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THE COMPOSITION OF SATURATED AND UNSATURATED FATTY ACIDS AND CHOLESTEROL IN MEAT OF BALI BULL FATTENED USING GREENLOT SYSTEM IN SMALL HOLDER FARMING

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ABSTRACT

The objective of this research was to analyze the composition of saturated fatty acid, unsaturated fatty acids, and cholesterol in meat of Bali bull fattened using forage crops in small holder farming. The experiment was conducted for 3 months using nine Bali bull aged 2.5-3.5 years old with the initial body weight of 227-290 kg in the feedlots of Bero Sembada Farmers Group, Laen Manen Sub District, Malaka Regency, East Nusa Tenggara. The experiment was conducted based on the fattening system of local farmers in terms of feeding, housing, and health management. Feed given during the experiment was *Centrosema pubences*, *Clitoria ternatea*, fresh corn straw, king grass, *Leucaena leucocepala*, natural grass, *Pennisteum purpureum*, and *Sesbania grandiflora*. At the end of this study, cattle were slaughtered and the composition of saturated fatty acid, unsaturated fatty acids and cholesterol were measured. Data were analyzed using a descriptive statistic. Results revealed that the total of saturated fatty acid and unsaturated fatty acids in meat were 36.628% and 26.739%, respectively, while the cholesterol reached 79.18 mg/100 g of meat. In conclusion, meat of Bali bull fattened using forage crops in small holder farming has higher saturated fatty acid than unsaturated fatty acids.

Key words: Bali bull, cholesterol, greenlot fattening system, saturated and unsaturated fatty acids

ABSTRAK

Penelitian bertujuan menganalisis kandungan asam lemak jenuh dan tak jenuh serta kolesterol pada daging sapi bali jantan yang digemukkan dengan hijauan di peternakan rakyat. Penelitian dilakukan selama 3 bulan di Kelompok Tani Bero Sembada, Laen Manen Sub District, Malaka Regency, Nusa Tenggara Timur menggunakan sembilan ekor sapi bali jantan umur 2,5-3,5 tahun dan kisaran bobot badan awal 227-290 kg. Eksperimen disesuaikan dengan kebiasan para peternak dalam menggemukan sapi bali jantan baik berupa tatalaksana pakan, perkandangan maupun kesehatan. Jenis pakan yang diberikan berupa Centrosema pubences, Clitoria ternatea, jerami jagung segar, kinggras, lamtoro, rumput alam, rumput gajah, dan turi. Pada akhir penelitian ternak dipotong untuk dianalisis komposisi asam lemak jenuh, asam lemak tak jenuh, serta kandungan kolesterol dagingnya. Data dianalisis menggunakan statistik deskriptif. Hasil penelitian menunjukkan total asam lemak jenuh dan tidak jenuh daging masing-masing adalah 36,628% dan 26,739%, sedangkan kadar kolesterol daging mencapai 79,18 mg/100 g daging. Dapat disimpulkan penggemukan sapi bali jantan fase finishing menggunakan hijauan di tingkat petani peternak menghasilkan rasio asam lemak jenuh lebih tinggi dari asam lemak tak jenuh.

Kata kunci: sapi bali jantan, kolesterol, penggemukan dengan hijauan, asam lemak jenuh dan tak jenuh

INTRODUCTION

Nowadays, the annual meat demand is increase due to population growth and income raising, education level, and awareness of nutrition sufficiency. Meat is enriched with protein, amino acid, fat, mineral, and other organic components which is important for the development of body. However, several issues regarding human health must be considered particularly the composition of fatty acids in meat.

Previous study has been reported that red meat caused some physical disorders such as heart problem, hypertention, and cholesterol, which related to the higher content of saturated fatty acid than unsaturated fatty acids of meat. Therefore, it is important to regulate the meat consumption in appropriate portion since unsuitable proportions lead to a negative effect on human health (Baltušnikienė and Jukna, 2011). High consumption of saturated fatty acid (SFA) is closely related to low-density lipoprotein (LDL)-cholesterol concentration in serum, the risking factor of coronary heart disease (Keys, 1970 as cited in French *et al*., 2000).

Saturated fatty acid has stable polyhydrocarbon chain, uneasily oxidized, and turned into unsaturated fatty acid or other harmful chemicals. Saturated fatty acid is found in animal products (e.g., meat, cheese, and milk) or plant products (e.g. palm oil and palm kernel oil). Saturated fatty acid can also increase LDL cholesterol that directly increased the total of cholesterol made up of LDL and HDL (Tuminah, 2009).

Meat contains more unsaturated fatty acid than SFA. Unsaturated fatty acid is essential for human health for instance in lowering blood pressure, better nutritious content, and antioxidant. Monosaturated fatty acid (MUFA) and some polyunsaturated fatty acid (PUFA) are antithrombonenic (Ulbricht and Southgate, 1991). Ruminant fat contains higher SFA than PUFA when compared to SFA in non-ruminant fat due to hydrogenation of rumen unsaturated fatty acid load. Therefore, the strategy to increase the ratio of PUFA over SFA in intramuscular fat will eventually lead in increasing consumers perspective on healthy meat.

Cholesterol is a vital substance in body life regulation therefore it is found in all cells of the body. In human, cholesterol plays an important role both as a precursor molecule of hormones and other molecules and as a structural element of cell membranes (Baltušnikienė and Jukna, 2011). According to Tabas (2002), cholesterol, cholesterol metabolites, and immediate biosynthetic precursors of cholesterol play essential roles in cellular membrane physiology,

absorption of dietary nutrient, reproductive biology, responses of stress, salt and water balance, and metabolism of calcium. However, if the levels of cholesterol in human blood more than 250 mg/dL, it can cause a negative impact on human health (Waloyo *et al.*, 2013). Meat, specifically red meat, is commonly identified as a major source of dietary cholesterol (Baltušnikienė and Jukna, 2011).

Feed is one of common factor influenced the quality of meat. The higher the feed quality given to cattle the higher the meat quality will be produced by cattle. French *et al.* (2000) reported that cattle undergoing green diet program less than 3 months had significantly higher unsaturated fatty acid than cattle fed on concentrate feed. Moreover, level of intramuscular fat was also lower, but it contains a higher unsaturated fatty acid, omega-3 fatty acid, and conjugated linoleic acid (Bowling *et al*., 1977 as cited in Kim *et al*., 2007).

The research above is a great concern in East Nusa Tenggara (NTT) mainly in West Timor because most of farmers only gave a green diet for Bali bull during fattening without a grain or concentrate feed addition. On the other hand, composition of saturated and unsaturated fatty acid, and cholesterol levels meat of Bali bull fattened using Greenlot system in NTT has not study yet.

MATERIALS AND METHODS

The experiment was conducted within 3 months using nine Bali bull aged 2.5-3.5 years old (average 3.0±0.46 years old based on teeth estimation), the initial body weight of 227-290 kg (average 257.40±23.60 kg) in the feedlots of Bero Sembada Farmers Group, Laen Manen Sub District, Malaka Regency, NTT. The experiment adopted the fattening system of local farmers in terms of feeding, housing, and health management. Feed given during the experiment was *Centrosema pubences*, *Clitoria ternatea*, *Zea mays* fresh straw, *Pennisetum purpuroides*, *Leucaena leucocepala*, natural grass, *Pennisteum purpureum,* and *Sesbania grandiflora*. Chemicals composition of feed are presented in Table 1. The observed variables were saturated fatty acid, unsaturated fatty acid, and cholesterol levels. Equipment in feedlots comprised nine of 1.5 x 2 m individual cages, cage cleaning kit, digital animal scale -2000 kg for body weight and slaughter weight, feed scale -25 kg, bucket, and machete. Carcass data collection required hanging scale -50 kg for carcass, and utensils for cattle slaughtering and cleaving carcass and meat.

Fatty Acid and Cholesterol Measurements

Fatty acid was analyzed using gas chromatography (GC Shimadzu 9 AM), whereas cholesterol was analyzed with spectophotometer (Optima SP. 300) at 680 nm wavelength. Meat sample was taken upon slaughtering in Kefamenanu slaughter house and then subsequently stored in cool box and transported to laboratory for fatty acid and cholesterol analysis.

The One-step fatty acid methylation was conducted based on Ulberth and Henninger (1992). 100-500 mg sample (containing 10-50 mg fat) was filled into a test tube and then added 1 mL of standard C19:0, 1 mL of toluene, and 1 mL of 5% fresh HCl-methanol according to procedure described by Christie (1982). Subsequently, the mixture was thoroughly mixed, and the test tube was added with nitrogen gas, sealed, and heated for 2 hours in water bath at 70° C. A total of 5 mL of K_2C0_3 5% and 2 mL of toluene was added to test tube, vortexed, and centrifuged at 1100 rpm for 5 minutes. Organic phase was transferred into other test tube and dried using Na_2S0_4 anhydride. One microliter of supernatant was analyzed with Gas Chromatography.

In situ transesterification was carried out based on Park and Goins (1994) method. The procedure was started with mixing 2 g meat with 4 mL distilled water to obtain 100 µL homogenate which then put into test tube. In situ transesterification was performed by adding 100 µL methylene chloride and 1 mL NaOH 0.5 N in methanol. Nitrogen gas was then given followed by sealed the tube and heated in 90° C water bath for 10 minutes, then cooled and added 1 mL BF3 14% in methanol. Heating was extended for 10 minutes, and finally, the test tube was cooled at room temperature. Subsequently, 1 mL distilled water and 200-500 µL hexane was added, then vortexed for 1 minute to extract the fatty acids methyl ester (FAME). After centrifugation, the upper layer was readily subject to Gas Chromatography.

Table 1. Nutrient compositions of various forage crops given to the Bali bull during greenlot fattening system in small holder farming system

Feed	Nutrient composition (% of DM)							
	Dry matter	Organic matter	Ash	Crude protein	Crude fat	Crude fiber	NFE[6]	Energy (cal/g)[5]
Pennisetum purpuroides[3]	21.27	86.37	13.63	8.47	4.11	29.11	44.69	4007.778
Natural grass	10.30	85.52	14.48	8.98	4.99	31.72	39.83	4202.087
Pennisteum purpureum[4]	21.00	88.30	11.70	10.20	1.60	34.20	32.60	
Leucaena leucocepala[1]	29.90	91.40	8.26	25.00	11.48	14.27	66.74	4903.413
Zea mays Fresh straw[1]	18.25	89.16	10.84	11.43	11.73	26.77	39.23	3966.922
Sesbania grandiflora[2]	27.87	91.50	8.50	27.37	3.93	7.30	52.90	4378.260
Centrosema pubences[1]	36.87	92.02	7.91	10.17	8.48	35.06	38.38	4080.413
Clitoria ternatea[1]	25.33	89.41	10.59	19.98	7.98	28.53	32.92	4374.276

[1]According to analysis result of Laboratory of Feed Biochemistry, Animal Science Faculty, Gadjah Mada University. [2]Result by Laboratory of Feed Biochemistry, Animal Science Faculty, Nusa Cendana University, Kupang.[3]Average result by Laboratory of Feed Biochemistry, Animal Science Faculty, Gadjah Mada University and Laboratory of Feed Chemistry, Animal Science Faculty, Nusa Cendana University, Kupang. [4]According to Rukmana (2005). [5]According to analysis result of Food and Nutrition Research Center, Gadjah Mada University; [6]Calculation result : NFE = 100-ash-CP-EE-CF

Cholesterol analysis with Lieberman-Urchard method (Plummer, 1977). One gram meat was added with 10 mL acetone-alcohol solution (1:1), boiled and stirred in boiling water, then cooled at room temperature to filter. The filtrate was centrifuged at 2500 rpm for 15 minutes, producing supernatant that was steamed in 100° C water bath to dry, then cooled and dissolved in 3 mL chloroform, added with 3 mL acetic anhydride- thick acid sulfate (30:1), homogenized and placed in dark room for five minutes until the solution turned bluish green. Blank solution was made following the same procedure. Absorbance was indicated at 680 nm wavelength to measure the sample's cholesterol content by comparing sample's cholesterol absorbance to standard cholesterol absorbance.

To prepare pure cholesterol solution, 10 mg pure cholesterol was weighed and dissolved in 10 mL acetone-alcohol solution (1:1). Then six test tubes containing 0.4 mL, 1.8 mL, 1.2 mL, 1.6 mL, 2.0 mL, and 0.0 mL pure cholesterol solution were prepared. The solutions were crystalized in water bath, and the leftover crystal was added with 2.1 mL chloroform and 2 mL acetate anhydride-acetate acid (30:1), vortexed and transferred into dark room for 30 minutes followed by signed at 625 nm wavelength.

Data Analysis

Data were analyzed using descriptive statistics (Steel *et al*.,1997).

RESULTS AND DISCUSSION

In this study, the total of saturated fatty acid and unsaturated fatty acid was 36.628% and 26.739%, respectively. The unsaturated fatty acid contains 10.643% of MUFA and 16.096% of PUFA. On the other hand, the average of cholesterol was 0.7918 mg/g (Table 2). Fatty acids are categorized into saturated and unsaturated fatty acids besides essential fatty acid and non-essential fatty acids. Essential fatty acids cannot be synthesized in human and animal bodies eventhough it is essential for the regulation of human and animal body functions. There are three major factors that influence the fatty acid composition of beef include age of animal, diet, and breed type (Smith *et al*., 2009).

Saturated Fatty Acid (SFA)

Composition of saturated fatty acid in meat of Bali bull fattened with forage crops was presented in Table 2 and Figure 1. From 36.628% of SFA, the major composition of SFA was palmitic acid (C16:0) and stearic acid (C18:0) that is 15.302% and 16.320 %, respectively. Other compound of the SFA was 0.217% of caprilic acid (C8:0), 0.556% of capric acid (C10:0), 0.769% of lauric acid (C12:0), 2.828% of myristic acid (C14:0), and 0.637% of arachidic acid (C20:0). The results indicate that meat of Bali bull fattened with forage crops contain lower fatty acid of capilaric acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and arachidate acid (C20:0). In contrast, meat of Bali bull fattened using greenlot system contains higher fatty acid of palmitic acid (C16:0) and stearic acid (C18:0) reaches 31.622%. According to Soeparno (2009), the major sequent of SFA was C16 and C18 (molecules with 16 and 18 carbon chains) and followed by C12, C14, and C16 in smaller amount.

The total of SFA in this study is lower compared to the total of SFA from Brahman cross meat reported by Soebagyo *et al.* (2000). The total of SFA in Brahman cross meat from different fattening period (2, 3, and 4 months) was 38.43%, 38.05%, and 43.16%, respectively; while the major SFA, palmitic acid, was 30.51%, 30.05%, and 31.75%, respectively, followed

Table 2. Average composition of fatty acids (%) and cholesterol (mg/g) of Bali bull fattened using forage crops (greenlot fattening system) in small holder farming*

Variables	Means
a. Saturated fatty acid	
Capilaric acid (C8:0)	0.217
Capric acid (C10:0)	0.556
Lauric acid (C12:0)	0.769
Myristic acid (C14:0)	2.828
Palmitic acid (C16:0)	15.302
Stearic acid (C18:0)	16.320
Arachidate acid (C20:0)	0.637
Total saturated fatty acid	**36.628**
b. Unsaturated fatty acid	
Mono Unsaturated Fatty Acid (MUFA)	
Palmitoleic acid (C16:1)	2.915
Oleic acid (C18:1)	7.728
Total MUFA	**10.643**
Polyunsaturated Fatty Acid (PUFA)	
Linoleic acid (C18:2)	11.730
Linolenic acid (C18:3)	1.883
Eicosa Pentaenoic Acid (EPA)	2.483
Total PUFA	**16.096**
Total Unsaturated Fatty Acid	**26.739**
c. Cholesterol	**0.7918**

*Result analysis of Laboratory Food Technology and Agricultural Product, Gadjah Mada University, Yogyakarta (2013)

by lauric acid 3.87%, 4.59%, and 7.30%, respectively.

The levels of palmitic acid (C16:0) and stearic acid (C18:0) from this study was also lower compared to the study conducted by Hwang and Joo (2017). The total of SFA in meat of Hanwoo cattle fed grass diet was 47.23±2.04% which palmitic acid (C16:0) and stearic acid (C18:0) were the major of fatty acid that is 29.29±0.57% and 13.32±1.67%, respectively. This differences of SFA composition from this study and previous studies could probably due to the differences of feed, age, and genetic factors.

Unsaturated Fatty Acid (UFA)

The percentage of unsaturated fatty acid (UFA) in meat of Bali bull in this study was 26.739% (Table 2, Figure 1) which is dominated by 11.730% of linoleic acid or omega-6 fatty acid (C18:2), 7.728% of oleic acid or omega-9 fatty acid (C18:1), 2.915% of palmitoleic acid (C16:1), 2.483% of eicosa pentaenoic acid (EPA), and 1.883% of linolenic acid (C18:3). From the number of doubled-chain unsaturated fatty acid, percentage of MUFA and PUFA was 10.643% and 16.096 %, respectively. In this study, the percentage of MUFA is lower, however the percentage of PUFA is higher compared to the study conducted by Hwang and Joo (2017) which reported that the levels of MUFA and PUFA in meat of Hanwoo cattle were 49.07±1.82% and 3.70±0.33%, respectively.

The major UFA of cattle fat or meat was palmitoleic acid (C16:1), oleic (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Oleic acid is the major UFA in cattle body (Soeparno, 2009). In contrast, in the present study the major UFA was linoleic acid (18:2) which indicate that green diet contained sufficient linoleic acid which influenced the composition of UFA in meat (Soeparno, 2009).

Palmitic acid can be converted further to 18-carbon stearic acid, which both serve as precursor of two unsaturated fatty acid with one double bond forming palmitoleic acid (C16:1) and oleic acid (C18:1), respectively. Mammals are unable to synthesize linoleic acid, thefore, it must be supplied from plant or feeds (Lehninger, 1982).

The average of saturated and unsaturated fatty acids in this study was lower compared to the castrated of Australian Commercial Cross (ACC) aged 2-3 years old reported by (Basuki, 2000) that experienced compensation growth with 10% green feed and 90% concentrate at high protein energy treatment (T), medium protein energy (S), and low protein-energy (R). The levels of fatty acids were 1.54-25.25% of palmitic acid, 7.17-36.29% of stearic acid, 1.38-7.76% of palmitoleic acid, 30.57-48.39% of oleic acid and 0.40-8.26% of linoleic acid. Saturated and unsaturated fatty acids in treatment T, S, and R were 43.32% and 46.56%; 35.60% and 50.54%; 40.33 and 49.92%, respectively. Total unsaturated fatty acid in this research was also lower compared to Brahman cross cattle fattened during 2, 3, and 4 months namely 61.67 %, 61.95 %, and 56.76 %, respectively as reported by Soebagyo *et al.* (2000). The differences of saturated and unsaturated fatty acids levels can be influenced by type of cattle, diet, period of fattening, and farming management.

Results of this study showed that Bali bull fed with 100% green diet resulted in the incease of saturated fatty acid level, particularly palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) 2.828 despite the relatively lower presentation. Parakkasi (1999) reported that palmitic acid and stearic acid were prevalent in cattle under a 180-day green diet. The findings above showed that the meat of cattle fed with green diet contained more saturated fatty acid compare to unsaturated fatty acid. According to Soeparno (2009), the unsaturated fatty acid level was affected by breed, species, age, weight, sex, condition, and feed.

Cholesterol

For human, **c**holesterol is essential for body growth, cell regeneration, producing bile acid that supports fat

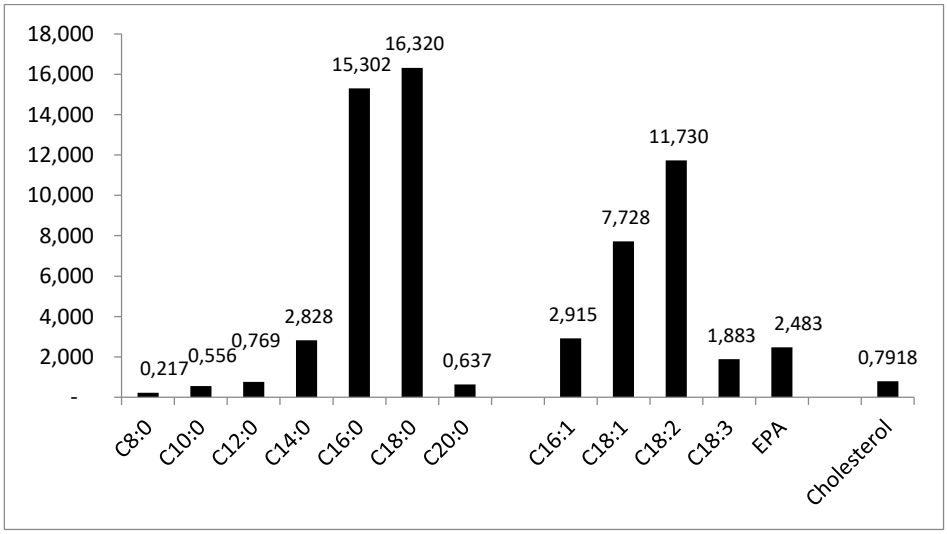

Figure 1. The average composition of fatty acid (%) and cholesterol (mg/g) in meat of Bali bull fattened using forage crops (greenlot fattening system) in small holder farming

absorption, and producing hormones. Although cholesterol has essential functions in body, several health references widely reported that consume excessive animal product such as mutton, meat, innards or brain, liver, heart, intestines and stomach have a negative impact on human health (Saidin, 2000).

Cholesterol levels of meat of Bali bull fattened with forage crops were 0.7918 mg/g or 79.18 mg/100 g meat (Table 2, Figure 1). The cholesterol level in this study was higher compared to results reported by Suryanto *et al.* (2014): 38.75-42.00 mg/100 g. Moreover, it was also higher compared to results reported by de Almeida *et al.* (2006) on the semimembranosus and biceps femoris muscle: 51.97 and 63.02 mg/100 g, respectively. Rusman *et al.* (2003) as cited in Soeparno (2011) reported that cholesterol levels of Angus cattle, Friesian Holstein, Australian Commercial Cross, and Murray Grey were 41.64 mg/100 g, 59.98 mg/100 g, 40.40 mg/100 g, 58.93 mg/100 g, and 51.01 mg/100 g, respectively. In addition, according to Rule *et al.* (2002), the cholesterol levels of longisimus dorsi muscle on fattened beef cattle reached of 52.7 mg/100 mg. Our results indicate that the use of 100% forage crops during fattening in Bali cattle can increase the levels of cholesterol.

Some factors related to cholesterol levels in fresh meat and cooked meat were the slices of carcass, preparation method, meat grade, meat type (red meat or white meat), species, breed of cattle, age, and marbling content. Cholesterol balance is controlled by liver that manages the circulation of cholesterol levels through lipoprotein particles (Soeparno, 2011). According to Muchenjeab *et al.* (2009), the differences breeds of cattle raised in the pastures do not affect on the content of meat cholesterol. Plasma cholesterol of pasteured animal in pastora during wet season is significantly lower than those fed on concentrate but still sufficient for normal growth. Some mammals have homeostatic plasma that increases fat absorption and eventually blood cholesterol level (Parakkasi, 1999).

CONCLUSION

Meat of Bali bull fattened with forage crops (greenlot fattening system) resulted in higher saturated fatty acid than unsaturated fatty acid. The major of saturated fatty acids were stearic acid and palmitic acid, whereas the major unsaturated acids were linoleic acid and oleic acid.

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