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The Effect of Protected Lemuru Fish Oil Supplementation on In Vivo Nutrient Digestibility and Sheep Blood Profile

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ABSTRACT

This research was intended to observe the effect of protected lemuru fish (*Sardinella longiceps*) oil for ruminants, especially for sheep. This study aimed to evaluate the digested nutrients and blood profile of sheep. This study was conducted in September-October 2020 in Dumbira Farm, Kalasan, Yogyakarta, using 12 sheep divided into 3 treatments with 4 replications each. Treatment P0 was Total Mixed Ration (TMR) without protected Lemuru fish oil (control), treatment P1 was TMR with 5% protected Lemuru fish oil, and treatment P2 was TMR with 10% protected Lemuru fish oil. The data were statistically analyzed using one way analysis of variance and continued with Duncan new Multiple Range Test for significant results. The results of this study indicated that the addition of 10% protected Lemuru fish oil in TMR feed had a significant effect ($P<0,05$) on the increased value of in vivo digestion of crude fiber and crude fat, but did not affect the digestibility of dry matter, organic matter, and protein. The addition of protected Lemuru fish oil did not cause hematological disorders showed by the blood profiles were in the normal range. In conclusion, protected lemuru fish oil supplementation had a favorable influence on the production performance without affecting blood profile of sheep.

Keywords: Blood profile, Digestibility, Lemuru fish oil protected, Sheep

Introduction

Increasing energy intake in ruminants can be done by increasing feed energy using fat (oil), while protein intake can be increased by providing protected protein that undegradable by rumen microbes (Pramono *et al*., 2013). One of the potential energy sources, widely available, and affordable is Lemuru fish oil. Lemuru fish oil contains high unsaturated fatty acids. Supplementation of unsaturated fatty acids has been shown to increase energy efficiency through increasing energy density and supporting increased efficiency of protein tissue synthesis via increasing the flow of non-ammonia nitrogen into the duodenum (Johnson *et al*., 2002). Constraints found when Lemuru fish oil is given directly in the feed are (1) possibility of hydrogenation process in the rumen which converts unsaturated fats into saturated fats; (2) application of oil can interfere the cellulolytic microbial activity, thereby reducing the rate of fermentation in the rumen; (3) Lemuru fish oil has a fishy smell due to its trimethylamine oxide compounds, and may lead to low palatability if it mixes directly to the feed rations (Pramono *et al*., 2013). Therefore, protective treatment is needed to obtain tangible benefits from the supplementation of energy and/or protein sources in the feed. In unsaturated fatty acid supplementation, protection is needed to prevent unsaturated fatty acids from double bond biohydrogenation by rumen microbes (Ashes *et al*., 1995). Protection is also helpful in eliminating the negative impact of unsaturated fatty acid supplementation at high levels, in the form of decreasing fiber degradability (Aharoni *et al*., 2004). Calcium soap (Ca-soap) is one of the technologies to protect fat that has been developed recently. Calcium soap is a form of protected fat and is an effective source of fat in ruminant feed ingredients because it can make the rumen fermentation system remains normal, high fatty acid digestibility, and this soap can be easily mixed with various types of feed ingredients (Jenkins and Palmquist, 1984). Through the saponification method with calcium salt ($CaCl_2$), it is expected that the use of fat will not have a negative impact on the rumen microbial ecosystem. Increased digestion of fatty acid in Ca soap as a result of decreased ruminal biohydrogenation and hence larger concentrations of UFA in intestinal mucosa was also observed to improve extract ether (EE) and crude fiber (CF) digestibility with calcium salt supplementation.

Lipolysis and biohydrogenation are common in unprotected fat that travels through the rumen. As the unsaturation of a fatty acid increases, so does its digestibility. As a result, the increased digestibility of EE and CP in protected fat in the diets could be related to reduced ruminal biohydrogenation and the presence of a considerable proportion of long chain unsaturated fatty acids in the small intestine for absorption (Behan *et al*., 2019).

The decrease in sheep's performance might be caused by the not optimum metabolic processes in the sheep's body. Metabolic processes that are not optimal can disturb the physiological condition of livestock, and one of the indicators that can determine the physiological condition of livestock is the livestock's blood profile (Astuti *et al*., 2008). Lipid profile in blood is one of the parameters used to determine livestock's health condition and productivity. Blood has a complex role in the body's physiological processes (Gross *et al*., 2016). Fatty acids, especially omega-3 in fish oil and omega-6 in plant oil, can affect fat metabolism. Therefore, the level of fat components (cholesterol, triacylglycerol, HDL, and LDL) in the blood can be used to indicate the effectiveness of fat protection in ruminant rations (Adawiah *et al*., 2006). Fatty acids that pass from the rumen and into the duodenum are typically connected to meal particles or microorganisms. Fatty acids will dissolved by bile salts.

Phospholipase enzymes hydrolyze lecithin, a microbial phospholipid, to produce lysolecithin. Micelles will be formed from fatty acids, salt bile, and lysolecithin (small circles). These micelles aid in the absorption of fatty acids in the intestine (jejunum). Fatty acids are esterified in epithelial cells of the small intestine, and triacylglycerol and phospholipid bind to chyclomicron and very low density lipoprotein (VLDL) and are transported to the lymph glands (Wina and Susana, 2013).

This study aimed to evaluate the protection of Lemuru fish oil by saponification method as a feed supplement based on in vivo nutrient digestibility and blood profile in sheep.

Materials and Methods

Protected lemuru fish oil making process

Lemuru fish oil was obtained from the waste of sardine canning industry by PT Sumber Yalasamudra in Muncar, Banyuwangi, East Java. Other ingredients were distilled water, caustic soda (technical NaOH), technical $CaCl_2$, and starch flour as a capsule.

The oil was heated and mixed with different concentrations of technical NaOH solution (caustic soda) while stirring and adding starch solution until a soft and elastic paste (gel) was formed. The ratio of the volume of oil with a solution of NaOH and starch was 1:2:1. The formed paste was left overnight (12 hours) to solidify. After the solidification, the gel was made into thin plates, sliced with a knife, and then immersed in a saturated $CaCl_2$ solution until the gel plate hardened. Subsequently, the dough was crushed (still immersed in a saturated $CaCl_2$ solution) by pressing until small granules (crystals) were formed. After the granules were formed, it was still immersed in a saturated $CaCl_2$ solution for approximately 1 hour so that the granules harden, and then the granules were filtered. The remaining liquid carried by the granules was removed by pressing. The granules obtained were dried under the sun (10 to 20% moisture content) to form fatty acid soap from Lemuru fish oil (Setyaningrum *et al*., 2015). The soap that has been formed then later mix into the Total Mixed Ration (TMR).

In vivo experiments on sheep

This study used 12 ewes age less than one year with an average body weight of 18 kg. The research location was the Dumbira Farm, Tamanmartani, Kalasan, Yogyakarta. The basal ratio used in this study was dried kale, and concentrate was given using the Total Mixed Ration (TMR) method. The concentrate given was the one commonly used by breeders. The constituent ingredients of the concentrate were coffee husk, cocoa husk, copra meal, palm cake, and pollard. The ingredient of each experimental diets is presented in Table 1. Drinking water was provided ad libitum. The materials used for taking sheep blood were 5 ml sterile syringe, 3 ml EDTA tube, venoject, vacutainer, and cooler box.

The treatment was P0 (control = without Lemuru fish oil supplementation), P1 (TMR containing 5% protected Lemuru fish oil supplementation), and P2 (TMR containing 10% protected Lemuru fish oil supplementation). The composition of feed nutrients is presented in Table 2.

The experiment was carried out for 8 weeks with an adaptation period of one week. Feeding was done twice in the morning and evening. Nutrient digestibility was calculated based on the total collection method carried out for 14 days in the fifth and sixth weeks of the experiment. The fecal collection was carried out by collecting all sheep feces during the total collection period. Every day (24 hours), the feces were weighed, and then 200 grams of feces were taken for drying. Feces for 14 days in each experimental unit were composited for nutrient levels analysis. Blood samples were taken at the sixth week of rearing before the sheep was fed. Blood was taken from the jugular vein using a 5 ml sterile syringe, then put into a sterile tube containing EDTA anticoagulant. The samples were then analyzed for levels of blood metabolites, including cholesterol, triglycerides, LDL, HDL, and glucose.

Experimental design and data analysis

The study used a completely randomized design with 3 treatments and 4 replications. The data obtained were analyzed using One way ANOVA. The observed variables included the

Table 1. The ingredients of each experimental diets

Feed ingredients	Nutrient content							
	Dry matter	Ash	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen free extract	Total digestible nutrient
Coffee husk	95.22	0.85	98.31	3.08	0.43	39.74	55.90	82.22
Cocoa husk	94.01	9.63	80.74	14.18	4.83	13.74	57.62	54.08
Copra meal	95.91	10.67	78.66	18.41	3.6	10.38	56.94	53.58
Palm cake	95.96	4.34	91.31	11.76	12.61	14.82	56.47	93.25
Pollard	95.71	5.74	88.52	11.98	3.82	8.33	70.13	66.95
Dried kale	91.97	11.67	76.66	7.2	2.1	23.32	55.71	53.69

Table 2. Nutrient composition of the total mixed ration (TMR)

Chemical composition (%)	Treatment		
	P0	P1	P2
Dry matter [1]	82.95	80.87	80.20
Organic matter[1]	89.57	87.94	87.12
Crude protein[1]	12.41	11.89	11.53
Crude fat[1]	4.33	5.12	6.14
Crude fiber[1]	25.62	28.17	26.55
Total Digestible Nutrient[2]	67.29	64.68	65.73

[1] Analysis Results from the Laboratory of Animal Feed Science, Faculty of Animal Science, UGM.
[2] Calculation results based on the formula of John (2005).

digestibility of the dry matter, organic matter, crude protein, crude fat, crude fiber, Nitrogen free extract (NFE), and TDN, and blood profiles consisting of cholesterol triglycerides, LDL, HDL, and glucose.

Results and Discussion

Nutrient digestibility

The average dry matter digestibility and nutrients of sheep are presented in Table 3. Dry matter digestibility, organic matter, crude protein, and TDN resulted from the three treatments showed no significant difference (P>0.05). The dry matter digestibility that did not show a difference was possible because of the protection of the oil. According to Tanuwiria *et al*. (2006), protection is a form of manipulation of feed in the rumen to maximize the nutrients intake. This causes regular microbial activity in the rumen due to the protected fat can go directly to the post-rumen. There was no difference between the amount of feed consumed and feed rate in the rumen between the three treatments due to the normal microbial activity. According to Abqoriyah *et al*. (2013), factors that affect dry matter digestibility include the ratio composition, the rate of travel through the digestive tract, and the physical form of the feed ingredients. The faster the flow rate of feed particles leaving the rumen causes a higher chance for feed ingredients to the shorter degradation, which leads to higher digestibility. The physical form of feed ingredients from P0, P1, and P2 in this study did not differ, so it was assumed that the feed flow rate in the rumen did not affect digestibility. According to the research of Kustantinah *et al*. (2007), the possible cause of the treatment did not provide a significant difference was the body weight and age of the livestock used in the study were almost the same, and there were not enough replications. The protection may prevent rumen microbial degradation, and the protection of lemuru oil will be ruptured post ruminally, so that it can be easily digested and absorbed post ruminally which in turn effects on nutrient digestibility.

Analysis of variance from the three treatments showed significantly different results (p<0.05) for digestibility of crude fat, crude fiber, and Nitrogen free extract (NFE). Fiber digestibility is related to the ability of rumen microbes to degrade fiber components. In this study, crude fiber digestibility of the ratio using protected fish oil was higher than the control one. This showed that the role of protection in the use of oil could maintain rumen microbial growth conditions. Protection can cover oil against feed particles so that microbial growth in rumen fluid is not inhibited and does not reduce fiber digestibility (Abqoriyah *et al*., 2013).

The treatment was P0 (control = without Lemuru fish oil supplementation), P1 (TMR containing 5% protected Lemuru fish oil supplementation), and P2 (TMR containing 10%

Table 3. The average in vivo nutrient digestibility of sheep

Digestibility	Treatment		
	P0	P1	P2
	----------%----------		
Dry matter	65.70±5.51	65.76±0.77	70.50±3.32
Organic matter	66.26±5.49	66.09±0.97	70.85±3.30
Crude protein	70.83±5.66	71.33±0.70	72.45±3.55
Crude fiber	53.70±9.02^{a}	61.83±2.00^{ab}	65.54±3.53^{b}
Crude fat	84.51±3.20^{a}	88.73±3.69^{ab}	92.06±2.09^{b}
Nitrogen free extract (NFE)	70.04±3.88^{a}	64.49±2.42^{b}	70.65±3.48^{a}
Total digestible nutrient (TDN)	64.09±5.07	64.20±1.34	69.19±3.21

Different superscript letters in the same line shows significantly different (p<0.05).
P0 (control=without protected Lemuru fish oil supplementation); P1 (TMR with 5% protected Lemuru fish oil supplementation) and P2 (TMR with 10% protected Lemuru fish oil supplementation).

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Sheep blood profile

The average cholesterol, triglycerides, LDL, HDL, and blood glucose of sheep from the study are presented in Table 4.

The cholesterol level in this study ranged from 52.95±8.66 to 66.68±16.69 mg/dl. This study has a lower cholesterol level than the study of Gagah *et al.* (2016), which was between 58 mg/dL–81 mg/dL. The results of the analysis of variance showed that the effect of treatment on the cholesterol level of sheep's blood was not significantly different (P>0.05), but descriptive calculations showed that there was a decrease in cholesterol level in the treatment that was given 10% protected Lemuru fish oil. The decrease in sheep blood cholesterol levels was caused by the inhibition of saturated fatty acids formation so that the production of unsaturated fatty acids increased. Fatty acids that pass to the small intestine will increase the production of bile. Increased bile fluid can indirectly reduce cholesterol in the blood (Sudarmi *et al*., 2012). Another possible reason for the insignificant blood profile level was the stress due to weighing, which was done once a week that may disturb the sheep's metabolic system. Cholesterol is a component of fat, and the otal cholesterol is a composition of many substances, including triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL) (Alam *et al*., 2010). Special receptors in peripheral tissues capture the cholesterol contained in LDL. Excess cholesterol in peripheral tissues is transported by HDL to the liver to be excreted through the bile ducts as bile acids (Cheng and Hardy, 2004). The triglyceride level in sheep in this study ranged from 23.68±5.64 to 33.40±7.02. This result was lower than the previous study by Hatta et al. (2018), ranged from 25,732 - 40.44 mg/dl. The low level of triglycerides in the blood was due to the body's energy needs being met. Soehardi (2004) stated that if cells need energy, the lipase enzyme will break down triglycerides into glycerol and fatty acids and release them into the blood vessels. Damron (2003) stated that the blood triglyceride level is influenced by the fat level digested from food or the amount of fat that enters from outside the body.

High-Density Lipoprotein (HDL) plays an essential role in binding the excess cholesterol and transporting it to the liver. High HDL level can prevent the risk of atherosclerosis. Hasanudin *et al.* (2013) stated that HDL is a lipoprotein that maintains the balance of cholesterol to not accumulate in cells by equalizing the sterol removed from the membrane and the cholesterol synthesized into the liver. Table 3 shows the HDL level of sheep blood ranged from 34.70 ± 1.97 mg/dl – 41.40 ± 6.65 mg/dl. The results of this study were almost the same as those of Faisal *et al.* (2017), which the HDL level ranged from 22.25 to 46.25 mg/dl, but lower than the study of Hatta *et al*. (2018) found that the HDL level in sheep serum ranged from 54.87 to 59.39 mg/dl.

Hasanudin *et al*. (2013) stated that LDL plays a role in providing cholesterol in body tissues because it is the primary carrier for cholesterol from the liver to body tissues. Table 3 shows the LDL level of sheep blood ranged from 14.40±2.61 – 19.22±5.43 mg/dl. This level was still lower than Prayitno and Heni (2021) research results, 31.78±1.16 – 43.94±15.36 mg/dl. Supported by Hasanudin *et al*. (2013) that there is a positive correlation between LDL and cholesterol. LDL plays a role in providing cholesterol in body tissues because it is the primary carrier for cholesterol from the liver to body tissues. The decrease in LDL was thought to be due to unsaturated fatty acids contained in protected Lemuru fish oil, which increased LDL catabolism's speed. This was supported by the statement of Pramitasari *et al.* (2012) that several hypotheses have explained the effect of unsaturated fatty acids in the form of stimulating cholesterol excretion into the intestine and stimulating the cholesterol oxidation becomes bile acids. LDL is often called bad fat, so its level must be lower than HDL.

The glucose levels in this study were still in the normal range, namely 55.22±11.09 to 54.65±8.19. According to Cynthia and Scott (2005), the normal level of sheep blood glucose is 44 – 81 mg/dL. Glucose serves as the fastest source of energy to be used as ATP for both major organs such as the brain and nervous system and other organs whose role cannot be replaced by other nutrients (Astuti *et al.,* 2006). The glucose level in this study was not affected by the addition of 5% and 10% protected Lemuru fish oil. The profile and blood metabolites level did not differ and were still in the normal range, a positive indication that the addition of 5% and 10% protected Lemuru fish oil did not affect the process of blood components formation and absorption of nutrients from metabolism, which indirectly also indicated that

Table 4. The average sheep blood profile levels

Blood profile	Treatment		
	P0	P1	P2
	----------mg/dl----------		
Cholesterol	63.73±4.92	66.68±16.69	52.95±8.66
Triglycerides (mg/dl)	23.68±5.64	24.95±4.20	33.40±7.02
HDL (mg/dl)	38.3±5.94	41.4±6.65	34.7±1.97
LDL (mg/dl)	17.45±0.34	19.22±5.43	14.40±2.61
Glucose	55.52±13.69	54.65±8.19	55.22±11.09

The analysis result from Laboratorium Penelitian dan Pengujian Terpadu (LPPT) UGM.

the health/physiological condition of sheep was not disturbed. This was in accordance with the statement of Astuti *et al.* (2008) that the hematological profile and blood metabolite status is one of the indicators that determine the physiological condition of livestock.

Conclusions

From this study, it can be concluded that 10% protected Lemuru fish oil supplementation in the TMR (P2) showed the best in vivo crude fiber digestibility and crude fat digestibility compared to P0 and P1 treatments. The addition of protected Lemuru fish oil in the TMR ration had no significant effect on the digestibility of the dry matter, organic matter, and crude protein. In general, the supplementation of protected Lemuru fish oil did not disturb the sheep's physiological condition, as reflected by the normal blood profile of the treated sheep.

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