Polymorphism of IGFBP7 gene (g.72351183 A>C) and its association with mineral content and cholesterol of Indonesian lamb meat

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Abstract. The genetic improvement of sheep for higher productivity and meat quality is strongly needed due to the increasing demand for meat sheep. Insulin growth factor binding protein 7 (IGFBP7) has been reported as a potential gene which has a substantial role in meat quality. The objective of the study was to identify IGFBP7 gene polymorphisms and their association with minerals and cholesterol in Indonesian lamb meat. The study used 130 rams consisting of local and crossbred sheep. The local sheep were Javanese thin-tailed, Javanese fat-tailed, and Jonggol sheep. The crossbred sheep were Compass agrinak, Composite garut agrinak and Bahtera agrinak sheep. The identification of polymorphism was using PCR-RFLP method. The association analysis was obtained using a general linear model. The results showed that IGFBP7 (g.72351183 A>C) was polymorphic in local and crossbred sheep. The IGFBP7 was significantly associated with cholesterol in crossbred ($P<0.01$), whereas the AA genotype had the lowest cholesterol. Cholesterol in crossbred sheep also was higher ($P<0.01$) compared to local sheep for all observed genotypes. IGFBP7 gene was not significantly associated with minerals and cholesterol ($P>0.05$) in local sheep. It is concluded that IGFBP7 gene seems promising as a genetic marker for cholesterol level in Indonesian crossbred sheep.

1 Introduction

Sheep is one of the important livestock in Indonesia. In the recent following years, the national demand for sheep meat showed an increased trend. The number of registered slaughtered sheep in 2018 was 1,389,619 heads, and it was increase to 1,695,996 heads in 2021 [1]. The trend would be a continuous increase with the increasing of the Indonesian population. The increasing purchasing power and awareness of health may also lead to increasing demand for high-quality and nutritional meat.

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In addition, meat quality is an essential factor that influences the preference of customers to buy meat [2-4]. Consequently, the increasing productivity and meat quality of Indonesian sheep are strongly needed. Several meat compositions that should be taken into account in Indonesian lamb meat are mineral content and cholesterol level.

Minerals from meat are essential nutrients for the human health and human immune system [5, 6]. In a meat quality perspective, mineral content in meat is an important element that influences meat quality [7, 8]. Iron (Fe), zinc (Zn), and potassium (K) were reported correlate with meat color, drip loss and marbling, respectively [7]. Minerals also have a correlation with the performance of animals [8]. The level of cholesterol in meat may influence perspective of customers for healthier meat. Cholesterol has an association with cardiovascular disease in humans [9], whereas a high level of cholesterol gives a high risk of cardiovascular disease [10]. One of the major sources of minerals and cholesterol for the human diet is meat. Therefore, genetic improvement through a selection of genetic markers for higher mineral content and lower cholesterol level in lamb meat is important for improving meat quality. One of the genes that suggested has a potential effect to mineral content and cholesterol level in meat is insulin growth factor binding protein 7.

Insulin growth factor binding protein 7 (IGFBP7) gene is a member of IGFBP family, which has an important role in regulating the activity of insulin-like growth factors (IGFs) [11]. IGFBP7 gene has important roles in growth, differentiation, and survival of cell. In sheep, IGFBP7 gene has been reported as one of the genes that have an association with meat quality [12, 13]. Several Indonesian local sheep breeds, such as Garut sheep (GS), Javanese thin-tailed sheep (JTTS), Javanese fat-tailed sheep (JFTS) and Jonggol sheep (JS) are common sheep breed raised by farmers and contribute substantially to the national meat sheep consumption [14]. In addition, to increase the productivity of local sheep, some improved breeds of sheep also have been developed and launched by the Indonesian government. These sheep are Composite garut agrinak sheep (25 % Garut sheep, 25 % St. Croix sheep, and 25 % Moulton Charolais sheep), Compass agrinak sheep (50 % Sumatra sheep, 25 % Barbados black belly sheep, and 25 % St. Croix sheep) and Bahtera agrinak sheep (50 % Sumatra sheep and 50 % Barbados black belly sheep) [15-17]. To our knowledge, the study of IGFBP7 has not been studied yet before in those Indonesian sheep breeds. Therefore, the objective of the study was to analyze the polymorphism of IGFBP7 gene and to describe its association with the mineral content in local sheep and cholesterol level in local and crossbred sheep.

2 Materials and methods

2.1 Animals and observed variables

A total of 130 rams were used in the study, consisting of local and crossbred sheep. The local sheep used in the study were 84 Javanese thin-tailed sheep (JTTS), 9 Javanese fat-tailed sheep (JFTS), and 15 Jonggol sheep (JS). While the crossbred sheep were 6 Compass agrinak sheep (CAS), 6 Composite garut agrinak sheep (CGAS), and 10 Bahtera agrinak sheep (BAS). The rams were taken from smallholders' farmer for JTTS and JFTS, and from research institute for JS, CAS, CGAS and BAS. Rams were slaughtered at 10 - 12 months old ages. The slaughtered procedures followed SNI 99003-2018 [18]. All experimental procedures in the study were approved by the Institutional Animal Care and Use Committee (IACUC), IPB University (approval ID: 117-2018 IPB).

The observed variables in the study were mineral content and level of cholesterol in lamb meat. The longissimus dorsi sample was used for analysis of observed variables. Minerals content in the study was potassium (K), selenium (Se), zinc (Zn), and iron (Fe). The content of mineral in meat was measured using protocol the LP-04.10-LT-1.0, referring to AOAC

(2015) official method 969.08 (AOAC 4.8.02). The cholesterol level in lamb was analyzed using the High Performance Liquid Chromatography (HPLC) method.

2.2 DNA extraction, PCR-RLFP amplification and genotyping

The *longissimus dorsi* samples from rams were used for DNA extraction using a genomic DNA mini kit (Geneaid Biotech, Taiwan). The primer3 online software was used to design specific primers (Table. 1). The PCR premix contained two µL DNA, 6.1 micro nuclease-free water, 0.4 µL of primers, and 7.5 µL MyTag Red Mix. The amplification of polymerase chain reaction (PCR) steps was subsequently: (1) initial denaturation at 95 °C for 1 min, (2) 35 cycles, which consisted of denaturation at 15 °C for 15 s, annealing of primer at 55 °C and DNA elongation at 72 °C for 15 s and (3) final elongation at 72 °C for 15 s. The detection of DNA amplicon was performed by electrophoresis in 1.5 % agarose gel. The restriction fragment length polymorphism (RFLP) method was used for genotyping. The DNA amplicon was digested using *Tsp*451 restriction enzyme, and it was incubated at 37 °C for 4 hours. The digested product was visualized using 2 % agarose gel under a UV transilluminator (Alpha Imager; Alpha Innotech, Santa Clara, CA, USA).

Table 1. Gene bank accession number, primers and restriction enzyme

Gene	Accession number	Size of PCR	Primer sequence	TA(°C)	Enzyme
IGFBP7 (g.72351183 A>C)	NC_019463.2	419 bp	F: 5'- GCC TTA TGC GTG CAA ACT GT- -3' R: 5'- GGT GAA GGT GCT GAG CTG TA- -3'	55	*Tsp*451

F= Forward; R= Reverse, TA = Temperature of annealing

2.3 Data analysis

The allele and genotype frequencies of IGFBP7 (g.72351183 A>C) gene were calculated using the formula described by Nei and Kumar [19]. The Hardy-Weinberg equilibrium was estimated using procedures described by Hartl and Clark [20]. The general linear model was used to analysis the association between IGFBP7 gene (g.72351183 A>C) polymorphisms with mineral content of lamb meat. The mathematic model for association analysis was:

$$Y_{ij} = \mu + G_i + e_{ij}$$

whereas Y_{ij} was value of observed variables, µ was the overall mean, G_i was the fixed effect of genotype and e_{ij} was the random error. In addition, the T-test was performed to test the differences levels of cholesterol between breeds on the same genotype and between genotypes on the same type of breed.

3 Results and discussion

3.1. IGFBP7 gene polymorphism

The amplification of IGFBP7 gene (g.72351183 A>C) was successfully performed using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) (Figure 1). The results of the genotype were in accordance with the simulation on the online program

(https://nc2.neb.com). The visualized genotypes were AA (426 bp), CC (147 and 279 bp), and AC (147, 279, and 426 bp).

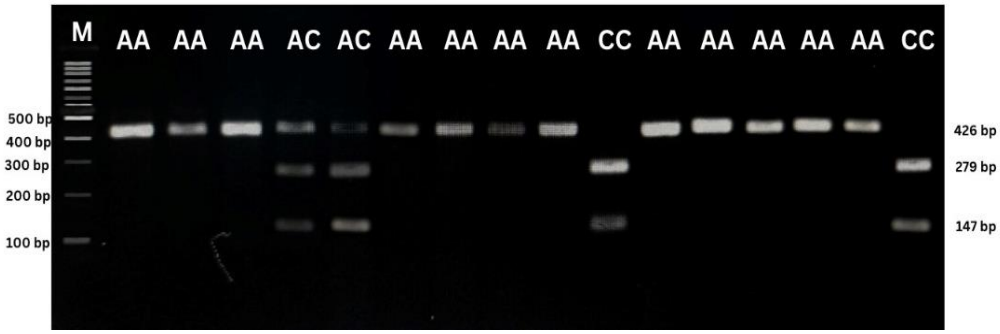

Fig. 1. PCR-RFLP results of IGFBP7 gene (g.72351183 A>C) using *Tsp*451 enzyme. M=Marker 100 bp.

The result of genotype frequency, allele frequency, and Hardy-Weinberg equilibrium of IGFBP7 gene in the study were shown in Table 2. The results showed that allele A was the dominant allele on local (90 %) and crossbred sheep (77 %). The allele distribution on both types of sheep showed on Hardy-Weinberg equilibrium. It suggested there was no natural selection, migration, and genetic drift from one generation to the next in the population.

Table 2. The number of sheep per genotype and allele frequency

Sheep	n	Genotype frequency			Allele frequency		Chi-square (X^2)
		AA (n)	AC (n)	CC (n)	A	C	
Local sheep	108	0.82 (89)	0.16 (17)	0.02 (2)	0.90	0.10	1.15 (ns)
Crossbred sheep	22	0.55 (12)	0.45 (10)	0.00 (0)	0.77	0.23	1.90 (ns)
Total	130	0.78 (101)	0.21 (27)	0.01 (2)	0.88	0.12	0.02 (ns)

n = number of samples
x^2 table = 3.84
ns = not significant

3.2. IGFBP7 gene association with minerals and cholesterol

The association analysis between IGFBP7 gene with mineral content in lamb meat (Fe, Zn, K, and Se) in local breed showed not significantly associated (P<0.05) (Table 3). The Fe content in lamb meat in the study ranged from 1.80 to 1.98 mg/100 g. While for Zn, K, and Se content were 2.07 - 2.72 mg/100 g, 243.42 - 299.33 mg/100 g, and 0.58 - 0.63 mg/100 g, respectively. The mineral content of lamb meat in the study was similar for Fe and Zn compared to Merino male lamb [21] and Australian lamb [22, 23].

Table 3. Association between IGFBP7 gene and mineral on local sheep

Mineral	**AA** ($\bar{x}$ ± sd) (n)	**AC** ($\bar{x}$ ± sd) (n)	**CC** ($\bar{x}$ ± sd) (n)	**P Value**
Fe	1.88 ± 0.82 (80)	1.80 ± 0.70 (17)	1.98 ± 0.39 (2)	ns
Zn	2.61 ± 0.95 (80)	2.72 ±1.18 (17)	2.07 ± 0.75 (2)	ns
K	267.55 ± 86.53 (80)	299.33 ± 79.25 (17)	243.42 ± 78.85 (2)	ns
Se	0.61 ± 0.3 (80)	0.58 ± 0.36 (17)	0.63 ± 0.25 (2)	ns

$\bar{x}$ = mean (mg/100 g)
sd = standard deviation
ns = not significant

The results of the association analysis between IGFBP7 gene and cholesterol level are shown in Table 4. The results showed that IGFBP7 gene was not associated with cholesterol level in lamb meat on local sheep (P>0.05). Potassium (K) was reported had a moderate to high correlation value with the level of cholesterol in beef [8]. The absence of IGFBP7 gene association with potassium might resulted in the absence of cholesterol association with IGFBP7 in local breed sheep.

However, IGFBP7 gene was significantly associated with cholesterol level in crossbred sheep (P<0.01). In crossbreed sheep, the sheep with the AA genotype (14.24 ± 9.20 %) had significantly lower cholesterol level compared to the AC genotype (26.83 ± 15.95 %) (P<0.01). Cholesterol is an important part of lipid fraction [24]. IGFBP7 gene had an important role in the differentiation of preadipocyte cells [11]. Preadipocyte cell is the precursor of fat cell and constitutes a large part of fat tissue. IGFBP7 gene also reported associated with growth and fat deposition in chicken [25, 26], and subcutaneous fat deposition in cattle [11]. Since IGFBP7 gene correlated with lipid, it also suggested that IGFBP7 gene have a role in the cholesterol level in meat.

Table 4. The association of IGFBP7 gene with cholesterol

Sheep	AA ($\bar{x}$ ± sd) (n)	AC ($\bar{x}$ ± sd) (n)	CC ($\bar{x}$ ± sd) (n)	P value[B]
Local sheep	6.74 ± 2.42 (75)	6.95 ± 1.98 (12)	na	ns
Crossbred sheep	14.24 ± 9.20 (12)	26.83 ± 15.95 (10)	na	**
P value[A]	**	**	na	

P value[A] : significancy between local and crossbred sheep on same genotype
P value[B] : significancy between genotype on same type of sheep breed
** : significant different (P<0.01)
na : not available

The level of cholesterol in crossbred was significantly different than in local breed for AA and AC genotypes. The cholesterol level of AA genotype on local breed (6.74 ± 2.42 %) was significantly lower than AA genotype on crossbred sheep (14.24 ± 9.20 %) (P<0.01). for genotype AC, cholesterol level on local sheep (6.95 ± 1.98 %) was also significantly lower compare to crossbred sheep (26.83 ± 15.95 %) (P<0.01). It showed that local breed had better cholesterol level in lamb meat compared to crossbred for all observed genotypes. The different level of cholesterol in meat, which is affected by breed have been reported in previous studies [27- 29].

4 Conclusion

The IGFBP7 gene was polymorphic in local and crossbred sheep populations in this study, with allele A was a dominant allele. It was not associated with mineral content and cholesterol level in local lamb meat. IGFBP7 gene seems promising as a genetic marker for cholesterol level in Indonesian crossbred sheep since it was associated with cholesterol level in crossbred, whereas AA genotype had lower cholesterol compared to AC genotype.

References

1. Directorate General of Livestock and Animal Health, *Statistik peternakan dan kesehatan ternak (*Directorate General of Livestock and Animal Health, Ministry of Agriculture, Jakarta, Indonesia, 2022)

2. D.T.N. Thu, VNUHCM Journal of Science and Technology Development **9**, 65–70 (2006)
3. A. Gunawan, K. Listyarini K, R.S. Harahap, Jakaria, K. Roosita, C Sumantri, I. Inounu, S.H. Akter, M.A. Islam, M.J. Uddin, PLoS ONE **16** (**12**), (2021)
4. M. Henchion, M. McCarthy, V.C. Resconi, D. Troy, Meat science **98** (**3**), 561-568 (2014)
5. P. M. D. C. C. Pereira, A. F. D. R. B. Vicente, Meat science **93** (**3**), 586-592 (2013).
6. C. Weyh, K. Krüger, P. Peeling, L. Castell, Nutrients **14** (**3**), 644 (2022)
7. G. Z. Ren, W. Ming, Z. T. Li, X. J. Li, J. F. Chen, Q.Q Li, Am. J. Anim. Vet. Sci **3**, 18-22 (2008)
8. N. Patel, M. Bergamaschi, L. Magro, A. Petrini, G. Bittante, Animals **9** (**12**), 1073 (2019)
9. F. Jiménez-Colmenero, J. Carballo, S. Cofrades. Meat science **59** (**1**), 5-13 (2001)
10. H. Ma, K. J. Shieh. The Journal of American Science 2 **1**, 46-50 (2006)
11. Z. Hu, J. Wu, L. Qin, H. Jin, Y. Cao, Y. Zhao, Animal Biotechnology **32** (**1**), 21-30 (2021)
12. S. Cheng, X. Wang, Q. Zhang, Y. He, X. Zhang, L. Yang, J. Shi. Genes **11** (**2**), 183 (2020)
13. K. Listyarini, C. Sumantri, S. Rahayu, M. A. Islam, S. H. Akter, M. J. Uddin, A. Gunawan, Animals **13** (**4**), 674. (2023)
14. R. S. Harahap, R.R. Noor, Y. C. Endrawati, H.S. Darusman, A. Gunawan, Animal Bioscience **36** (**6**), 840 (2023)
15. Directorate General of Livestock and Animal Health, *Pelepasan rumpun domba compass agrinak (*Directorate General of Livestock and Animal Health, Ministry of Agriculture, Jakarta, Indonesia, 2014)
16. Directorate General of Livestock and Animal Health, *Pelepasan rumpun domba komposit garut agrinak (*Directorate General of Livestock and Animal Health, Ministry of Agriculture, Jakarta, Indonesia, 2020)
17. Directorate General of Livestock and Animal Health, *Pelepasan rumpun domba bahtera agrinak (*Directorate General of Livestock and Animal Health, Ministry of Agriculture, Jakarta, Indonesia, 2020)
18. National Standardization Agency of Indonesia, *SNI 99003-2018 Pemotongan Halal Pada Hewan Ruminansia* (National Standardization Agency of Indonesia, Jakarta, Indonesia, 2018)
19. M. Nei, S. Kumar, *Moleculear Evolution and Phylogenetics* (Oxford University Press, USA, 2000)
20. D.L. Hartl, A. G. Clark, *Principle of Population Genetic* (Sinauer Associates, Sunderland, UK, 1997)
21. B. Panea, G. Ripoll, M. J. Alcalde, Animals **13** (**17**), 2756 (2023)
22. S. I. Mortimer, J. H. J. Van der Werf, Robin H. Jacob, D. L. Hopkins, L. Pannier, K. L. Pearce, G. E. Gardner, R. D. Warnerg, G.H. Geesink, J.E.H. Edwards, E. N. Ponnampalam, A.J. Ball, A.R. Gilmour, D.W. Pethick, Meat science **96** (**2**), 1016-1024 (2014)
23. S.M. Fowler, S. Morris, D.L. Hopkins, Meat science **154**, 126-132 (2019)
24. H.K. Walker, W.D. Hall, J. W. Hurst, *Clinical methods, 3rd edition-The history, physical and laboratory examinations* (Butterworths, Boston, USA, 1990)
25. H. B. Wang, H. Li, Q. G. Wang, X. Y. Zhang, S. Z. Wang, Y. X. Wang, X. P. Wang. *Bmc Genomics* **8** (**1**), 1-14 (2007)
26. F. Z. Lu, J. Chen, X. X. Wang, H. L. Liu, Asian-Australasian Journal of Animal Sciences 22 (**4**), 471-482 (2009)

27. G. Arsenos, D. Zygoyjannis, D. Kufidis, N. Katsaounis, C. Stamataris, Small Ruminant Research **36** (**3**), 275-283 (2000)
28. T.D. Bunch, R.C. Evans, S. Wang, C. P. Brennand, D. R. Whittier, B. J. Taylor, Small Ruminant Research **52** (**3**), 239-245 (2004)
29. K, Belhaj, F. Mansouri, A. Benmoumen, M. Sindic, M. L. Fauconnier, M. Boukharta, C.H. Serghini, A. Elamrani, Archives Animal Breeding **63** (**2**), 471-482 (2020)