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## Physicochemical Profiles of Goat Meat: Influence of Unsaturated Fatty Acid-Enriched Diets

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### ABSTRACT

The aim of this research was to determine the physicochemical profiles of meat from goat given a diet containing unsaturated fatty acids. The material used in this research was local male goats aged  $\pm$  12 months. Treatment used in the present study was R0, as a control diet, R1: R0 added with 2.5% of tuna fish oil (TFO), R2: R0 added with 5% of tuna fish oil (TFO), R3: R0 added with 2.5% of soybean oil (SO) and treatment R4: R0 added with 5% of soybean oil (SO). This research was conducted for three months. After the feeding trial, the goats were slaughtered, and the quality of the longissimus dorsi was analyzed. The research design used was a completely random design and four replications. The results showed that the feed containing TFO and SO at a level of 2.5% - 5% did not cause a significant difference ( $p > 0.05$ ) in the carcass components and the physical quality of the meat. Meanwhile, other parameters such as meat cholesterol, stearic acid, oleic acid, and linoleic acid were affected significantly ( $p < 0.05$ ). It should be concluded that the giving 2.5% - 5% of feed containing an unsaturated fatty acids profile (tuna fish oil and soybean oil) has not shown changes in the physicochemical quality of meat. But at the level of tuna oil and soybean oil, up to 5% of dry matter are able to increase the unsaturated fatty acid profile in meat.

Keywords: *Unsaturated fatty acids, Physicochemical, Goat meat, Diets*

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### Introduction

Society often perceives goat meat as a healthier alternative to other red meats. However, it is crucial to recognize that despite its several advantages, an important aspect of goat meat is its saturated fatty acid (SFA) content. According to research conducted by Mirdhayati *et al.* (2014), goat meat comprises approximately 28-29% saturated fatty acids of its total fat content, primarily consisting of palmitic acid, stearic acid, and myristic acid. High consumption of saturated fatty acids can contribute to a range of health issues, such as cardiovascular diseases. Data from the World Health Organization (WHO) indicates that excessive intake of saturated fatty acids may elevate LDL cholesterol (often referred to as "bad" cholesterol) levels in the bloodstream, posing a risk for atherosclerosis.

Fatty acid biohydrogenation is the mechanism responsible for the ruminant meat's content of saturated fatty acids. During rumen fermentation, the hydrogenation process converts unsaturated fatty acids into their saturated counterparts (Baumann and Hanzelmann, 2018; Toral *et al.*, 2024). There exist various strategies mitigate the hydrogenation in ruminant livestock.

One such approach involves reducing methane production during ruminal fermentation. As indicated by Sondakh *et al.* (2015), suppression of methane production inhibits methanogenic bacteria from utilizing hydrogen (H) to form methane. The hydrogen (H) that is not used for methane synthesis can be redirected to enhance the accumulation of propionate.

Numerous studies have explored the enhancement of propionate as a consequence of decreased methane during ruminal fermentation, particularly focusing on the supplementation of feed sources rich in unsaturated fatty acids. In their *in vitro* research, Sondakh *et al.* (2017) examined the effects of tuna oil and soybean oil as sources of unsaturated fatty acids, revealing that both oils can elevate propionate levels in ruminal fluid. Moreover, Sondakh *et al.* (2015) noted that an increase in propionate signifies a suppression of the biohydrogenation process of fatty acids. However, these investigations are primarily confined to *in vitro* studies, and there remains a dearth of in-depth research concerning the implications of propionate enhancement attributed to tuna oil and soybean oil-rich sources of unsaturated fatty acids on the quality of the resulting meat. While considerable research has

been conducted on the content of unsaturated fatty acids in feed, it has mainly focused on non-ruminant livestock that do not undergo biohydrogenation. An intriguing aspect of this study lies in the examination of unsaturated fatty acids in feed and their effects on the physicochemical qualities of ruminant meat, a topic that has yet to be extensively explored.

The purpose of this investigation is to determine the physicochemical profiles of meat from goat given a diet containing unsaturated fatty acids.

## Materials and Methods

The material used was 20 local goats (*Capra aegagrus hircus*), aged twelve months and weighing approximately 12 kg.

### Dietary treatment

The feed consists of grass and concentrate, with a comparison of 60%: 40% of tuna fish oil (TFO) and soybean oil (SO). The concentrate given consisted of soybean cake and rice bran. Treatment used in the present study was R0, as a control diet, R1: R0 added with 2.5% of TFO; R2: R0 added with 5% of TFO; R3: R0 added with 2.5% of SO; and treatment R4: R0 added with 5% of SO.

The study was conducted in several stages, including preparation, adaptation, and raising, which included data collection and sample analysis. TFO and SO were produced chemically using nitrogen (N) extraction method (AOAC, 2019). These oils were used as the treatment material.

### Procedure research

Goats were weighed and grouped on each drill for feed adaptation and environmental conditions. Each treatment consisted of four goats or replication. The adaptation stage was carried out for two weeks with the aim that the goat could adapt to the given feed and the existing environmental conditions.

The next stage was the maintenance phase. At this stage, raising was done until the goat would be slaughtered. This stage was conducted for ten weeks. Slaughtering was done to all the experimental goats (twenty goats).

### Meat analysis

*Longissimus dorsi* muscle was sampled for analysis of meat quality. Meat quality assessment encompasses the chemical properties of the meat, which include fat, protein, moisture, and cholesterol; fatty acid content of meat; and quality of physical properties of meat consisting of pH, tenderness, and water holding capacity, drip loss (Yuan *et al.*, 2012). Moisture, protein, fat, and cholesterol were determined according to AOAC (2019). Fatty acids of meat were analyzed with gas chromatography (AOAC, 2019). Muscle pH was measured using a pH meter (AOAC, 2019). Tenderness was measured in terms of using the Warner-Bratzler Shear Force, kg/cm<sup>2</sup> (Soeparno, 2016).

### Statistical analysis

To investigate the data, we employed variance analysis. With four replications and five treatments, this study used a completely randomized experimental design. Should an analysis reveal a discrepancy, the analysis would proceed using the contrast orthogonal test (Torrie and Steel, 1980).

## Results and Discussion

### Carcass characteristic

This result of the studies, the variables measured as listed in Table 1 consisted of feed intake, initial weight, slaughter weight, ADG, FCR, carcass weight, lean, bone, fat, The thickness of back fat, dressing percentage, and rib eye area did not exhibit statistical significance ( $p < 0.05$ ). Utilizing soybean oil and tuna fish oil in the ration has not caused a change in carcass characteristics.

Table 1. The average of growth performance and quality of carcass in goat fed tuna fish oil (TFO) and soybean (SO) in ration

| Variables                       | Treatments |             |              |             |             |
|---------------------------------|------------|-------------|--------------|-------------|-------------|
|                                 | R0         | R1          | R2           | R3          | R4          |
| Feed intake (g/day)             | 493± 4.24  | 498.5± 4.50 | 501.25± 3.59 | 494.75±4.57 | 499.75±3.30 |
| ADG (g)                         | 53.32±0.61 | 54.71±1.26  | 54.79±1.15   | 52.88±1.17  | 54.50±1.29  |
| Feed conversion ratio           | 6.49±0.10  | 6.59±0.20   | 6.56±0.17    | 6.41±0.17   | 6.54±0.17   |
| Carcass weight (kg)             | 10.83±0.47 | 11.09±0.10  | 11.4±0.13    | 11.19±0.16  | 11.50±0.49  |
| Dressing percentage (%)         | 49.75±2.27 | 50.57±1.33  | 52.60±1.08   | 50.88±0.84  | 52.69±2.11  |
| lean (kg)                       | 7.43±0.49  | 7.62±0.39   | 7.84±0.32    | 7.74±0.60   | 7.95±0.13   |
| Bone (kg)                       | 2.12±0.09  | 2.08±0.09   | 2.06±0.05    | 2.19±0.05   | 2.12±0.09   |
| fat (kg)                        | 1.17±0.13  | 1.39±0.15   | 1.49±0.07    | 1.26±0.07   | 1.43±0.11   |
| lean (%)                        | 68.59±0.49 | 68.66±0.43  | 68.79±0.32   | 69.15±0.60  | 69.19±0.13  |
| fat (%)                         | 10.81±1.13 | 12.52±0.63  | 13.09±0.65   | 11.27±0.71  | 12.32±0.84  |
| bone (%)                        | 19.59±0.95 | 18.81±0.51  | 18.12±0.49   | 19.57±0.88  | 18.49±1.31  |
| Back fat thickness (mm)         | 1.95±0.02  | 2.03±0.12   | 2.12±0.16    | 1.97±0.14   | 2.06±0.03   |
| Rib eye area (cm <sup>2</sup> ) | 4.34±0.36  | 5.46±0.31   | 5.53±0.48    | 5.24±0.36   | 5.28±0.42   |

Note: R0: as control diet, R1: R0 added with 2.5% TFO, R2: R0 added with 5% TFO, R3: R0 added with 2.5% SO and treatment R4: R0 added with 5% SO. ADG=average daily gain

Optimal nutrition, the primary determinants of weight gain in animals are intake

and digestibility. Feed composition, availability, palatability, and feedback mechanisms all affect

how much feed livestock eat (Yousuf *et al.*, 2014). Feed efficiency of animals is an important factor that determines profitability in livestock production systems. The insignificant differences in consumption, weight gain, feed conversion, carcass production, and carcass quality indicated a similarity in growth performance. This indicates that the balance of energy and crude protein from the feed is sufficient. It also demonstrated that there was no negative impact on the growth of goats fed TFO and SO. Majewska *et al.* (2016) have reported that the use of sunflower oil containing high saturated fatty acids did not cause changes in carcass weight and carcass percentage. As Roy *et al.* (2013) discovered, using vegetable oils up to a level of 4.5% had not caused a change in the percentage of goat carcasses.

Feeding containing tuna fish oil and soybean oil up to 5% has not changed the rib-eye and back fat thickness. Feeding containing

unsaturated fatty acids has been investigated by Miltko *et al.* (2019), which uses vegetable oil as an unsaturated fatty acid source, and the outcome had no bearing on the thickness or the slaughter weight of the back fat and hot carcass weight. According to Park *et al.* (2018), back fat thickness is predominantly influenced by breed, management practices, the age of the livestock, and animal nutrition.

### Physical and chemical quality of meat pH value

The study's findings indicated that the meat's pH fell between 5.95 and 6.01, as indicated in Table 2. There wasn't a significant difference, according to the analysis of variance in the pH values of all treatments. Likewise, there was no difference in the pH value of the goats fed with the control treatment (R0).

Table 2. The average of physical quality of meat goat that given tuna fish oil and soybean oil in ration

| Variables                        | Treatments |            |            |            |            |
|----------------------------------|------------|------------|------------|------------|------------|
|                                  | R0         | R1         | R2         | R3         | R4         |
| pH                               | 5.95±0.06  | 6.01±0.04  | 5.95±0.04  | 6.00±0.02  | 6.01±0.08  |
| Water Holding Capacity (%)       | 43.52±0.25 | 43.08±0.66 | 43.85±0.35 | 43.29±0.12 | 43.33±0.08 |
| Tenderness (kg/cm <sup>2</sup> ) | 1.88±0.15  | 1.7±0.21   | 1.80±0.13  | 1.74±0.08  | 1.76±0.10  |
| Cooking loss (%)                 | 34.01±0.23 | 34.00±0.09 | 33.98±0.20 | 34.09±0.12 | 33.59±0.16 |

Note: R0: as control diet, R1: R0 added with 2.5% TFO, R2: R0 added with 5% TFO, R3: R0 added with 2.5% SO and treatment R4: R0 added with 5% SO

According to Soeparno (2016), Meat has a pH between 5.4 and 5.8. Meat's high glycogen content is what determines the pH level. Arshad *et al.* (2018) claim that meat glycogen has a major effect on the pH of meat. The quantity of glycogen in the meat will decompose by glycolytic enzymes into lactic acid, resulting in a low pH.

### Water holding capacity (WHC)

Results of WHC value can be seen in Table 2. Treatment of feed containing tuna fish oil and soybean oil has not resulted in changes in WHC value compared to feed without treatment. Likewise, the WHC value of meat from goats fed with tuna oil is no different from that of meat containing soybean oil.

No effect on WHC of meat was found in this study, probably due to a relatively similar pH of meat for all treatments. The pH level of the meat greatly influences the WHC. Major influences on WHC in meat include muscle pH and protein denaturation (Li *et al.*, 2017). As the final pH rises, the higher the binding the water, the smaller the free water percentage. The rate of postmortem pH decrease will affect the water binding (Berahun *et al.*, 2022).

### Tenderness

The study's tenderness value was in the range of 1.7-1.88 as listed in Table 2. The meat tenderness of goats that are fed with tuna oil and soybean oil of 2.5% - 5% was in the range of 1.7-1.80 and 1.74-1.76, respectively. The results showed no difference between the outcomes of the variance analysis and the two treatments.

Likewise, this value did not differ from goats given the control diet (R0) as well.

No different texture of meat from goats fed on tuna and soybean oil, probably due to a relatively similar pH of meat for all treatments. The meat's tenderness highly depends on how acidic the meat is. According to Bhat *et al.* (2018), the following factors affect how tender the meat is: the enzymes calpain and cathepsin. These enzymes worked at a certain pH. Saturno *et al.* (2020) stated that treatments, Diets had no discernible impact on the goat's tenderness of goats given feed supplemented.

### Drip loss

The results of the percentage of drip loss can be seen in Table 2. Data showed that the goats fed on tuna oil had the same effect as that of soybean oil. Ration containing both tuna fish oil and soybean oil has not provided changes in the drip loss when compared to the control treatment. Feeds containing tuna oil and soybean oil have not affected the cooking losses of meat. Meat cooking losses in this study were relatively similar; this was presumptively due to the composition of crude fat (Table 3), which was relatively similar.

In this study, cooking loss in lamb given different amounts of supplemental fatty acid soaps NaOH concentrations was 34.18±3.86 to 38.05±0.62%, which is less than that of Setyaningrum *et al.* (2015).

### Moisture

Data on the moisture Table 3 shows the amount of meat. The outcome demonstrated that goats fed soybean and tuna fish oils did not

significantly affect the meat's moisture content. Likewise, the control ration still has a relatively the same effect on the moisture of meat as that of both tuna oil and soybean oil. Moisture of meat given

tuna oil and soybean oil with a level of 2.5 - 5%, and with the control diet ranged between 73.01 - 73.33.

Table 3. The average of chemical quality of meat goat that given tuna fish oil and soybean oil in ration

| Variables   | Treatments               |                          |                          |                          |                          |
|-------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|             | R0                       | R1                       | R2                       | R3                       | R4                       |
| Moisture    | 73.00±0.11               | 73.09±0.13               | 73.11±0.21               | 73.27±0.30               | 73.33±0.22               |
| Protein     | 18.47±0.45               | 18.31±0.08               | 18.67±0.24               | 18.75±0.21               | 18.62±0.14               |
| Lipid       | 5.2±0.31                 | 5.10±0.40                | 4.95±0.36                | 4.99±0.27                | 5.01±0.10                |
| Cholesterol | 80.50 <sup>b</sup> ±0.66 | 78.43 <sup>a</sup> ±0.86 | 77.75 <sup>a</sup> ±0.41 | 78.05 <sup>a</sup> ±0.48 | 77.32 <sup>a</sup> ±0.28 |

Note: R0: as control diet, R1: R0 added with 2.5% TFO, R2: R0 added with 5% TFO, R3: R0 added with 2.5% SO and treatment R4: R0 added with 5% SO. Superscript in the same row shows significantly different ( $p < 0.05$ )

This water content conforms to Soeparno's (2016) assertion that meat has a water content ranging from 68% to 75%. Meat's water content was more impacted by livestock dehydration rate before slaughtering, environmental and genetic factors, and age conditions.

### Crude protein

Data in Table 3 shows the meat's crude protein content. The investigation findings into meat protein content from goats given tuna oil and soybean oil are still relatively the same. Likewise, between the treatment ration and the control ration, there was no discernible difference between the tuna and soybean oil.

### Crude fat

The results of research on meat fat Table 3 displays the content. The findings indicated that there was no discernible variation in meat fat between the control and treatment rations. Likewise, there was no difference between rations of tuna and soybean oil. Rations containing unsaturated fatty acid substrate, tuna fish oil, and soybean oil have not changed in fat content. The lipid content in this study ranged from 4.9% to 5.2%..

### Cholesterol levels

In this study, there was a change in meat cholesterol content as shown in Table 3. Meat

cholesterol from animals fed with tuna oil and soybean oil was significantly lower than meat cholesterol from that of control rations.

Yaseen *et al.* (2021) reported a decrease in meat cholesterol caused by animals consuming vegetable oil in their diet. In rumen fermentation, there is a relationship between decreased methane and the meat cholesterol. Sondakh *et al.* (2017) that feed containing tuna fish and soybean oil significantly increases the propionic acid content of meat in *in vitro* rumen fermentation.

Furthermore, Sondakh *et al.* (2017) assert that the elevation of propionate leads to a reduction in the cholesterol content of meat. The cholesterol of meat in this study is higher than that of Adeyemi *et al.* (2015), which ranges from 45.96 – 50.63 mg/100 g in goats that were given a dietary blend of palm and canola oils.

### Fatty acid profile

The study's findings can be seen in Table 4. Feed containing up to 5% tuna fish oil (TFO) and up to 5% soybean oil (SO) had no effect on lauric, myristic, palmitic, palmitoleic, arachidic, and linolenic acids of meat ( $p > 0.05$ ). Meanwhile, stearic, oleic, linoleic, eicosapentaenoic, and docosahexaenoic had a significant effect when goats were fed with TFO and SO ( $p < 0.05$ ). Feed containing 5% of TFO and 2.5% of SO had higher oleic acid and linoleic fatty acid than the control diet ( $p < 0.05$ ). The same feed composition can reduce the stearic acid content of meat.

Table 4. Profile of fatty acids of meat goat that given tuna fish oil and soybean oil in ration

| Fatty acids                | Treatments               |                            |                            |                            |                            |
|----------------------------|--------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                            | R0                       | R1                         | R2                         | R3                         | R4                         |
|                            | g/100 g crude fat        |                            |                            |                            |                            |
| Lauric (C12:0)             | 0.10±0.01                | 0.12±0.01                  | 0.14±0.02                  | 0.13±0.03                  | 0.12±0.01                  |
| Myristic (C14:0)           | 2.28±0.04                | 2.25±0.03                  | 2.27±0.02                  | 2.29±0.04                  | 2.24±0.07                  |
| Palmitic (C16:0)           | 14.10±0.10               | 14.14±0.20                 | 14.12±0.46                 | 14.06±0.17                 | 14.10±0.29                 |
| Palmitoleic (C16:1)        | 0.18±0.01                | 0.19±0.02                  | 0.19±0.02                  | 0.20±0.01                  | 0.19±0.02                  |
| Stearic (C18:0)            | 3.39 <sup>b</sup> ±0.66  | 2.32 <sup>a</sup> ±0.28    | 2.42 <sup>a</sup> ±0.08    | 2.41 <sup>a</sup> ±0.10    | 2.89 <sup>a</sup> ±0.17    |
| Oleic (C18:1)              | 15.77 <sup>a</sup> ±0.18 | 16.42 <sup>ab</sup> ±0.96  | 17.36 <sup>bcd</sup> ±0.08 | 18.81 <sup>d</sup> ±0.29   | 17.08 <sup>abc</sup> ±0.22 |
| Linoleic (C18:2)           | 4.87 <sup>a</sup> ±0.30  | 5.28 <sup>a</sup> ±0.14    | 5.69 <sup>c</sup> ±0.38    | 5.53 <sup>bc</sup> ±0.44   | 5.49 <sup>b</sup> ±0.25    |
| Linolenic (C18:3)          | 0.28±0.05                | 0.31±0.08                  | 0.32±0.07                  | 0.33±0.03                  | 0.28±0.04                  |
| Arachidic (20:0)           | 0.12±0.01                | 0.12±0.01                  | 0.11±0.02                  | 0.11±0.01                  | 0.11±0.01                  |
| Eikosapentaenoic (C20:5n3) | 0.18 <sup>a</sup> ±0.02  | 0.20 <sup>ab</sup> ±0.02   | 0.21 <sup>b</sup> ±0.01    | 0.22 <sup>b</sup> ±0.02    | 0.23 <sup>bc</sup> ±0.02   |
| Dokosaheksaenoic (C22:6n3) | 0.14 <sup>a</sup> ±0.02  | 0.16 <sup>b</sup> ±0.02    | 0.15 <sup>b</sup> ±0.02    | 0.16 <sup>b</sup> ±0.02    | 0.16 <sup>b</sup> ±0.02    |
| Total of fatty acids       | 59.29±0.94               | 58.74±3.06                 | 60.23±0.73                 | 61.49±1.46                 | 60.23±0.83                 |
| Total SFA                  | 38.56 <sup>d</sup> ±0.73 | 36.95 <sup>bcd</sup> ±0.29 | 33.07 <sup>bcd</sup> ±0.56 | 34.02 <sup>b</sup> ±0.16   | 37.47 <sup>a</sup> ±0.18   |
| Total UFA                  | 21.35 <sup>a</sup> ±1.61 | 22.46 <sup>a</sup> ±1.19   | 23.82 <sup>bc</sup> ±0.87  | 24.16 <sup>bcd</sup> ±0.98 | 23.44 <sup>bc</sup> ±0.43  |



| SFA/UFA | 1.79±0.11  | 1.64±0.08 | 1.39±0.06 | 1.41±0.03 | 1.59±0.03 |
|---------|--|-----------|-----------|-----------|-----------|
| Note:   | R0: as control diet, R1: R0 added with 2.5% TFO, R2: R0 added with 5% TFO, R3: R0 added with 2.5% SO and treatment R4: R0 added with 5% SO. Superscript in the same row shows significantly different (p<0.05) |           |           |           |           |

Likewise, levels up to 5% tuna fish oil and soybean oil cause a decrease in stearic acid, which in turn causes the saturated fatty acids to decrease in meat. In ruminant livestock, the process of hydrogenation occurs when cattle consume polyunsaturated fats. Unsaturated fatty acids consumed will be converted into saturated fatty acids, especially on the c-18 chain, through a process known as trans-cis hydrogenation (Mauger *et al.*, 2021). The current study demonstrated that feeding fish oil and soybean oil to animals could prevent the production of stearate and palmitic acid because the unsaturated fatty acids could withstand the hydrogenation process. Similarly, oleic acid increased its production in meat. This increase was also due to the feed given contained unsaturated fatty acid substrate. Unsaturated fatty acids in animal rations were inhibited by the hydrogenation process. Sondakh *et al.* (2017) state that unsaturated fatty acids can withstand the hydrogenation process because they are capable of producing high amounts of propionic acid. It was also confirmed by Sondakh *et al.* (2012) that increases in propionic acid in rumen fermentation result in increased unsaturated fats, particularly linoleic and oleic, in sheep meat.

Additionally, the decrease in the content of stearate and palmitate was due to saturated fats (SFA). Stearic acid predominates as the saturated fat type in goat meat. Similarly, an increase in the quantity of linoleic and oleic fatty acids led to an increase in total unsaturated fatty acids (UFA). Miltko *et al.* (2019) found that the use of vegetable oil in the ration can decrease the SFA of lambs.

In this study, the proportion of acid that is unsaturated compared to saturated has dramatically dropped. Because oleic and linoleic acid levels rise with stearate production, unsaturated fatty acid-containing substrates like soybean oil and fish oil can lower palmitic acid, the proportion of saturated to unsaturated fatty acids in goat meat.

According to Bond *et al.* (2016), the decrease in SFA in muscles was most likely brought on by a greater proportion of exogenous FA in the metabolic pool, which inhibited de novo synthesis. According to Ameer *et al.* (2014), it is a complicated and tightly controlled metabolic process. According to this study, the biohydrogenation process may also be responsible for decreased SFA in the muscles taken from goats fed soybean and tuna fish oil. According to Gallardo *et al.* (2015), providing UFA supplementation (linseed oil) in ration causes a decrease in the SFA content of the lamb's biceps femoris and longissimus dorsi muscles. According to Urrutia *et al.* (2015), there were no appreciable variations in the SFA value of the fed lambs' muscle diets varying in the amount of unsaturated fatty acid (linseed oil).

## Conclusion

The giving 2.5% – 5% of feed containing unsaturated fatty acids (tuna fish oil and soybean oil) has not shown changes in the meat's chemical and physical characteristics. However, at 2.5% to 5%, soybean oil and tuna oil can raise the quantity of the meat's unsaturated fatty acids.

## Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

## Author's contribution

EHB, JK, and MW conceived and designed the experiments. EHB performed the experiments. All authors analyzed the data. MS and FR writing of the manuscript. HL translating of the manuscript. All authors critically revised the manuscript and approved the final version.

## Ethics approval

All the animal care management and procedures were approved by Animal Ethical Clearance Fakultas Peternakan Universitas Sebelas Maret, No. 14/UN27.20/PT01.01/2023.

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