Chemical characteristic and cholesterol level of local sheep with intensive fattening

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Abstract. Red meat is a livestock product with high nutrition. Red meat obtained from sheep had high fat and cholesterol content. Fat and cholesterol in the body of livestock could be reduced by feed manipulation through the gastrointestinal system approach. This research was aimed to examine the effect of feed containing shrimp waste on unsaturated fat and fatty acids, blood cholesterol, internal fat, and subcutaneous fat of local sheep in Bogor. The research was designed using Completely Randomized Design with four feed formulas as the treatments of four replicates. Sheep were slaughtered after being kept in individual cages for 3 months. Further, the percentage of internal fat, total cholesterol, triglycerides, HDL and LDL in the blood were analyzed. The parameters measured were the percentage of internal fat and the iodine number of subcutaneous fat, total cholesterol, triglycerides, HDL and LDL level in the blood. The results showed that the higher percentage of shrimp waste reduced blood cholesterol, triglycerides, and LDL level and increased blood HDL level and iodine number (unsaturated fat) of lamb meat.

1. Introduction

Red meat is a livestock product with high nutritional value. Red meat can be obtained from cattle, buffaloes, goats, sheeps, camels, horses and others. Sheep is a potential red meat producer [1]. Lamb meat has a high fat and cholesterol content, Aberle et al. [2] stated that fat content in beef, lamb, pork and chicken meat were 6.16, 7.0, 6.75 and 1.2 mg/100g, respectively; while Lawrie [3] stated that cholesterol content in beef, lamb and pork were 59, 79 and 69 mg / 100g, respectively. Nowadays, public awareness had increased for fat and cholesterol consumption, including those originated from meat that contains lots of fat such as lamb meat [4]. Excessive consumption of lamb can lead to coronary heart disease, cancer, diabetes, and high blood pressure and atherosclerosis [5]. Many efforts have been made to reduce fat and cholesterol levels in meat. The feed manipulation with the gastrointestinal system approach is one of which, with the aim of removing fat and cholesterol through excreta. The mechanism of action of the presence of fibers in the digestive tract is to bind most of the bile salts to excretion. Once most bile salts are released, the body needs to synthesize bile salts derived from the body's cholesterol, this can reduce the body cholesterol.

Shrimp waste contains high chitin, resembles cellulose and is analogous to a fiber that can bind bile acids and further, the emulsified fatty acids are also bound, cannot be absorbed and eventually released through feces. This process can reduce the cholesterol content in the muscles of livestock and lead to low
cholesterol meat product. Shrimp waste not only contains high fiber but also has a fairly high crude protein content of 41.58% and metabolic energy of 2427 kcal/kg [6], making it available for protein source in animal feed [7]. Researches on the use of shrimp waste as ruminant feed ingredients had not been widely done. In mice feed, 15% and 20% of shrimp waste can increase body weight and reduce levels of LDL (low-density lipoprotein), both in male and female.

This research was aimed to examine the effect of feed containing shrimp waste on unsaturated lipids and fatty acids, and cholesterol in serum, internal fat, and subcutaneous fat of local sheep in Bogor, Indonesia. Shrimp waste in the feed was used as a source of fiber and chitin content was expected to reduce fat and cholesterol content in sheep.

2. Materials and methods

2.1. Shrimp waste preparation
Shrimp waste was taken from the shrimp freezing company in Muara Baru, Cilincing District, North Jakarta, and transported using a cool box containing ice gel. The waste was then dried and milled to make flour. The flour was then hydrolyzed by autoclaving at a temperature of 121 °C with a pressure of 1 Atm for 6 hours, then mixed with feed ingredients provided (table 1) and made into 8 mm pellets.

2.2. Livestock management
Sixteen local rams from Jonggol Animal Husbandry Education and Research Unit, Bogor Agricultural University (UP3J-IPB) were used in this research. The rams were 8 months old of age and 15 kg of body weight. The animals were kept in 1.0m x 1.2m x 0.75m individual cage and were given the anthelmintic drug to prevent bias and vitamins to reduce stress. The first week was cage and feed adaptation (preliminary period).

Weighing body weight is done every 2 weeks. The feed consisted of four levels of shrimp waste flour, with 0%, 10%, 20%, and 30% of concentration. Feed nutrients (table 1) were adjusted to ram protein needs 14.7% and metabolism energy 2,500 kcal/kg [8]. Feed and drinking water were given ad libitum. Feed quantity was weighed before given and the unconsumed feed was weighed the day after. Body weight evaluation was conducted every two weeks.

2.3. Animal slaughtering
The rams were slaughtered after being kept for 3 months. Body weights measurement were done previously to determine the slaughtering weight. Carcass and non-carcass marking and cutting were done shortly after slaughter. Further, cholesterol, triglycerides, HDL, and LDL level were measured [9].

2.4. Measured variables
Cholesterol, triglycerides, HDL and LDL levels of serum and triglyceride and subcutaneous fat cholesterol levels. Abdominal fat percentage was calculated based on the total amount of internal fat contained in the body, divided by the empty weight, then multiplied by 100%. Subcutaneous fat is analyzed as follows: (1) analysis of total cholesterol, triglycerides and HDL using the Kit and Humalyser Spectrophotometry method [9]; and (2) analysis of saturated and unsaturated fats using the method of counting iodine number/Hanus method [10].

2.5. Data analysis
This research was designed using Completely Randomized Design with four feed formulas as the treatments of four replicates. The data obtained were analyzed using analysis of variance (ANOVA), followed by the Duncan test according to the Steel and Torrie procedure [11].
Table 1. Feed Composition and Nutrition

| Raw materials          | Feed (kg) | P0 | P1 | P2 | P3 |
|------------------------|-----------|----|----|----|----|
| Natural grass          | 40.0      | 40.0| 40.0| 40.0|
| Shrimp waste           | 0.0       | 10.0| 20.0| 30.0|
| Soybean mill           | 13.5      | 9.0 | 4.5 | 0.0 |
| Molasses               | 15.0      | 15.0| 15.0| 15.0|
| Corn                   | 2.8       | 0.5 | 2.4 | 4.7 |
| Pollard                | 2.8       | 3.4 | 1.9 | 0.3 |
| Onggok                 | 18.6      | 14.3| 7.4 | 0.2 |
| Crude Palm Oil (CPO)   | 3.9       | 4.9 | 5.9 | 6.9 |
| Urea                   | 2.0       | 2.0 | 2.0 | 2.0 |
| Salt (NaCl)            | 0.4       | 0.4 | 0.4 | 0.4 |
| Calcium Carbonate (CaCO3) | 0.5   | 0.0 | 0.0 | 0.0 |
| Premix                 | 0.5       | 0.5 | 0.5 | 0.5 |
| TOTAL                  | 100.0     | 100.0| 100.0| 100.0|

Nutrition (%):

|                     | P0  | P1  | P2  | P3  |
|---------------------|-----|-----|-----|-----|
| Crude Protein       | 14.70| 14.70| 14.70| 14.70|
| Crude Fiber         | 13.51| 15.26| 16.86| 18.39|
| Crude Fat           | 5.74 | 7.01 | 8.61 | 10.28|
| Calsium             | 0.89 | 1.34 | 2.26 | 3.16 |
| Phosphor            | 0.44 | 0.52 | 0.59 | 0.67 |
| Metabolic Energy (Kkal/Kg) | 2.501 | 2.503 | 2.500 | 2.500 |

Description: P0=0% of shrimp waste flour, P1=10% of shrimp waste flour, P2=20% of shrimp waste flour, and P3=30% of shrimp waste flour

3. Results

3.1. Fat

Fat consists of C, H and O elements which were not soluble in water, but soluble in organic matter such as ether, spirit petroleum, hexane, chloroform. Fat also had functioned as a solvent for vitamins such as vitamins A and D, E and K. In general, fat was defined as triglycerides which are in conditions of solid space temperature, especially fat in meat. Fat accumulation may occur if the energy consumed exceeds energy for metabolism. The fat was stored in adipose tissue as an energy reserve. Cholesterol was a typical fat substance that results in metabolism, found in many human and animal body structures. Cholesterol was therefore widely found in foods that come from animals such as meat, brain liver and egg yolk [12]. Approximately half of the cholesterol needs came from the body's own biosynthesis that takes place in the intestine, skin and especially in the liver (approximately 50%), the rest of the cholesterol was taken from food consumed.
Table 2. Blood serum fat and cholesterol levels in local rams

| Variable          | Treatments |
|-------------------|------------|
|                   | P0       | P1       | P2       | P3       |
| Internal fat (%)  | 5.33     | 5.02     | 4.22     | 4.77     |
| Subcutaneous fat: |          |          |          |          |
| Cholesterol (mg/g)| 0.53     | 0.41     | 0.41     | 0.20     |
| Triglyceride (mg/g)| 2.55  | 1.81     | 1.18     | 0.71     |
| Blood serum:      |          |          |          |          |
| Cholesterol (mg/dl)| 112.10a | 97.22b   | 89.04c   | 84.49c   |
| Triglyceride (mg/dl)| 40.44a| 35.489b  | 29.371c  | 25.732d  |
| HDL (mg/dl)       | 54.887b  | 58.831a  | 59.392a  | 59.392a  |
| LDL (mg/dl)       | 49.129a  | 31.29b   | 23.777c  | 19.7c    |

The number followed by the different letter on the same row showed significant different (p<0.05).

P0=0% of shrimp waste flour, P1=10% of shrimp waste flour, P2=20% of shrimp waste flour, and P3=30% of shrimp waste flour

The average percentage of internal fat and triglyceride levels of total cholesterol in subcutaneous fat of local rams was shown in table 2. Statistical analysis showed that feeding the sheep with different levels of shrimp waste had no significant effect on the percentage of internal fat, total cholesterol, and triglycerides of subcutaneous fat. This might be caused by the type of feed used for all treatments having the same energy and protein levels and according to the NRC recommendations [8]. Nutrient intake still exceeds the needs of the metabolic process causing internal fat deposits.

The internal fat weight percentage based on treatments P0, P1, P2, and P3 were 5.33, 5.02, 4.22 and 4.77, respectively. The result indicated that the use of shrimp waste to the level of 30% containing chitosan had not been able to reduce internal fat significantly (p<0.05). These were caused by high protein, energy levels, and complete amino acids of shrimp waste [13]. The feed given had good quality in accordance with the standards of livestock needs [8]. Coarse energy and protein levels were the same for all treatments (iso-energy and iso-protein). Fat occurs when energy consumption exceeds the need for metabolism [14]. The excess was stored in adipose tissue in the form of free pure fat or triglycerides. While cholesterol can be synthesized alone by the body approximately 70–80% of the daily needs, the rest was obtained from food consumed. Fulfillment of nutrients for growth needs was supported by high palatability. Proven by the high KBKR, which ranges from 901.8–1007 g/head/day or about 4% of body weight equivalent to dry matter. This was higher compared to the results of Hudallah et al. [1], which ranged from 611–651 g/head/day.

Total cholesterol, triglycerides, HDL and LDL in the blood serum of local sheep fed with different levels of shrimp waste were showed in table 2. The average cholesterol levels of sheep based on treatments P0, P1, P2, and P3 were 112.1 (mg/dl), 97.22 (mg/dl), 89.04 (mg/dl) and 84.49 (mg/dl), respectively. The results of the statistical analysis showed that the feed intake with different levels of shrimp waste had a significant effect (P<0.05) on total serum blood cholesterol in local sheep as well as on triglycerides, HDL and LDL. The total control cholesterol level or P0 was significantly higher than P1, P2 and P3, as well as P1 was significantly higher than P2 and P3, whereas between P2 and P3 was not significantly different. Triglyceride levels in each treatment were significantly different (P <0.05), P3 was lower than P2, P2 was lower than P1 and P1 was lower than P0. While LDL P2 was lower than P1 and P1 was lower than P0, but P2 was not significantly different from P3. Conversely, in HDL, the higher the level of shrimp waste resulted in increased HDL level. HDL P2 levels were significant (P<0.05) higher than P0. This means that the provision of shrimp waste containing chitin and chitosan was able to increase HDL levels in the blood serum of local rams. Increasing the level of shrimp waste resulted in increased levels of chitosan in the feed, which increased triglycerides, cholesterol, and lipids absorbed to be excreted.
with feces, thereby increasing fat and cholesterol in the stool. Conversely, chitosan was able to reduce the absorption of triglycerides and cholesterol in the intestine [15].

3.2. Iodine Number
Iodine number is the number of grams of iodine which can react with 100 grams of fatty acids or numbers that show the level of fat saturation. The more double bonds, the higher the iodine number. The more iodine is used, the higher degree of unsaturation since each double bond in fatty acids will bind two iodine atoms. The composition of fatty acids contained in animals and plants were measured by iodine number [10].

![Iodine Number (g/100g)](image)

Figure 1. Iodine number of fat. P0=0% of shrimp waste flour, P1=10% of shrimp waste flour, P2=20% of shrimp waste flour, and P3=30% of shrimp waste flour

The average iodine number obtained in this study was shown in figure 1. Statistical analysis showed that feeding with different levels of shrimp waste did not significantly affect the iodine number. However, the higher level of shrimp waste resulted in higher iodine number. This might be influenced by chitosan contained in the feed which was able to bind fat and excreted it through digestive tracts with feces. This result indicated that higher level of shrimp waste increased the unsaturated fat. Total cholesterol, triglycerides, and LDL P2 were significantly lower than P1 and P0 (P <0.05). This result indicated that the presence of chitosan in feed reduced cholesterol levels, triglycerides and LDL serum blood of local rams. Whereas HDL serum blood levels P2 were higher than P0, but not significantly different from P3. Chitin and chitosan have the ability to reduce fat levels, cholesterol, triglycerides, and LDL blood serum of local rams so that the use of shrimp waste in feed will produce low cholesterol lamb meat. Decreased cholesterol, triglycerides and LDL level in blood serum of local rams indicated a decrease in cholesterol, triglycerides and LDL level in lamb meat.

Chitosan had advantages compared to other fiber sources such as cellulose and agar. Chitosan could not be degraded by lipase enzymes, was insoluble in water and ordinary organic solvents but was biodegradable which could be broken down by the enzyme chitinase produced by microorganisms. Chitosan was able to bind bile acids, as of fatty acids emulsified by bile acids are bound [16]. Chitin and chitosan have the ability to reduce fat levels, cholesterol, triglycerides, and LDL blood serum of local rams so that the use of shrimp waste in feed will produce low cholesterol lamb meat. Decreased cholesterol, triglycerides and LDL level in blood serum of local rams indicated a decrease in cholesterol, triglycerides and LDL level in lamb meat. Supadmo [4] stated that serum level of cholesterol, triglycerides, and LDL always higher than those in meat.
4. Conclusion
The use of shrimp waste in local sheep feed reduced the internal fat, decreased blood cholesterol, triglycerides and LDL level of local sheep, and increased blood HDL level and unsaturated fats.

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