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Effectiveness of Lemuru Fish (*Sardinella longiceps*) Oil Supplementation on Nutrient Digestibility, Fiber Fraction and Rumen Fluid Fermentability

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ABSTRACT

The objective of this research was to identify the most effective quantity for incorporating fish oil microcapsules that do not interfere with the degradation of nutrients and rumen fluid characteristics (pH, VFA, NH_3) *in vitro* to produce livestock products that are high in omega-3 fatty acids. In this study, a randomized block design experimental method was employed, involving 7 distinct treatment conditions consisting of 3 rumen fluid collection groups. The seven treatments were A: control diet, B: control diet + 4% fish oil microcapsules (≈0.8% fish oil), C: control diet + 8% fish oil microcapsules (≈1.6% fish oil), D: Control ration + 12% fish oil microcapsules (≈2.4% fish oil), E: control ration + 0.8% fish oil, F: control ration + 1.6% fish oil, and G: control ration + 2.4 % Fish oil. Variables observed were: nutrient digestibility (dry matter, organic matter, crude fat, and crude fiber), digestibility of fiber fractions (NDF, ADF, cellulose, and hemicellulose), and fermentability of rumen fluid (pH, VFA and NH_3). The findings indicated that the treatment did not have a significant impact ($P>0.05$) on the digestibility of dry matter, organic matter, crude fiber, NDF, ADF, cellulose, hemicellulose, pH, and NH_3. However, there was a significant difference ($P<0.05$) observed in the digestibility of crude fat and VFA. The conclusions drawn from the research imply that the addition of fish oil microcapsules up to 12% in the ration does not interfere with nutrient digestibility, fiber fraction, and rumen fluid fermentability (pH, VFA, NH_3).

Key words: Omega-3, Fish oil microcapsules, Rumen fluid fermentability, *In vitro*, Digestibility.

INTRODUCTION

The cardiovascular diseases, including heart disease and stroke, continue to rise annually and remain the leading cause of death in Indonesia, especially at productive ages (Mahiroh et al. 2019; Mandagi et al. 2019). The main factor causing heart disease is the high consumption of foods that contain saturated fatty acids or cholesterol (Sartika 2008: Visser 2019; Perna and Hewlings (2022). Products from livestock, both eggs, milk, and meat contain high levels of saturated fatty acids and cholesterol, the saturated fatty acid content in meat is around 40-50% (Moloney 2012). Various attempts have been made to reduce saturated fatty acids and cholesterol. One of them is by adding fish oil which contains a lot of unsaturated fatty acids to livestock rations so that the resulting livestock products are expected to contain significant amounts of unsaturated fatty acids. According to Scollan et al. (2001), the application of 3% fish oil without protection in concentrate rations caused an increase in muscle phospholipids from 2.3% content of Eicosa Pentaenoic Acid (EPA) in control rations to 4.8% EPA after administration of unprotected fish oil. The same effect of giving fish oil in concentrate rations was observed in sheep by Wachira et al. (2002). Both Scollan and Wachira obtained Poly Unsaturated Fatty Acid (PUFA) which was not incorporated into muscle triglycerides. Efforts to increase PUFA levels in meat and milk have had limited success. This is due to the presence of rumen microbes which hydrogenate PUFA during digestion (Carreño et al. 2019). In the rumen, when unprotected fat sources are present, it is possible to predict the loss of unsaturated fatty acids. Specifically, approximately 86% of linoleic and oleic acids and 82% of linolenic acids can be expected to be lost (Jenkins and Bridges 2007).

Therefore, in order to shield unsaturated fatty acid sources from disruptions caused by rumen microbial imbalances, it is possible to utilize microcapsules as a means to deliver and protect omega-3 fatty acid sources. Omega-3 fatty acids are studied to have a protective effect on the cardiovascular system and have a good effect on blood pressure, triglycerides, inflammation, thrombosis and antiarrhythmic (Setiawan and Halim 2022). Lee et al. (2002)

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provided protection against soybean oil containing linoleic and linolenic acids by the encapsulation method. From this study, linoleic and linolenic acids were protected against degradation in the rumen, causing increased deposition of fat in muscle and adipose tissue.

The business of adding fish oil to the ration has experienced several obstacles including fish oil is easily oxidized so it is difficult to store it for a long time before being given to livestock, then the fish oil has a fishy aroma so if it is added to the ration or if given to livestock it can cause the product to smells fishy. This condition is a common condition that occurs in mixing into ruminant livestock rations and poultry rations. The special condition that occurs in mixing into ruminant rations is the disruption of these fatty acids in the rumen.

Fats, especially unsaturated fatty acids, undergo lipolysis and hydrogenation by microbial activity in the rumen. This condition limits the use of fat, only 3-4% of fat can be incorporated into food (Gulati et al. 2000). Gulati et al. (2000) conducted a study aiming to enhance the bypass level of fat through the utilization of diverse processing techniques. Sara et al. (2020) have shown that the most effective method for producing a fat feed additive is through the encapsulation of fat in a protein-protected matrix. Providing feed additives containing fat that can By-pass in the rumen will provide benefits including (1) optimal rumen protection or bypass >75% *in vitro*, (2) reducing free fat levels in the rumen, (3) reducing acid levels trans-fat, (4) the level of consumption of fat in the ratio can be increased up to 12% and (5) maximize the bioavailability of fatty acids in the small intestine for absorption.

The addition of sources of omega-3-rich fatty acids such as fish oil in ruminant rations causes biohydrogenation in the rumen, besides that high levels can also interfere with fiber digestibility (Frank et al. 2022). Biohydrogenation taking place in the rumen leads to the direct conversion process where unsaturated fatty acids are converted into saturated fatty acids without being absorbed by the small intestine or stored in body fat before being used for target organs. Adding fish oil as a source of omega-3 fatty acids to ruminant diets with the aim of producing livestock products, including meat and dairy items that are high in omega-3 fatty acids, may not yield the desired results due to the process of rumen biohydrogenation. One effort that can be done to overcome this problem is to protect the omega-3 fatty acids from fish oil products from the threat of rumen biohydrogenation. Efforts that can be made to protect it are to take fish oil with the encapsulation process.

The provision of encapsulated fish oil (fish oil microcapsules) into ruminant livestock rations is expected to produce meat products with a substantial amount of omega-3 fatty acids, low in cholesterol, and do not affect the aroma of these meat products. Meat products with a substantial amount of omega-3 fatty acids are very beneficial for health because they can increase intelligence, help eyesight, and mitigate the chances of developing coronary heart disease (Zhang 2022). Consuming meat products is expected to improve the nutrition of the people who consume them.

The research that will be carried out wants to see how fish oil microcapsules can be utilized in ruminant livestock rations so that they can produce products rich in omega-3 fatty acids and low in cholesterol. The administration of microcapsules directly to ruminants needs to be preceded by *in vitro* testing. *In vitro* testing was carried out first to determine the condition of the fish oil and fish oil microcapsules in the rumen and what level should be added to the ration. Microcapsule levels that do not interfere with the digestibility of fiber and the digestibility of other nutrients are used in future in *in vivo* studies.

The characteristics of the rumen fluid were measured in order to determine the microbial activity involved in feed fermentation. In the rumen, the fermentation process results in the production of volatile fatty acids (VFAs) and ammonia (NH_3) through microbial activity. This study aims to assess the effectiveness of fish oil microcapsules compared to encapsulated fish oil in ruminant livestock diets. The objective is to determine whether fish oil microcapsules can protect omega-3 fatty acids from degradation during rumen microbial fermentation, potentially increasing their presence in livestock products. Based on this, a study was carried out to determine the level of use of fish oil microcapsules in livestock rations and non-decapsulated fish oil as a comparison to see the success rate of the fish oil microencapsulation process, by looking at its effect on the characteristics of rumen fluid (pH, VFA NH_3) *in vitro*. The objective of this research is to establish the most effective concentration of fish oil microcapsules. that do not affect the digestibility of nutrients, fiber fraction, and rumen fluid fermentability (pH, VFA, NH_3).

MATERIALS AND METHODS

Animal Ethics

Research does not use experimental animals, so ethical animals are not needed.

Materials

In this study, the materials employed were basal rations consisting of Brachiaria decumbens, fine bran, coconut meal, soybean meal, refined corn, and minerals. Fish oil was obtained from lemuru fish canning waste in the Muncar area, Banyuwangi Regency. Fish oil microcapsules are fish oil that is encapsulated using a coating material from a mixture of meat meal and coconut meal. The process of making fish oil microcapsules can be seen in Fig. 1. The nutritional composition of both the feed ingredients incorporated in the ration and the basal ration can be observed in Tables 1 and 2. The equipment used was general laboratory equipment, such as measuring cups, Erlenmeyer, flasks, gauze, ovens, shaker water baths, centrifuges, pH meters, measuring pipettes, tissues, Conway cups, and other equipment used to analyze pH, VFA, and NH_3.

Methods

In this study, an experimental approach was employed, utilizing a randomized block design that incorporated 7 different treatment types and 3 groups of rumen fluid collection, namely:

Treatment A = Basal Ration as control
Treatment B = A + 4% fish oil microcapsules (≈0.8% fish oil)
Treatment C = A + 8% fish oil microcapsules (≈1.6% fish oil)
Treatment D = A + 12% fish oil microcapsules (≈2.4% fish oil)

Table 1: Nutritional content of feed ingredients composing the ration (%)

Feed Ingredients	Food Substances in Dry Matter (%)					
	Dry Matter	Crude Protein	Crude Fiber	Crude Fat	Nitrogen Free Extracts	Total Digestible Nutrient
Brachiaria decumbens	24.40	10.10	27.79	2.01	54.30	57.05
Fine bran	90.76	13.46	4.03	13.02	60.50	94.31
Coconut cake	87.90	15.90	9.40	3.46	67.24	75.70
Mineral	100	-	-	-	-	-
Fine corn	85.84	7.73	0.91	3.43	87.01	80.12
Soybean meal	91.75	41.38	12.41	0.91	19.61	75.23

* TDN is calculated based on Sutardi (1980): 1. For feed ingredients with CF < 18% and CP < 20%: TDN = 2.29 + 1.17 CP + 1.74 Cfat – 0.295 CP + 0.810 NFE: 2. For feed ingredients with SK < 18% AND PK > 20%: TDN = 25.6 + 0.530 CP + 1.70 Cfat – 0.474 CF + 0.732 NFE: 3. For feed ingredients with CF > 18% and CP < 20%: TDN = 70.6 + 0.259 CP + 1.01 Cfat – 0.760 CF + 0.0991 NFE: 4. For feed ingredients with SK > 18% and PK > 20%: TDN = 3.17% + 0.640 CP + 2.08 Cfat – 0.0675 CF + 0.940 NFE

Table 2: Nutrient content of control ration (%)

Feed Stuff (%)	Treatment						
	A	B	C	D	E	F	G
Brachiaria decumbens	60	60	60	60	60	60	60
Fine bran	17	17	17	17	17	17	17
Coconut cake	15	15	15	15	15	15	15
Mineral	5	5	5	5	5	5	5
Fine corn	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Soybean meal	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil	-	-	-	-	0.8	1.6	2.4
Fish oil microcapsules	-	0.8	1.6	2.4	-	-	-
	Chemical Composition						
Dry matter	90.36	90.36	90.36	90.36	90.36	90.36	90.36
Crude Protein	12.15	12.15	12.15	12.15	12.15	12.15	12.15
Crude Fiber	19.12	19.12	19.12	19.12	19.12	19.12	19.12
Crude Fat	4.13	4.93	5.73	6.53	4.93	5.73	6.53
Nitrogen Free Extracts	57.79	57.79	57.79	57.79	57.79	57.79	57.79
Total Digestible Nutrient	63.63	63.63	63.63	63.63	63.63	63.63	63.63

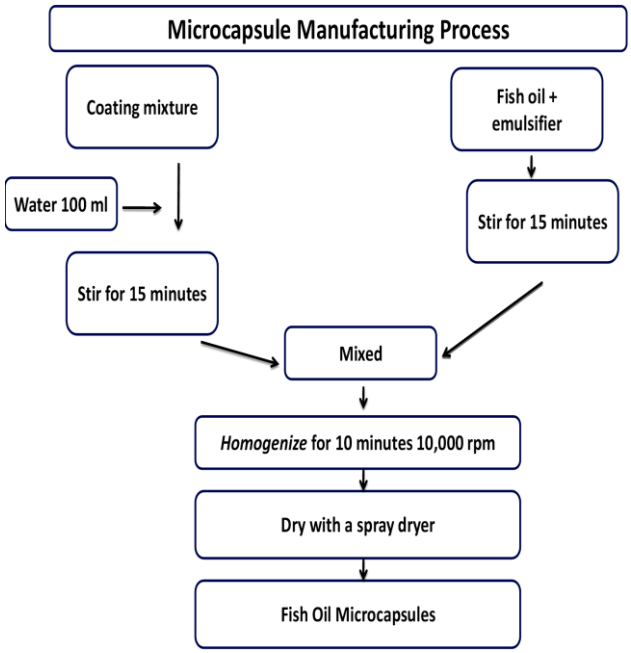

Fig. 1: Working procedure for making fish oil microcapsules.

Treatment E = A + 0.8% fish oil
Treatment F = A + 1.6% fish oil
Treatment G = A + 2.4% fish oil

The data obtained from the calculation results were analyzed statistically using analysis of variance. When there was a statistically significant difference in the effect ($P<0.05$), the subsequent step involved conducting the Duncan Multiple Range Test (Steel and Torrie 2021).

Parameters

The parameters measured were nutrient digestibility (dry matter digestibility, organic matter digestibility, crude fat digestibility, crude fiber digestibility, NDF digestibility, ADF digestibility, cellulose digestibility and hemicellulose digestibility), rumen fluid characteristics (pH, VFA and NH_3).

Procedure

The rations were prepared according to the treatment. Rations A were control rations without treatment, rations B, C, and D were control rations plus fish oil microcapsules according to the treatment level and rations E, F, and G were control rations plus fish oil according to the treatment level.

Rumen fluid was taken in the morning from the cutting site, then put into a flask which was previously filled with hot water to maintain the temperature at 39°C and maintain anaerobic conditions, then brought to the laboratory for *in vitro* evaluation (Tilley and Terry 1963). The rumen liquid was filtered using 4 layers of cheesecloth. All ingredients for Mc Dougall's buffer were dissolved with distilled water to 2L. This buffer solution was prepared the day before fermentation, placed in a water bath shaker with a temperature of 39°C, and CO_2 gas was flowed for 40min so that the anaerobic conditions and pH were adjusted to close to 7 using 20% NaOH or 20% H_3PO_4. The inoculum was prepared by mixing 4 parts of buffer with 1 part of rumen fluid. Samples that had been weighed as much as 2.5g were put into an Erlenmeyer tube, and then rumen fluid which had been mixed with buffer solution was added. Immediately after administration of rumen fluid, CO_2 gas flowed for ± 30sec so that the conditions remained

anaerobic and the pH was neutral (6–7). The Erlenmeyer tube was then covered with a rubber lid with ventilation, then placed in a water bath shaker to incubate for 48h.

pH measurements were promptly taken following the conclusion of each incubation period using a pH meter. Before use, the tool was standardized with a buffer solution between pH 7 and 4, after which the sample was centrifuged at 1200rpm for 20min. The supernatant was taken to analyze the levels of VFA and NH_3. While the residue was used for the analysis of food substances and fiber fractions. Food substances were analyzed using the AOAC method (2016) and fiber fractions using the Van Soest et al. (1991) method. Nutrient digestibility was determined by following these equations:

$$\text{DM}= \frac{\{[DM\ samples-(DM\ residue-DM\ blanks)]}{DM\ sample\}} x\ 100\%$$

$$\text{OM}= \frac{\{[OM\ samples-(OM\ residue-OM\ blanks)]}{OM\ sample\}} x\ 100\%$$

$$\text{CP}= \frac{\{[CP\ samples-(CP\ residue-CP\ blanks)]}{CP\ sample\}} x\ 100\%$$

$$\text{NDF}= \frac{\{[NDF\ samples-(NDF\ residue-NDF\ blanks)]}{NDF\ sample\}} x\ 100\%$$

$$\text{ADF}= \frac{\{[ADF\ samples-(ADF\ residue-ADF\ blanks)]}{ADF\ sample\}} x\ 100\%$$

$$\text{Cellulose}= \frac{\{[Cellulose\ samples-(Cellulose\ residue-Cellulose\ blanks)]}{Cellulose\ sample\}} x\ 100\%$$

$$\text{Hemicellulose}= \frac{\{[\text{Hemicellulose}\ samples-(\text{Hemicellulose}\ residue-\text{Hemicellulose}\ blanks)]}{\text{Hemicellulose}\ sample\}} x\ 100\%$$

The VFA concentrations in the samples were calculated using the following formula:

$$\text{VFA concentration (mM)}= \frac{Sample\ area\ x\ standard\ VFA\ concentration}{Standard\ VFA\ concentration}$$

The concentration of NH_3 was calculated using the following formula:

$$NH_3\ (\text{mM})= \frac{mL\ H2SO4\ x\ N\ H2SO4\ x\ 1000}{g\ sample\ x\ DM\ sample}$$

RESULTS

Digestibility of Nutrients

According to Table 3, the digestibility of dry matter, organic matter, and crude fiber was not significantly influenced by the treatment (P>0.05). However, a significant difference (P <0.05) was observed in the crude fat digestibility. After the DMRT test was carried out, the G treatment, namely 2.4% fish oil supplementation, showed the lowest digestibility. The highest digestibility of crude fat was obtained by supplementing fish oil microcapsules of 8% (Treatment C) but not different (P>0.05) with treatments A, B, D, E and F.

Digestibility of Fiber Fraction

Table 4 shows that the digestibility of NDF, ADF, cellulose, and hemicellulose was not significantly influenced by the treatments A, B, C, D, E and F (P>0.05).

Rumen Fluid Fermentability

Based on Table 5, the treatment did not have a significant effect (P>0.05) on pH and NH_3 levels. However, there was a significant difference (P<0.05) observed in the production of VFA. There was a significant reduction (P<0.05) observed in the microcapsule supplementation treatments (B, C and D) than the fish oil supplementation treatments (E, F and G). The graph of the difference in VFA and NH_3 concentration between fish oil supplementation and microcapsules can be seen in Fig. 2 and Fig. 3.

DISCUSSION

Digestibility of Dry Matter

According to Table 3, it is evident that the average *invitro* treatment dry matter digestibility ranged from 62.15 to 70.65%. The results of the analysis of variance indicated that there was no significant effect (P>0.05) of the treatments on the *in vitro* digestibility of dry matter. This

Table 3: Effect of treatment on the digestibility of dry matter, organic matter, crude fat, and crude fiber

Treatment	Digestibility of			
	Dry matter (%)	Organic matter (%)	Crude fat (%)	Crude fiber (%)
A	62.15±0.45	64.08±0.23	66.37±0.23a	40.81±0.21
B	63.93±0.34	66.19±0.36	69.54±0.41a	41.92±0.41
C	65.13±0.36	66.93±0.54	71.50±0.36a	36.19±0.19
D	70.65±0.53	69.94±0.23	69.22±0.41a	41.72±0.23
E	64.43±0.45	64.60±0.34	62.91±0.43a	32.73±0.34
F	63.51±0.29	63.97±0.43	67.73±0.32a	29.68±0.22
G	62.93±0.33	63.58±0.21	47.85±0.38b	32.39±0.34
Average	64.68	65.61	63.59	36.49
SE	1.89	2.27	2.88	3.14

Values (mean±SD) having different alphabets within the same column are significantly different at P<0.05.

Table 4: Effect of treatment on the digestibility of NDF, ADF, cellulose and hemicellulose

Treatments	Digestibility of			
	NDF (%)	ADF (%)	Cellulose (%)	Hemicellulose (%)
A	62.04±0.32	60.68±0.25	61.01±0.24	62.76±0.23
B	60.15±0.18	59.14±0.27	63.18±0.32	63.44±0.41
C	61.42±0.48	61.38±0.31	63.90±0.33	64.49±0.32
D	62.61±0.36	62.46±0.29	63.97±0.54	65.28±0.33
E	60.24±0.32	57.82±0.38	60.92±0.43	63.41±0.34
F	58.12±0.33	57.90±0.33	62.44±0.32	62.47±0.23
G	57.89±0.42	55.41±0.26	60.97±0.18	62.85±0.17
Average	60.38	59.26	62.34	63.57
SE	1.15	1.98	0.66	0.43

There is no significant difference between treatments (P>0.05). NDF= neutral detergent fiber, ADF= acid detergent fiber.

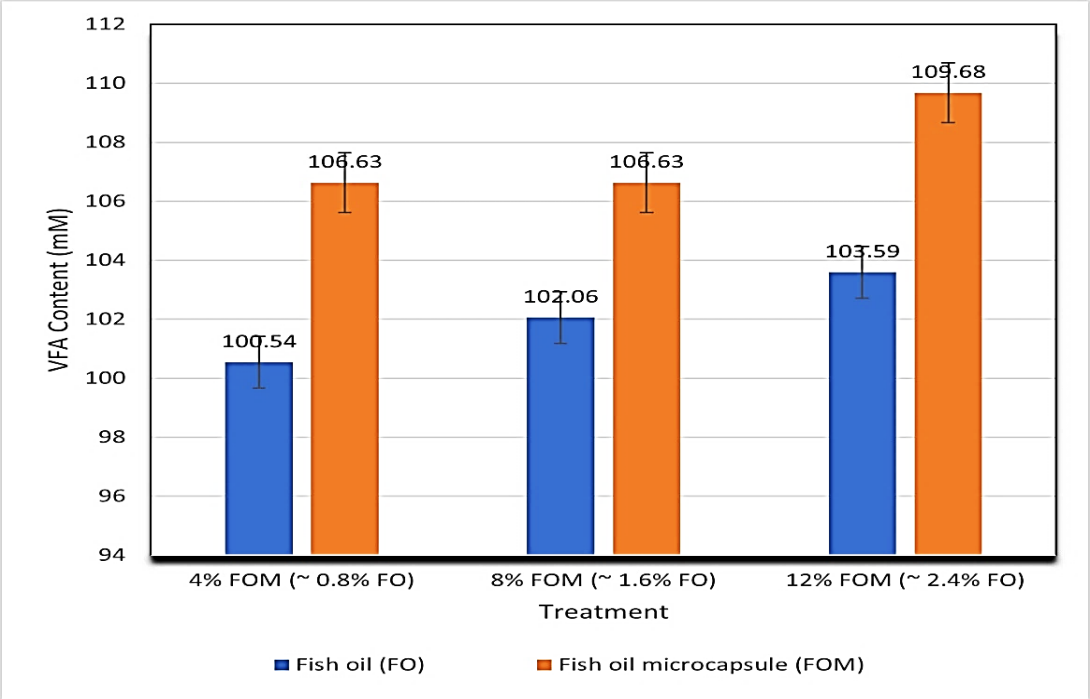

Fig. 2: Effect of various treatments on VFA; FO = fish oil; FOM = Fish oil Microcapsule.

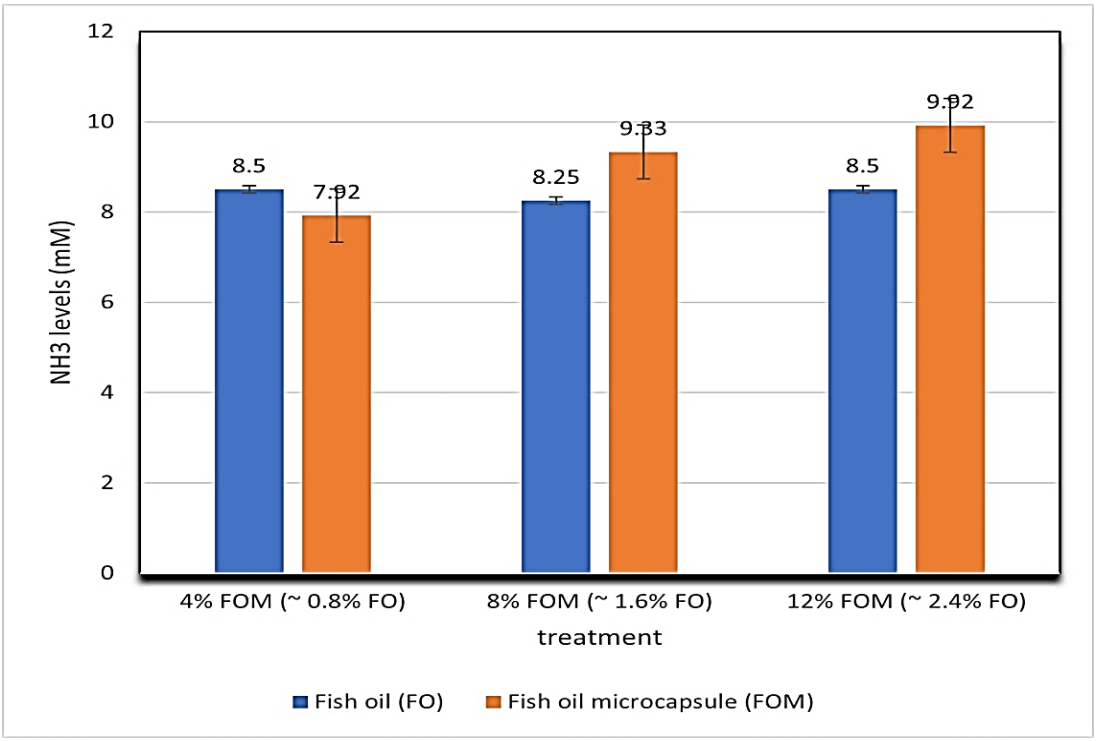

Fig. 3: Effect of various treatments on NH_3; FO = fish oil; FOM = Fish oil Microcapsule.

Table 5: Effect of treatments on pH, VFA and NH_3

Treatment	pH	VFA (mM)	NH_3 (mM)
A	6.87±0.45	101.11^{a} ±1.44	8.33±0.28
B	6.85±0.08	100.54^{a}±1.49	8.50±0.52
C	6.89±0.35	102.06^{a}±1.34	7.92±0.72
D	6.84±0.32	103.59^{a}±1.21	8.25±0.45
E	6.86±0.03	106.63^{b}±1.24	9.33±0.36
F	6.90±0.27	106.63^{b}±1.34	8.50±0.73
G	6.88±0.29	109.68^{b}±1.32	9.92±0.36
SE	0.03	1.57	0.57

Values (mean±SD) having different alphabets within the same column are significantly different at P<0.05.

lack of significance suggests that the addition of fish oil and fish oil microcapsules did not impact the activity of rumen microbes in digesting the feed. Additionally, the crude fiber content in each treatment ration was relatively consistent (Table 2), which likely contributed to the comparable digestibility of dry matter across the rations.

The high proportion of fiber in the feed allows the availability of adequate feed particles to facilitate the biohydrogenation of unsaturated fatty acids which is a detoxification mechanism for these compounds (Alzahal et al. 2009). Biohydrogenation is also enhanced in high-fiber

feeds by the high population of bio hydrogenating microbes (Salami et al. 2021). This is reflected in the absence of significant changes in dry matter digestibility in the treatment group, with or without protection.

Organic Matter Digestibility

The average dry matter digestibility, each treatment added fish oil microcapsules and fish oil *in vitro* can be observed in Table 3. The data presented in Table 3 reveals that the average digestibility of organic matter treated *in vitro* varied between 64.58 and 69.94%. Results analysis of the variance showed that treatments showed no significant (P>0.05) effect on the digestibility of organic matter *in vitro* rations. This non-significant difference is related to the digestibility of the dry matter. Pazla et al. (2022) and Sari et al. (2022) have suggested that the digestibility of dry matter can have an influence on the digestibility of organic matter. The digestibility of organic matter refers to the extent to which nutrients from feed are available and can be utilized by livestock.

The connection between organic matter and dry matter is intricately linked, as a proportion of the dry matter consists of organic matter. If the digestibility of the dry matter remains constant, it logically implies that the digestibility coefficient of the organic matter will also remain constant (Pazla et al. 2021a). The nutrient substances found within organic matter are the constituent elements of dry matter. This aligns with the viewpoint expressed by Pazla et al. (2018a) and Pazla et al. (2021b) which asserts that there is a positive correlation between the consumption of dry matter and the consumption of organic matter.

Table 3 shows that the treatment with added fish oil microcapsules led to a numerical improvement in organic matter digestibility compared to other treatments. This improvement can be attributed to the relatively higher dosage of fish oil microcapsules as opposed to fish oil alone.

Crude Fat Digestibility

Table 3 presents the average digestibility of crude fat for each treatment, encompassing the inclusion of fish oil microcapsules and fish oil *in vitro*. The analysis results indicated that the treatments had a significantly varied effect (P<0.05) on the digestibility of crude fat. The average digestibility of crude fat in the *in vitro* treatment ranged from 47.85-71.50%. The highest average crude fat digestibility (71.50%) was found in treatment C, namely the basal ration plus 8% fish oil microcapsules. This is due to the high content of ration fat which will affect rumen microbial metabolism and the post-rumen digestive system.

According to Jenkins (1993), rumen bacteria have a strong ability to lipolyze ration fat. Nevertheless, the presence of ration fat resulted in a reduction of the growth of specific rumen bacteria, particularly cellulolytic ones. The decrease was sharper with increasing C_{18} fatty acid unsaturation in the ration, while the amylolytic species had less effect. However, the addition of fat in the ration did not change the concentration and total number of bacteria in the rumen (Azmi et al. 2020).

The average crude fat digestibility in treatment A did not show a significant difference (P>0.05) compared to the average crude fat digestibility in treatments B, C, D, E, F, and G. This was due to the oil or fat added to the ration, controlling the rumen protozoa population. Under conditions of covering protozoa with fat, protozoa, just like bacteria, do not possess lipolytic activity. In addition, many protozoa are involved in phospholipid hydrolysis, as a result, the metabolic activity of protozoa is disrupted, resulting in numerous consequences Tiven et al. (2021).

Administration of fish oil microcapsules to treatments B, C, and D compared to fish oil treatments E, F, and G did not show a significant difference effect (P<0.05). This is attributed to the inclusion of fish oil and fish oil microcapsules in the diets, both rich in omega-3 fatty acids. However, adding fish oil to the diets can pose challenges like rapid hydrogenation and clumping, leading to non-uniform ration mixing. These issues can impact the levels of unsaturated fatty acids, notably EPA and DHA. Rations containing fish oil microcapsules serve to safeguard the omega-3 fatty acids within fish oil against oxidation, transforming the fish oil into a form that can influence digestibility (Keogh et al. 2001).

The administration of fish oil microcapsules at a 4% level (treatment B) demonstrated a statistically significant difference (P<0.05) compared to treatments C and D, which were administered at 8 and 12% levels, respectively. This is because the omega-3 fatty acids contained in fish oil can be protected from oxidation and processing by suppressing or slowing down oxidation (Heinzelmann et al. 2000; Kolanowski 2004; Sun et al. 2005). The presence of a high-fat content in the ration negatively impacts the growth of rumen microbes. The addition of fat in beef and sheep rations reduces fiber digestibility because long-chain fatty acids inhibit rumen microbial metabolism (Palmquist 1986). According to the findings of Kook et al. (2002), the administration of unprotected fish oil in cattle resulted in an increase in blood serum cholesterol levels.

The administration of fish oil microcapsules at 8% (C) level was not significantly different from the administration of 12% fish oil microcapsules (D). This is caused by the administration of fish oil microcapsules at a level of 8-12% which can affect the digestibility of crude fat. According to Shirley (1986), a fat content of 3-4% can be given to beef cattle rations if given above 5% will reduce the performance of livestock. Makmur et al. (2019) reported that rumen lipid concentration influences biohydrogenation, methanogenesis, overall digestibility, carbohydrate fermentation, protein deamination, and microbial cell synthesis in the rumen.

The administration of fish oil in the ration at the level of 0.8% (E), was not significantly different (p>0.05) from the F and G treatments (1.6%) and (2.4%), respectively. Meanwhile, treatment F did not show a significant difference (P<0.05) compared to treatment G. This was caused by mixing fish oil in rations above the 5% level affecting microbial activity in the rumen. According to Shirley (1986), a fat content of 3-4% is usually given to beef cattle rations and if given above 5% will reduce the performance of livestock.

Crude Fiber Digestibility

The average crude fiber digestibility of each treatment with added fish oil microcapsules and fish oil *in vitro* can be seen in Table 8. The analysis of variance results revealed

that there was no statistically significant effect (P>0.05) of the treatments on the digestibility of crude fiber. The average digestibility of crude fiber treatment *in vitro* ranged from 29.68-41.92%. The highest average digestibility of crude fiber was 41.92% in treatment B, namely the basal ration added with 4% fish oil microcapsules. This shows that protected fats can suppress or reduce the negative effects on fiber digestibility, and protected fats can protect omega-3 fatty acids contained in fish oil from oxidation by suppressing or slowing down oxidation (Heinzelmann et al. 2000; Kolanowski 2004; Sun et al. 2005).

The existence of covering the feed particles with fat causes microbial access to these feed particles to be hampered and will ultimately reduce rumen microbial metabolism. The digestibility of fiber tends to decrease as the amount of fat in the ration increases (Arief et al. 2023). The digestibility of fiber also depends on the composition of fatty acids contained in the fat. The digestibility of fiber decreases more if what is added is fat rich in unsaturated fatty acids. Protected fat can suppress or reduce the negative effects of fiber.

The Effect of Treatment on the Degradation of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF)

The research results provide information on the average degradation of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) for each treatment, as shown in Table 4.

By referring to Table 4, it is apparent that the degradation of NDF in this study varied between 57.89 and 62.61%, while the degradation of ADF ranged from 55.41 to 62.46%. The diversity analysis results revealed that there was no significant effect (P>0.05) observed between the treatments involving the addition of fish oil microcapsules (FOM) and fish oil (FO) on the *in vitro* degradation of NDF and ADF.

The effect of adding FOM and FO was not significant because the crude fiber content in each treatment ration was relatively the same, so it did not affect the degradation of NDF and ADF. In addition, the chemical composition, amount, and type of concentrate from each treatment ration were also the same so that the availability of the energy and nutrients that can support the digestive activity of microbes in the rumen were also the same in all of these treatments. This is in accordance with the opinion of Zain et al. (2019) that the amount and type of concentrate in the ration mix will affect the digestibility of nutrients.

Administration of FOM at a level of 4% (treatment B) up to a level of 12% (treatment D) had no significant effect on the degradation of NDF and ADF. This is because fish oil that has been protected by encapsulation is still safe to use in livestock bodies up to a level of 12% because 12% fish oil microcapsules contain 2.4% fish oil which is still safe to use. After all, it has been protected. The findings align with Gulati et al.'s (2000) perspective, which suggests that the provision of unsaturated fatty acids in the ration is only 3-4%, but if more is given, it is better to protect it first.

Despite statistical tests indicating no significant (P>0.05) effect on the degradation of NDF and ADF, there was a numerical increase observed in the administration of fish oil microcapsules compared to the control treatment (treatment A). This increase can be observed in treatments B, C, and D.

Providing fish oil at concentrations ranging from 0.8% in treatment E to 2.4% in treatment G did not result in a significant difference in its impact on the degradation of NDF and ADF. This is because mixing fish oil in rations up to a level of 2.4% does not affect microbial activity in the rumen, because the fat contained therein is still safe to use. But numerically in the E, F, and G treatments, there was a decrease in the degradation of NDF and ADF compared to the control.

NDF degradation was higher than ADF degradation, this was because NDF had a fraction that was easily soluble in the rumen, namely hemicellulose. The hemicellulose content will greatly determine the rate of food in the rumen, the higher the hemicellulose content, the higher the degradation so that the rate of food in the rumen will be faster (Indah et al. 2020). ADF degradation is reduced due to the presence of components in ADF that are inherently more challenging to digest, particularly cellulose and lignocellulose.

Effect of Treatment on Cellulose and Hemicellulose Degradation

Table 4 displays the average breakdown of cellulose and hemicellulose in each treatment, as indicated by the study's findings. Based on Table 4, it can be observed that the degradation of cellulose in this study ranged from 60.92 to 63.97% and the degradation of hemicellulose ranged from 62.74 to 65.28%.

The analysis of variance results indicated that there was no significant effect (P>0.05) between the addition of FOM and OM on the degradation of cellulose and hemicellulose *in vitro*. Digestibility of cellulose is an active role for these microbes to break bonds of 1,4 glucosides and digest cellulose as an energy supply.

The addition of protected fish oil (FOM) up to a level of 12% in treatment D did not yield a significantly different effect (P>0.05) on the degradation of cellulose and hemicellulose. This lack of significant difference can be attributed to the maintained safety and integrity of the protected fish oil. This is also due to the fact that the chemical composition of each treatment ration is also relatively the same. The effect of the treatment on the degradation of cellulose and hemicellulose was not significantly different, presumably because the crude fiber content remained consistent across all treatments produced, with no variation observed.

Giving MI to treatments E, F, and G also had no significant effect on the degradation of cellulose and hemicellulose, this was because the fish oil given up to a level of 2.4% did not affect microbial activity in the rumen. Hemicellulose degradation was higher than cellulose degradation. This is due to the constituent components of hemicellulose consisting of carbohydrate polymers containing hexose, pentose, harban, xylan, and polyuric sugars which are less resistant to chemical solvents or enzymatic reactions than cellulose (Jamarun et al. 2021)

Despal et al. (2021) further added that hemicellulose is a fraction that dissolves easily in the rumen, so its digestibility is higher. According to Jamarun et al. (2017) and Pazla et al. (2020), the degradation of cellulose is generally more challenging compared to the degradation of

hemicellulose. This is due to several factors, including the presence of specific bacteria in the rumen, the percentage of lignin and silica, and the crystallization of lignocellulosic bonds. Cellulose degradation is influenced by the number of bacteria thriving in the rumen, as well as the levels of lignin and silica present. Additionally, hemicellulolytic bacteria are unable to break down cellulose, whereas cellulolytic bacteria possess the ability to degrade hemicellulose (Pazla et al. 2021c). With all the comparisons of NDF and ADF, the degradation of hemicellulose has no significant effect because hemicellulose is obtained from the results of the difference between NDF and ADF (Liu et al. 2020).

Effect of treatment on the characteristics of the rumen fluid

Rumen Fluid pH

The impact of each treatment, which involves the addition of fish oil in the form of fish oil microcapsules and fish oil, on the rumen fluid acidity (pH) can be observed in Table 4. The obtained average rumen fluid pH values ranged from 6.84 to 6.90. According to the results of this study, the obtained pH value falls within the optimal range for digestive activity and protein synthesis in the rumen. Pazla et al. (2018b) states that the optimum pH required for microbial protein synthesis ranges from 6-7. Belanche et al. (2021) stated that rumen microbial activity requires certain pH conditions related to ongoing rumen environmental conditions. Van Soest (1994) stated that the activity of cellulolytic bacteria was inhibited when the pH of the rumen fluid falls below 6.2, and the activity would be optimal in the rumen at a pH of 6.7±0.5. Belanche et al. (2021) stated that if the rumen pH is less than 6 then it can inhibit the process of proteolysis and deamination because the growth of rumen bacteria will be hampered.

Based on the statistical tests conducted, the results demonstrated that increasing the level of fish oil microcapsules and fish oil in the control ration combination did not have a significantly different effect on rumen fluid pH compared to the control. The rumen fluid pH was not significantly affected by the increase in fat content in the ration resulting from the addition of fish oil microcapsules and fish oil, indicating that the pH remained within a range that was tolerable for rumen microbes. This tolerance can be seen from the activity of rumen microbial fermentation which produces VFA and NH_3 which are still within the optimum range to support microbial growth and activity. Based on the perspective of Ikhlas et al. (2023), which highlights one of the factors that affect pH is fermentation activity and fermented products, namely VFA and NH_3 levels.

The insignificant difference in rumen pH was also caused by artificial saliva from Mc Dougall's buffer sea which functioned as a pH neutralizer and the amount given to each treatment was the same. The saliva present in each treatment will maintain the rumen pH by buffering the fermented acids (VFA) resulting in a more or less constant rumen pH (Pazla et al. 2023a). In addition, the factors that influence the rumen fluid pH are not significantly different because the conditions given to each treatment are the same, such as the ratio between buffer, and inoculum, livestock source of inoculum, and incubation time. Factors that affect *in vitro* results are the source of inoculum, sample particle size, the ratio of the amount of inoculum to buffer and the length of incubation (Pazla et al. 2023b).

Rumen Fluid VFA Levels

The effect of each treatment of adding fish oil in the form of fish oil microcapsules and fish oil to rumen fluid VFA concentrations is shown in Table 4. The average rumen fluid VFA levels, as depicted in Table 4, varied from 100.54 to 109.68mM. The statistical analysis revealed a significant difference ($P<0.05$) in the effect of treatment with fish oil and fish oil microcapsules on rumen fluid VFA production.

The rumen fluid VFA results from this treatment were still within the range that did not interfere with rumen microbial growth activity. The increase in ration fat content after the addition of fish oil and fish oil microcapsules was still within limits that did not interfere with carbohydrate degradation. The amount of VFA required for optimum rumen microbial activity and growth ranged from 80-160mM (Jamarun et al. 2017a, b, c).

The addition of non-encapsulated fish oil, specifically in treatments E, F, and G, resulted in an increase in VFA values compared to the addition of fish oil microcapsules. This disparity is notable even though the fat content in the treatments involving fish oil microcapsules was equivalent to that of fish oil (Fig. 2).

In Fig. 2, it can be seen that increasing the level of addition of fish oil microcapsules and fish oil both increase VFA levels. In comparison to these two treatment groups, the addition of fish oil microcapsules exhibited a lower VFA content compared to the addition of non-encapsulated fish oil.

The low VFA content in the addition of fish oil microcapsules is due to the microencapsulation process which causes less degradation of the fish oil so that the VFA contribution is less. In comparison to the addition of non-encapsulated fish oil, the VFA levels were found to be higher when fish oil microcapsules were used. The high levels of VFA from the addition of encapsulated fish oil is caused by rumen microbes hydrolyzing the fat content into fatty acids and glycerol. Furthermore, the fatty acids will undergo a biohydrogenation process from unsaturated fatty acids to saturated fatty acids and glycerol will be fermented by rumen microbes to become propionic acid which increases rumen fluid VFA levels. This is in accordance with the statement of Makmur et al. (2019) that glycerol resulting from fat lipolysis will be fermented by rumen microbes to become propionic acid.

Rumen Fluid Ammonia (NH_3) Level

The effect of each treatment with the addition of fish oil in the form of fish oil microcapsules and fish oil on the rumen fluid ammonia (NH_3) concentration can be observed in Table 8. From Table 8, it can be observed that the average rumen fluid NH_3 level ranged from 7.92 to 9.92 mM. The results of the statistical tests indicated that the level of addition of fish oil and fish oil microcapsules in the ration formulation did not have a significant effect on the concentration of rumen fluid NH_3.

The difference was not significant that the NH_3 value of the above treatment was due to the fact that the level of 12% fish oil microcapsules did not interfere with rumen microbial activity in producing fermented products in the

form of NH_3. Based on the NH_3 content produced, it is known that incorporating fish oil produces higher NH_3 compared to the NH_3 produced from adding microencapsulated fish oil. The difference in NH_3 levels can be seen in Fig. 3.

The high levels of NH_3 produced in the addition of fish oil were probably due to the high levels of VFA so these VFAs were used by microbes as a carbon skeleton for their body's protein synthesis. Due to the large number of microbes growing, more enzymes will be produced to degrade proteins, so the NH_3 produced is also higher when compared to the treatment with the addition of fish oil microcapsules (Fig. 6). The NH_3 levels from the treatment involving the addition of fish oil microcapsules are observed to be lower in comparison to the NH_3 levels from the addition of encapsulated fish oil. The reduced NH_3 level in the treatment with fish oil microcapsules can be attributed to the lower levels of VFA in comparison to the addition of non-encapsulated fish oil.

The low level of NH_3 is possibly due to less degradation of fish oil microcapsules compared to fish oil degradation so that fish oil microcapsules can pass as bypass proteins to the omasum. This is in accordance with the opinion (Putri et al. 2019; 2021) that the feed protein which is not degraded will be a bypass protein for livestock.

Increasing the level of addition of fish oil to the ration, the production of NH_3 will increase. Where in the digestive system of ruminants, most of the protein that enters the rumen will undergo decomposition (degradation) by enzymes with proteolytic activity synthesized by microbes in the rumen into ammonia (NH_3). NH_3 production depends on the solubility of ration protein, and the amount of ration protein, where the more protein is degraded by microbes the higher the NH_3 production and the duration of food in the rumen (Zain et al. 2023).

Conclusion

The addition of fish oil microcapsules up to 2.4% in the ration did not interfere with nutrient digestibility, fiber fraction, and rumen fluid fermentability (pH, VFA, NH_3).

Author contributions

Conceptualization: Montesqrit and Roni Pazla, Data Curation: Montesqrit. Formal analysis: Montesqrit, Rusmana WSN, and Roni Pazla. *In Vitro* Treatment: Rusmana WSN and Roni Pazla, Funding acquisition: Montesqrit. Methodology: Montesqrit and Rusmana WSN. Project administration: Roni Pazla. Supervision: Montesqrit. Validation: Rusmana WSN. Writing-original draft: Montesqrit. Writing-review and editing: Roni Pazla.

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