry matter content was preceded by the analysis of moisture content. The clean porcelain dish was dried in an oven at 105°C for 1 hour and cooled in a desiccator for 15min and weighed. A sample of 1g was weighed and placed into a porcelain dish with a known weight, then put into an oven at 105°C for 8 hours and cooled in a desiccator for 15min and then weighed.

$$^{DMD} = \frac{(sample\ weight x DM\ sample) - (residue\ weight x DM\ residue) - (blank\ weight x DM\ blank)}{(sample\ weight x DM\ sample)} x\ 100\%$$

Organic Matter Digestibility (OMD)

The samples were placed in the electric furnace at 600°C for 6 hours on a scale between 3 and 4. After turning off the furnace and waiting for the temperature to drop to 200°C, they were placed in a desiccator for 30min and weighed.

$$^{OMD} = \frac{(sample\ weight x OM\ sample) - (residue\ weight x OM\ residue) - (blank\ weight x OM\ blank)}{(sample\ weight x OM\ sample)} x\ 100\%$$

Crude Protein Digestibility (CPD)

The Kjeldahl method was used to determine the crude protein content. This method involves three main steps: digestion and dilution of the sample, distillation to isolate the nitrogen and finally, titration to quantify the protein.

$$^{CPD} = \frac{(sample\ weight x BK\ sample x CP\ sample) - (residue\ weight x DM\ residue x CP\ residue)}{(sample\ weight x DM\ sample x CP\ sample)} x\ 100\%$$

Crude Fiber Digestibility (CFD)

2 grams were placed into a 500mL beaker, then, 100mL of 0.3N H2SO4 were added to the beaker and boiled for 30min. The H2SO4 solution was filtered with ordinary filter paper using a vacuum. Next, rinsed with 100mL of hot aquades three times. The filter paper was removed and rinsed with 100mL of 0.3N NaOH into a beaker, then boiled for 30min. The liquid was filtered using a vacuum through Whatman No. 41 filter paper. The filter paper and the residue were washed successively three times with 100mL of hot aquades and 25mL of acetone. The filter paper containing the residue was folded and placed into a clean porcelain crucible. The dish containing the sample was dried in an oven at 105°C for 1 hour. Cooled in a desiccator and weighed. Then, the dish and its contents were placed in an electric furnace at 600°C for 3-4 hours. Wait until the temperature drops to 105°C, cooled in a desiccator for 1 hour and weighed.

$$^{CPD} = \frac{(sample\ weight x BK\ sample x CF\ sample) - (residue\ weight x DM\ residue x CF\ residue)}{(sample\ weight x DM\ sample x CF\ sample)} x\ 100\%$$

Rumen fermentation characteristic analysis

pH measurement, VFA partials and NH3

The pH measurement of rumen fluid was conducted using a pH meter. The VFA partial concentrations were measured using a Gas Chromatograph (GC: Shimadzu GC-2010, Japan) fitted with an SGE BP21 30µm×530µm×1.0µm wide-bore capillary column. NH3 production was determined using the Conway microdiffusion procedure (General Laboratory Procedure, 1966). 1mL of supernatant was dropped on the right side of the Conway dish, and 1mL of Na_2CO_3 on the left side. In the center of the Conway dish, 1mL of H_2BO_3 was dropped, and the dish was tightly closed. Vaseline was applied to the edges of the dish and stored for 24 hours. After 24 hours, it was titrated with 0.005N H_2SO_4 until the color changed to reddish-green occurs.

$$NH3 = \frac{ml\ titration\ x\ NH2SO4\ x\ 17\ x\ 100}{ml\ sample}\ x\ 100$$

Protozoa population

The number of protozoa in the rumen fluid was counted using a hemocytometer with staining using MFS (Methyl green Formalin Saline) solution (Ogimoto and Imai 1981). The components of the MFS solution consisted of 35% formaldehyde solution 100mL, aquades 900mL, Methylgreen 0.6g and NaCl 8.0g. From 1mL of the rumen fluid sample that had been incubated for 4 hours, 1mL of MFS Solution was added and left for 1 hour. The total protozoa population was counted under a microscope (10x) using a Neubauer counting chamber in 4 boxes, each measuring 1mmx1mmx0.2mm.

$$NH3 = \frac{total\ cells\ in\ counting\ room}{ml\ sample}\ x\ dilution$$

Microbial protein synthesis

The measurement of microbial protein synthesis begins with *in vitro* testing. The sample was incubated at 39°C for 3 hours. The sample was centrifuged at a speed of 3.000rpm for 15min to obtain the supernatant. The supernatant was centrifuged again at a speed of 8.000rpm at room temperature for 15min to obtain the microbial pellet.

Microbial protein synthesis was measured using the Lowry method (Plummer 1987). The steps are as follows: the centrifuged sample was added to 1mL of 0.1N NaOH solution and homogenized. The sample was placed in a test tube, heated at 90°C for 15min and then cooled to room temperature for 10min. 1mL of the sample was taken, 5mL of Lowry B solution was added, and then vortexed. The sample was left at room temperature for 10min. Next, 0.5mL of Lowry A solution was added, vortexed, and left for 30min. The sample was read using a digital spectrophotometer with a wavelength of 700nm.

RESULTS

Unencapsulated oil, encapsulated oil and efficiency encapsulation

Treatment has significant (P<0.01) effect on unencapsulated oil, encapsulated oil and efficiency encapsulation (Table 3). The average of unencapsulated oil was getting lower as the *Uncaria Gambir Roxb* level was added (11.33-22.54%). Otherwise, the average of encapsulated oil and efficiency encapsulation were getting higher as *Uncaria Gambir Roxb*. added up to 12%.

Nutrient digestibility

Dry matter digestibility (DMO), Organic matter digestibility (OMD), crude protein digestibility (CPD) and crude fiber digestibility (CFD) were not affected (P>0.05) by both treatments whether the fish oil in the diet without any protection and with protection using *Uncaria Gambir Roxb*. The range of DMD (49.20-62.95%), the range of OMD (52.43-63.43%), the range of CPD (79.87-84.32%), and CFD (74.07-81.71%) were obtained from this research (Table 4).

Rumen characteristic fermentation

FOM using UG did not affect pH, NH3, VFA branches, butyrate, and protozoa population, yet acetate, propionate, and microbial protein synthesis were affected (P>0.05). The microencapsulations using *Uncaria Gambir Roxb*. Were judged to be applicable based on a relative number of pH, NH3, VFA (acetate, propionate and butyrate), iso VFA, microbe protein synthesis, and protozoa population. In general, the chemical composition of the basal ratio (Table 1) is typical, with the exception of level fish oil supplementation, which is relatively different in each treatment. As expected, average rumen characteristics do not have a negative value due to the level of fish oil protected by *Uncaria Gambir Roxb.* residues.

DISCUSSION

Unencapsulated oil, encapsulated oil and efficiency encapsulation

Unencapsulated oil is the oil present on the surface of the microcapsule product. Thus, the lower the unencapsulated oil content, the higher the amount of oil successfully coated by the coating materials. The increasing percentage of GLR causes a decrease in the unencapsulated oil content in each treatment. The residue of Gambir leaves contains a total tannin concentration of 12.5% (Ningrat et al. 2017). The tannins are phenolic compounds forming a protective structural matrix when coating the oil in the microencapsulated product. This is supported by Ozyurt et al. (2022) research, which compared the microencapsulation of fish oil using olive leaf extract rich in polyphenolic compounds with a control treatment, which was microencapsulation without olive leaf extract. Based on that research, the addition of olive leaf extract resulted in a higher content of encapsulated fatty acids compared to the control treatment. The content of fatty acids C14:0, C15:0 and C16:0 in the control treatment were 7.45, 1.36, and 29.22%, respectively.

Meanwhile, with the addition of olive oil extract, they were 9.18, 1.52 and 29.75%, respectively. The lowest unencapsulated oil content was in treatment 4 at 11.33%. This indicates that treatment 4 is the best treatment for encapsulating oil. Due to its low oil content, it was able to penetrate the coating wall and diffuse to the surface of the microcapsule. Furthermore, the components of the coating materials combined with GLR form a strong bond in the form of a protective structure that prevents oil from escaping to the surface layer of the microcapsule. The highest unencapsulated oil content was in treatment 1 at 22.54%. In this treatment, the Gambir leaf residue content was only 3%. This resulted in a weaker bond between the coating material and the tannin compounds in the GLR compared to other treatments, allowing more oil to escape.

Table 3: The average of unencapsulated oil, encapsulated oil and efficiency encapsulation (%)

Level of *Uncaria Gambir Roxb.*	Unencapsulated oil	Encapsulated oil	Efficiency encapsulation
3	22.54±0.61a	6.09±0.13a	19.52±0.33a
6	16.13±0.18b	7.76±0.11b	26.00±0.41b
9	15.04±0.05b,c	11.11±0.53c	39.00±0.20c
12	11.33±0.60d	17.29±0.61d	63.02±0.40d

Values (Mean±SE) bearing different letters in a column differ significantly (P<0.05).

Table 4: The average nutrient digestibility of the five-level of fish oil microencapsulation using *Uncaria Gambir Roxb.* incubated with rumen fluid (%)

The level of oil	DMD	OMD	CPD	CFD
2 (without protection)	49.20±1.39	52.43 ±1.38	79.87±0.83	79.69±1.21
2 (protected using *Uncaria Gambir Roxb.*)	54.50±1.86	57.30±1.29	80.70±0.96	79.85±1.17
4 (protected using *Uncaria Gambir Roxb.*)	55.73±1.58	60.73±0.76	81.94±0.36	80.32±1.12
6 (protected using *Uncaria Gambir Roxb.*)	62.95±0.95	63.43±1.08	84.32±1.16	81.71±0.92
8 (protected using *Uncaria Gambir Roxb.*)	49.39±1.28	53.00±0.87	83.16±1.19	74.07±1.10

The value of unencapsulated oil obtained in this study is lower compared to the research conducted by Alvarez et al. (2024) on the microencapsulation of coffee bean oil using gum Arabic as a coating material. In that study, the value of unencapsulated oil ranged from 15 to 44%. This indicates that the addition of Gambir leaf residue up to 12% can reduce the unencapsulated oil content in the microencapsulation of Lemuru fish oil. The encapsulated oil content increased in each treatment as the percentage of added GLR increased. This indicates that the resulting microcapsule product has good stability due to the addition of Gambir leaf residue. GLR can protect oil droplets from disintegration and disruption during the microencapsulation process through the spray drying technique. This proves that the addition of Gambir pomace has a positive impact on microencapsulation by preventing the transfer of oil to the surface of microcapsule particles. Thus, more oil is encapsulated. The lowest encapsulated oil content was in treatment 1 at 6.09%. Although there was a balance of protein and carbohydrates capable of forming a stable emulsion for the microencapsulated product, the low addition of Gambir leaf residue in this treatment resulted in a weaker coating wall compared to other treatments. The highest encapsulated oil content was in treatment 4, which was 17.29%. This was due to the interaction between the coating material, which contained a carbohydrate-to-protein ratio of 1:3, and tannin compounds. The interaction resulted in a coating wall with a dense structure and fewer pores, thereby minimizing oil diffusion out of the coating shell. Fig. 1 showed that the higher protective effect observed in treatment 4 corresponds to its higher Gambir residue content.

The values obtained in this study are higher compared to the research conducted by Khamidah et al. (2019) on the microencapsulation of patin fish oil with a coating material composition of maltodextrin and sodium caseinate in a ratio of 70:30, which had the best-encapsulated oil content of 6.23%. The values in this study are higher because there is a bond between the coating material composition and the compounds found in Gambir leaf residue, precisely tannin compounds. Gimenez et al. (2023) proved that the interaction between protein compounds and tannins can form a more effective coating shell compared to using only protein compounds as the coating material. The study reported the composition of C18:1, C18:2, and C18:3 in the coating material of soy protein isolate alone as 66.43, 202.80 and 612.44%, respectively. However, when tannin compounds were added to the coating material, the compositions increased to 71.64, 204.48 and 632.17%, respectively. This proves that the addition of Gambir leaf residue plays a role in forming a more robust coating that effectively maintains the encapsulated oil.

The lowest encapsulation efficiency was in treatment 1 at 19.52%. The low encapsulation efficiency value in treatment 1 indicates a high presence of Lemuru fish oil on the surface of the microcapsule particles. Adding 3% Gambir residue resulted in a higher unencapsulated oil content in this treatment. Thus, the encapsulation efficiency value obtained becomes lower. The highest encapsulation efficiency was in treatment 4 at 63.02%. The results obtained in this study are not much different from the microencapsulation of Lemuru oil using gum arab and maltodextrin as coating materials, which resulted in an encapsulation efficiency of 64.71% (Selim et al. 2021).

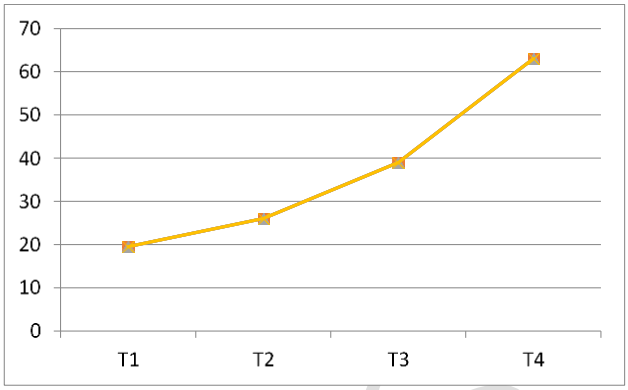

Fig. 1: The level of efficiency encapsulation. The X-axis of this figure represents the treatment variables applied in the study, while the Y-axis shows the percentage results of the measured parameter (T1=3% *Uncaria Gambir Roxb*., T2=6% *Uncaria Gambir Roxb*., T3=9% *Uncaria Gambir Roxb*., T4=12% *Uncaria Gambir Roxb*).

However, it is higher than the encapsulation efficiency of catfish oil, as determined by Khamidah et al. (2019), which was 60.93%, using maltodextrin and sodium caseinate as coating materials. Gimenez et al. (2023) conducted a cross-linking reaction between soy protein isolate and pure tannin compounds as the coating material. The 10% tannin compound used in that study yielded an encapsulation efficiency value of 54.62%. This result is lower than the encapsulation efficiency value in this study. Therefore, the addition of Gambir residue to the coating material composition showed the best results at 12% Gambir leaf residue (Table 3) as the coating material for the microencapsulation of Lemuru fish oil.

Nutrient digestibility

This study's average dry matter digestibility values ranged from 49.20% to 62.95%. This indicates that Gambir leaf residue can protect Lemuru fish oil. The values obtained in this study are not much different from Hasyaftala (2021), who added microencapsulated fish oil to sheep feed using Whey Protein Isolate and gum Arabic as coating materials. In that study, the dry matter digestibility values obtained ranged from 49.81 to 56.02%. The coating of Lemuru fish oil with Gambir leaf residue was able to form an oiled sheath around the core of the microcapsule, preventing the oil from leaking out and covering the feed. Gambir leaf residue is suspected to form a high emulsion viscosity, which strengthens the resulting skin layer, thereby optimizing the protective strength of the core material. This is in line with the opinion of Sugindro et al. (2008), who stated that low emulsion viscosity can reduce the protection of the core material.

Based on the results of the variance analysis, it was found that there was no significant difference ($P>0.05$) in the digestibility of organic matter. There were no significant differences between treatments in this study due to the same feed being given to each treatment, except for the addition of microencapsulated Lemuru fish oil. This causes the supply of nutrients to the livestock to be the same. Since the supply of fish oil given has been protected

first using a coating material made from Gambir leaf residue, the supply of fish oil does not contribute as a nutrient source that is degraded in the rumen. Adding fish oil through the microencapsulation method, which has been transformed into powder form, does not negatively affect the *in vitro* nutrient digestibility.

The organic matter digestibility values between the control treatment and the treatment with the addition of microencapsulated Lemuru fish oil did not differ significantly, indicating that the administration of microencapsulated Lemuru fish oil using Gambir leaf residue does not interfere with fermentation activity in the rumen.

The values obtained in this study ranged from 52.43 to 63.43%. These results are not significantly different from the study conducted by Pramono et al. (2016) on the supplementation of protected Lemuru fish oil, which resulted in organic matter digestibility values ranging from 59.21 to 88.23%. This indicates that the use of Gambir leaf residue in the microencapsulation of Lemuru fish oil has a positive impact on organic matter digestibility.

Based on the results of the variance analysis, it was found that there was no significant difference (P>0.05) in the digestibility of crude protein. This indicates that the addition of Lemuru fish oil (Table 4), whether directly or protected using Gambir leaf residue, can maintain the balance of rumen microorganisms, resulting in digestibility outcomes that are not significantly different from the control treatment. However, the digestibility of crude protein tends to increase in the treatment of microencapsulated Lemuru oil compared to unprotected Lemuru oil. This indicates that the microencapsulation method can inhibit the negative effects of oil on the feed. Suharti et al. (2018) explains that the use of fat protection methods can eliminate the adverse effects on bacterial populations, thereby increasing the digestibility of crude protein.

The values obtained in this study ranged from 79.87 to 84.32%. These values are slightly higher than those of Fadhillah et al. (2018) study on the microencapsulation of canola oil, which produced an average crude protein digestibility ranging from 59.57 to 63.31%. This difference is because Fadhilah's study was conducted *in vivo*, where the digestibility values were influenced by various factors related to livestock consumption, such as fat supplementation in the diet exceeding 70 g/kg dry matter (Palmquist and Jenkins 1980). This indicates that the use of Gambir leaf residue in the microencapsulation of Lemuru fish oil has a positive impact on the digestibility of crude protein.

Based on the results of the variance analysis, it was found that the different effects on the digestibility of crude fiber were not significant (P>0.05). The digestibility of fiber is related to the ability of rumen microbes to degrade the fiber components present in the diet. The insignificant differences in the results of this study indicate that the protective role of oil usage can maintain the growth conditions of rumen microbes. The addition of Gambir leaf residue in the microencapsulation method was able to coat Lemuru fish oil up to 8% level, thus not inhibiting the growth of microbes in the rumen fluid. This is in line with Tanuwiria et al. (2005), which states that protection can cause rumen microbial activity to remain normal because the protected fat can directly reach the post-rumen.

The values obtained in this study ranged from 74.07 to 81.71%. These values are higher compared to Sudibya et al. (2022) study on adding 4% Lemuru oil directly to livestock, which was 60.68%. The digestibility value of the control treatment in this study was higher than in that study because the level of Lemuru fish oil given in the control treatment was only 2% Lemuru fish oil. This indicates that the use of Gambir leaf residue in the microencapsulation of Lemuru fish oil has a positive impact on the digestibility of crude fiber.

Rumen characteristic fermentation

Based on the results of the variance analysis, it was found that the different effects on pH were not significant (P>0.05). This is suspected to be because the increase in the microencapsulated content of Lemuru fish oil in the feed is still within the limits that can be tolerated by the microbes in the rumen. Several factors, such as fermentation activity in the rumen and the presence of saliva as a pH neutralizer, determine the pH value. In this study, the artificial saliva used was a McDougall's buffer solution in equal amounts for each treatment. This solution functions to buffer the fermentation products in the form of VFA, which are acidic so that the rumen pH can be maintained in a neutral state for each treatment.

From Table 5, it is known that the average pH value ranges from 6.86 to 6.95%. The results obtained in this study align with the findings by Nurdin and Fitrimawati (2018) regarding the addition of Gambir leaf residue to dairy cattle rations, which produced pH values in the

Table 5: The average rumen characteristic fermentation of the five levels of fish oil microencapsulation using *Uncaria Gambir Roxb*. incubated with rumen fluid

Parameters	Level of fish oil (%)				
	2 (without protection)	In microencapsulation using *Uncaria Gambir Roxb*. residues			
		2	4	6	8
pH	6.86±0.04	68.4±0.08	6.88±0.03	6.95±0.05	6.86±0.07
NH3 (mg/100mL)	24.65±0.55	24.01±0.45	27.62±0.21	23.58±0.49	24.44±0.63
Protozoa population (x105 mM)	4.18±0.07	4.1±0.05	4.24± 0.01	4.40± 0.16	4.33±0.17
Acetate mmol/L	41.34±0.89a	41.64±0.85b	49.30±0.55c	63.17±0.82d	66.05±0.66d
Propionat mmol/L	34.87±0.60a	23.34±0.42b	23.91±0.62b	20.83±0.49c	19.44±0.39c
Iso-butyrat mmol/L	1.74±0.20	1.24±0.24	1.84±0.67	1.2±0.44	1.67±0.60
Butyrate mmol/L	10.05±0.51	6.24±0.55	10.96±0.38	6.84±0.46	7.95±0.30
Iso-valerat mmol/L	2.57±0.27	1.84±0.50	2.79±0.41	1.71±0.67	2.22±0.30
Valerat mmol/L	3.69±0.21	2.43±0.26	4.29±0.16	2.62±0.66	2.30±0.01
SPMmg/100mL	97.26±0.31a	98.16±0.29b	137.14±0.40c	160.26±0.39d	187.00±0.45d

Values (Mean±SE) bearing different letters in a row differ significantly (P<0.05).

range of 6.66-7.08%. These values are not significantly different (P>0.05), indicating that the Gambir leaf residue coating material can be used in the microencapsulation of Lemuru fish oil.

There is no significant difference (P>0.05) in NH3 levels. This is suspected to be because the addition of microencapsulation at the level of 8% Lemuru fish oil using Gambir leaf residue does not interfere with the activity of rumen microbes in converting protein to NH3. NH3 production is influenced by the solubility of protein in the ration, the amount of protein in the ration and the duration of food in the rumen (Rudi 2017). The more protein that is degraded in the rumen, the higher the NH3 production. Additionally, NH3 is a primary nitrogen source for microbial protein synthesis, so its concentration in the rumen is an important factor to consider. From Table 5, it is known that the average NH3 value ranges from 24.43 to 31.87mg/100mL. This indicates that Gambir leaf pomace has a good coating strength for Lemuru fish oil, resulting in an NH3 value within the normal range. The optimum concentration of NH3 in the rumen ranges from 85 to 300mg/L, equivalent to 6 to 21mM (McDonald et al. 2002).

The obtained NH3 value indicates the balance between rumen microbes' degradation and protein synthesis processes. The use of Gambir leaf residue in the microencapsulation of Lemuru fish oil in this study shows that Gambir leaf residue can protect the oil in the microcapsule product, thereby not hindering the work of rumen microbes in degrading nutrients. The values were not significantly (P>0.05) different indicating that the coating material of Gambir leaf residue can be used in the microencapsulation of Lemuru fish oil.

Based on Table 5, it is known that the protozoa population differs significantly between treatments (P<0.04). The values obtained ranged from 4.16 to $4.40x10^5$mM. The values obtained in this study were lower compared to Sazili et al. (2016), who reported the effect of supplementing a mixture of palm oil and canola oil at a 4% level on protozoa, which was $7.16x10^5$ mM. This is because this study used animal oil, specifically Lemuru fish oil. The type of supplementation of vegetable and animal oils used also affects the protozoa population (Yilmaz and Kara 2022). The availability of unsaturated and saturated fatty acids also affects this. As mentioned in Ivan et al. (2001), linoleic acid is the most toxic fatty acid to protozoa. The low availability of protozoa has a positive impact on bacterial activity. This is because protozoa can prey on bacteria, disrupting the rumen's fermentation activity.

The protozoa populations did not differ significantly in this study. However, the protozoa population tends to increase with the addition of Gambir leaf residue levels in the microencapsulation of Lemuru fish oil. Haitian et al. (2018) proved that the protozoa population responded linearly to readily fermentable carbohydrates (RFC). The nutrient content of the feed for each treatment is the same, with the only difference being the level of added Gambir leaf residue as a coating material. This shows that the tannin content in Gambir leaf residue maintains the structural matrix in the microencapsulation product and binds RFC, so the increase in Gambir leaf residue levels contributes to the reduction of RFC and the decrease in protozoa population. The increase observed in this study also aligns with the increase in protozoa population by Yilmaz and Kara (2022) with the supplementation of animal fat at levels of 4 and 6%. The results obtained in this study prove that the microencapsulation of Lemuru fish oil up to 8% using Gambir leaf residue as a coating is effective because it does not have a negative effect on protozoa. The fish oil content coated with Gambir leaf residue does not detach during the fermentation process in the rumen. This indicates that adding up to 8% fish oil can be done by coating Gambir leaf residue using the microencapsulation method.

In this study, it was found that the partial VFA branches and butyrate obtained had no significantly different effects on each treatment, which is in line with Yilmaz and Kara (2022) research. This indicates the success of using Lemuru fish oil even at levels up to 8% with coating using Gambir leaf residue. Compared to the other treatments, treatment one had the highest amount of acetate and the lowest amount of propionate. Otherwise, the lowest acetate and the highest propionate were on treatment four. VFA is a product of nutrient digestion in the rumen by rumen microbes. The partial VFA values that show different but insignificant effects indicate that the oil coated up to 8% is not detached from the structural matrix formed by Gambir leaf residue, allowing the nutrient digestion by rumen microbes to proceed optimally.

The partial VFA values, both branched and unbranched, are not much different from the results obtained by Ala et al. (2021), who conducted a study on the supplementation of tea leaf extract in feed *in vitro*. However, in that study, the total acetic acid decreased with each treatment, whereas in this study, there is a tendency for acetic acid to increase with each treatment. The addition of Gambir residue levels is suspected to influence the tendency for increased acetic acid. The previous studies showed that the amount of polyphenols in feed plants has been shown to affect this in several studies. The polyphenols used in the Seradj et al. (2014) study were reported to reduce acetate content, while the Paula et al. (2017) study reported that polyphenols extracted from propolis honey increased acetate content *in vitro*.

Based on Table 5, it is known that the use of Gambir leaf residue on microbial protein synthesis (MPS) showed a significant difference. On the other hand, Adeyemi (2016) research showed that oil supplementation in the diet at a level of 4% did not significantly affect MPS. The values obtained in this study ranged from 99.07 to 157.95mg/100mL. The highest MPS was on treatment 4, and the lowest was on treatment one. MPS is influenced by several factors, especially ammonia, which is the primary source of nitrogen and energy from carbohydrate metabolism (Orskov 1992) for MPS. The values obtained in this study are higher than those reported by Sazili et al. (2016) study on supplementing palm oil and canola oil *in vivo*. This is because using animal fats in feed supplementation results in higher ammonia and VFA values than vegetable oils (Yilmaz and Kara 2022).

Conclusion

Based on the efficiency encapsulation, degradability, and fermentation parameters results of this study, 12% of *Uncaria Gambir Roxb*. can be used as a coating material and protect fish oil up to 8% in rumen fermentation.

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