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THE EFFECT SNPs R25C AND R25H LEPTIN GENE TO CHOLESTEROL AND TRIGLYCERIDE LEVEL ON BLOOD OF ONGOLE CROSSBREED CATTLE

(Pengaruh Mutasi Snps R25c Dan R25h Pada Leptin Gen Terhadap Tingkat Kolesterol Dan Triglserid Dalam Darah Sapi Peranakan Ongole)

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ABSTRACT

Single Nucleotide Polymorphisms (SNPs) on the Leptin gene may alter the quality and quantity of Leptin hormone, which plays an active role in energy metabolism and it can contribute to fat deposition in mammals. This study proposed to determine the effect of SNPs R25C and R25H on Cholesterol, and Triglycerides levels in the blood of Ongole Crossbreed cattle. The research was carried out experimentally with a Completely Randomized Design using the sixteen heads of Ongole Crossbreed. There were three different genotypes based on SNPs R25C and R25H: CC, TT, and AA. The cattle were reared intensively for two months. The feed consists of corn kernels and concentrates, and It is calculated based on the Dry Matter (DM) intake, which is 3% of body weight. The result showed SNPs R25C and R25H on the Leptin gene did not have a significant effect on blood Cholesterol, and Triglyceride levels of Ongole Crossbreed cattle. Those SNPs in the Leptin gene cannot be used as selection markers in Ongole Crossbreed cattle. Further research is needed to include SNPs in other genes contributing to Cholesterol and Triglycerides on blood cattle

Keywords: *Leptin, Mutation, Cholesterol, Triglyceride, Cattle*

ABSTRAK

Mutasi titik *Single Nucleotide Polymorphisms* (SNPs) pada gen Leptin dapat mengubah kualitas dan kuantitas hormon Leptin, yang berperan aktif dalam metabolisme energi dan dapat berkontribusi terhadap deposisi lemak pada mamalia. Penelitian ini bertujuan untuk mengetahui pengaruh SNP R25C dan R25H terhadap kadar Kolesterol, dan Trigliserida dalam darah sapi Peranakan Ongole (PO). Penelitian ini dilakukan secara eksperimental dengan Rancangan Acak

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Lengkap menggunakan enam belas ekor sapi PO. Terdapat tiga genotipe berbeda berdasarkan SNP R25C dan R25H: CC, TT, dan HH. Sapi dipelihara secara intensif selama dua bulan. Pakan terdiri dari biji jagung dan konsentrat, dan dihitung berdasarkan konsumsi Bahan Kering, yaitu 3% dari berat badan. Hasil penelitian menunjukkan SNP R25C dan R25H pada gen Leptin tidak memberikan pengaruh yang signifikan terhadap kadar Kolesterol dan Trigliserida darah sapi PO. Mutasi titik R25C dan R25H pada gen Leptin tersebut tidak dapat digunakan sebagai penanda seleksi pada sapi PO. Perlu dilakukan penelitian lebih lanjut untuk mengikutsertakan SNP pada gen lain yang berperan terhadap konsentrasi cholesterol dan trigliserida dalam darah sapi.

Kata kunci: *Leptin, Mutasi, Sapi PO, Kolesterol, Trigliserid*

INTRODUCTION

Meat production from local beef cattle contributes sufficiently to meet the needs of Indonesia. One of them is the Ongole Crossbreed (OC) cattle, which are widespread almost throughout the Indonesian area. This breed exhibits excellent performance in terms of production and reproduction. It is very adaptable to harsh environments and possesses resilience to diseases. Optimal utilization of OC cattle is not only related to increasing productivity. It is necessary to consider meat quality and its fat content, which will contribute to consumer health.

Meat quality can be evaluated by considering the amount of fat (adipose). The deposition of animal adipose tissue substantially impacts the quantity of meat obtained from individual carcasses and the perception of its palatability by customers (Schumacher *et al.,* 2022). Meanwhile, cholesterol and triglyceride levels in the bloodstream are measurable factors that impact meat quality.

Leptin is a hormone that actively influences productivity and fat metabolism in mammals. The leptin hormone is essential for various bodily activities, including energy metabolism, regulation of body weight, control of feed intake, reproductive processes, immune system functionality, and neuroendocrine functions (Sainz *et al.,* 2015; Upadhyay *et al.,* 2015; Mota *et al., 2017* Maurya *et al.,* 2018). The leptin endocrine system has a crucial evolutionary function in maintaining the stability of adipose tissue mass (Friedman, 2019). Therefore, disruptions in leptin signaling have been linked to a range of digestive system activities, including fat metabolism (Silva *et al.,* 2014). The leptin concentrations were negatively correlated with cholesterol content and were not correlated with sensory indexes including tenderness, juiciness, and like flavor (*Geng et al.,* 2020)

A previous study found that mutations in the Leptin gene alter the leptin hormone's quantity and functionality, impacting its effects on metabolism. The missense mutation Single Nucleotide Polymorphism R25C in exon 2 of the Leptin gene has the potential to alter the functionality of protein molecules, resulting in modifications to the biological function of this gene (Buchanan *et al.,* 2002). Animals with a congenital shortage in leptin have abnormalities in macronutrient metabolism, such as disruption of glucose, lipid, and protein metabolism. They appear as increased amounts of circulating free fatty acids, elevated triglycerides, higher LDL and HDL cholesterol, and noticeable fat accumulation in the liver (Prieur *et al.,* 2008).

Information about the association of SNPs R25C and R25H as a specific SNP found in Indonesian local cattle (Hilmia *et al.*, 2018), in the Leptin genes with Cholesterol, and Triglyceride in blood has yet to be carried out in Ongole Crossbreed cattle. Based on these, the research aims to determine the effect of SNPs R25C and R25H on

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Cholesterol, and Triglycerides levels in the blood of Ongole Crossbreed cattle. In further the SNPs in the leptin gene are expected to be a genetic marker for the beef quality of Ongole Crossbreed.

MATERIALS AND METHODS

Materials

Sixteen female Ongole crossbreed cattle, approximately 2-2,5 years old, were selected for this study. The genotypes of these cattle were determined using Single Nucleotide Polymorphism (SNP) markers R25C (resulting in a change from Arginine to Cysteine) and R25H (resulting in a change from Arginine to Histidine). Three genotypes were identified: CC, TT, and AA (Hilmia *et al.,* 2018). The cattle were housed in separate enclosures and reared intensively for 75 days. The feed utilized comprises corn kernels and concentrated supplements. The feed demand is determined by calculating the Dry Matter Intake (DMI), equivalent to 3% of body weight (Perry and Cecava, 1995). The feed was provided at a proportion of 25% forage and 75% concentrate. Feeding occurs twice daily, and water is provided as a adlibitum.

Research methods

The research was conducted using a Completely Randomized Design (CRD) in an experimental setting (Gaspersz, 1991). . There were three different genotypes, namely CC, TT, and AA, as treatment, which were repeated at 6, 2, and 6 heads respectively, for parameters of Cholesterol and Triglyceride level in blood.

Parameter

Cholesterol and Triglyceride in Blood Cholesterol levels were analyzed using the photometric test method, while triglycerides were determined by the Calorimetric Enzymatic Test using the glycerol-3-phospateoxidas method. Analysis of these two parameters was carried out using a spectrophotometer.

Data analysis

Differences of Cholesterol, and triglyceride levels based on genotype (CC, TT and AA) were calculated using the Analysis of Variance (ANOVA) (Gaspersz 1991),

RESULTS AND DISCUSSION

Cholesterol and Triglyceride

Cholesterol is critical in mammalian cells, and its balance is carefully regulated. Among the primary plasma lipids, triglycerides and cholesterol are the most significant. In addition to its crucial function in cellular structure and stability, cholesterol is a foundational component for producing steroid hormones, vitamin D, oxysterols, and bile acids (Rohrl & Stangl, 2018). Elevated cholesterol levels in the bloodstream may indicate elevated cholesterol levels in the body's tissues and muscles. The data on cholesterol levels from the blood of Ongole Crossbreed based on different Genotypes of Leptin genes based on SNP R25C and R25H are presented in Table 1.

According to the data in Table 1, the cholesterol levels observed in this study align with the findings of Faza *et al.,* (2017), who reported an average blood cholesterol level of 250.16 ± 21.84 mg/dL in cattle, along with a triglyceride level of 56.11 ± 6.13 mg/dL. The differences data of parameter based on difference genotype, indicated no significant impact of genotype differences ($P>0.05$) on blood cholesterol and triglyceride levels. The mutations, specifically SNPs R25C and R25H, were found to change the amino acid code from Arginine to Cysteine and Arginine to Histidine, respectively. However, these mutations do not impact blood cholesterol and triglyceride levels. This study aligns with the findings of Giblins *et al.,* (2010), who observed no correlation between LEP-1238, LEP-963, R25C fat metabolism.

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Table 1. Cholesterol and Triglyseride Levels in Blood of Ongole Crossbreed Based on Different Genotype of Leptin Gene based on SNP R25C and R25H

Individu	Cholesterol Levels			Triglyceride levels		
	CC	TT	AA	CC	TT	AA
	…….....mg/dL…….....					
1	285.5	124.0	223.5	17.1	5.7	13.0
2	297.2	261.5	284.9	29.3	25.2	30.9
3	200.6		276.0	8.9		30.9
4	216.8		223.5	5.7		17.1
5	247.5		253.6	24.4		5.7
6	216.8		287.2	26.8		20.3
Average	244.1	192.7	258.1	18.7	15. 4	19.7

Cholesterol and triglycerides are by-products of the complex mechanism of fat metabolism, which involves multiple hormones, including leptin (Schumacher et al., 2022). The levels of triglycerides are significantly affected by the amount of fat and carbohydrates consumed in the diet. Triglycerides function as fuel for the body and any excess is stored in the liver as energy reserves (Röhrl and Stangl, 2018). According to the previous understanding of metabolic processes, catabolism, and mechanisms of body fat biosynthesis, at least three sets of genes are involved. The three categories are Triglyceride lipase, Fatty acid synthase, and Fatty acid binding protein (FABP) (Hamed & Ali, 2011). According to Altiner *et al.,* (2017), the hormones adiponectin, insulin, ghrelin, and leptin impact the fat production and deposition process. The PPAR and AMPK signaling pathways are important examples of lipid metabolism in Shandong black cattle. In addition, FABP4 and ADIPOQ have seven overlapping regulatory factors that affect many genes. The regulatory variables encompass PLIN1, PLIN2, PPARGC1A, RXRA, PCK1, LEPR, and LEP. These genes regulate lipolysis in adipocytes, the signaling pathways of adipocytokines, and the PPAR signaling pathway (Liu *et al.,* 2020).

Hormone regulation has a significant role in triglyceride production. The primary hormone involved in the process of lipogenesis is insulin. Whenever the amounts of triglycerides entering the body exceed the energy requirements, they are stored as fat depots, serving as reserves for energy (Schumacher et al. 2022). The triglyceride lipase gene is the primary set of genes linked to adipose tissue. The triglyceride lipase genes encode the lipase enzyme. The gene group comprises carboxyl ester lipase (CEL), endothelial lipase (LIPG), hepatic lipase (LIPC), adipose triglyceride lipase (ATGL), and lipoprotein lipase (LPL) (Sato et al., 2010). The CEL gene encodes a lipase enzyme that catalyzes the early breakdown of dietary fat into fatty acids during metabolism. The lipase enzyme synthesized by CEL exhibits active functionality only upon stimulation by bile salts. The LPIG, LPIC, and ATGL genes encode lipase proteins that catalyze the hydrolysis of fat or triglycerides derived from endothelium, liver, and adipose tissue, yielding glycerol and fatty acids. Furthermore, Kigoshi *et al.,* (2019) reported polymorphism in the *STARD3* gene, which is involved in cholesterol distribution and cell homeostasis. They investigated the effects of the *STARD3* gene polymorphism on carcass traits and fatty acid composition.

Analysis using multiple markers suggests the existence of genetic networks

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that are potentially associated with the Reverse Cholesterol Transport (RCT) pathway. These networks are linked to phenotypes such as kidney-pelvic-heart fat percentage, ribeye area, and subcutaneous fat depth. The markers utilized are obtained from paraoxinase 1, apolipoproteins A1, and E. These findings indicate that genes that regulate cholesterol levels could be important for investigating human obesity and improving meat quality in cattle farming (Daniel *et al.*, 2010). The mRNA abundance of genes involved in cholesterol synthesis was *SREBF-2*, *HMGCS1*, and *HMGCR* (Kessler *et al.*, 2014).

CONCLUSION

SNPs R25C and R25H on the Leptin gene did not significantly blood Cholesterol, and Triglyceride level of Ongole Crossbreed cattle. Those SNPs in the Leptin gene cannot be used as selection markers in Ongole Crossbreed cattle. Further research is needed to include SNPs in other genes contributing to productivity.

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