Livestock and Animal Research
Accredited by Directorate General of Strengthening for Research and Development No. 10/E/KPT/2019

Open Access
Livest. Anim. Res., July 2021, 19(2): 238-246
p-ISSN 2721-5326 e-ISSN 2721-7086
https://doi.org/10.20961/lar.v19i2.48557

Original Article

Effect of polymorphism of *Insulin-induced gene 1* (*INSIG1*) (A4366G) on slaughter characteristics in unproductive Kebumen Ongole Grade cows

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Received: February 15th, 2021; Accepted: June 1st, 2021; Published online: July 30th, 2021

Abstrak

Tujuan: Sapi Peranakan Ongole Kebumen diketahui mempunyai ukuran tubuh dan bobot badan lebih tinggi dibanding SNI Nasional Sapi PO. Produktivitas dipengaruhi oleh faktor genetik dan faktor lingkungan. Gen *Insulin-induced gene-1* (*INSIG1*) merupakan salah satu kandidat gen yang berhubungan dengan karkas dan berperan dalam metabolism lemak (adipogenesis and or lipogenesis). Tujuan dari penelitian ini yaitu mengidentifikasi SNP A4366G pada gen *INSIG1* dan asosiasinya dengan karakteristik pemotongan pada sapi PO Kebumen betina tidak produktif.
Metode: Penelitian ini menggunakan 44 sampel sapi PO Kebumen betina tidak produktif dari RPH/TPH Kebumen. Parameter karakteristik pemotongan sapi meliputi bobot sebelum dipotong, bobot karkas dan persentase karkas. Sebanyak 3 ml sampel darah diambil dari *vena jugularis* dan selanjutnya DNA diekstraksi menggunakan metode *salting out*. *Genotyping* gen *INSIG1* (A4366G) menggunakan metode PCR-RFLP dengan enzim restriksi *Taq*I. Data keragaman genetik populasi meliputi frekuensi alel, genotipe, heterozigositas, dan Keseimbangan Hardy-Weinberg. Asosiasi karakteristik pemotongan dengan genotipe gen *INSIG1* dianalisis menggunakan ANOVA model.
Hasil: Polimorfik gen *INSIG1* SNP A4366G ditemukan pada populasi sapi PO Kebumen betina tidak produktif. Terdapat tiga varian genotipe (AA, AG, GG) dan dua varian alel (A dan G) dengan frekuensi alel G sebesar 0,795. Populasi ternak dalam keadaan keseimbangan genetik. Asosiasi bobot hidup sebelum dipotong, bobot karkas dan persentase karkas pada sapi PO Kebumen betina tidak produktif terhadap genotipe gen *INSIG1* (A4366G) menunjukkan tidak signifikan ($p > 0,05$).
Kesimpulan: Polimorfik gen *INSIG1* (A4366G) ditemukan pada populasi sapi PO Kebumen betina tidak produktif dengan alel dominan G. Populasi dalam keadaan keseimbangan genetik. Hubungan antara bobot hidup sebelum dipotong, bobot karkas dan persentase karkas dengan genotipe gen *INSIG1* ditemukan tidak signifikan.

Kata Kunci: Gen *INSIG1*; SNP A4366G; bobot potong; bobot karkas; persentase karkas; Sapi PO Kebumen betina tidak produktif

Abstract

Objective: Kebumen Ongole Grade (Kebumen OG) are known as good performance cattle with body weight and body measurement higher than National Standard of Ongole Grade cattle. Productivity is influenced by genetic and environmental factors. *Insulin-induced gene-1* (*INSIG1*) gene is one of many genes that are considered important in influencing carcass characteristics and playing an important role in lipid metabolism (adipogenesis and or lipogenesis). So, the aim of this study was to identify of SNP A4366G in *INSIG1* gene and associated with slaughter characteristics of unproductive Kebumen OG cows.
Methods: In this study used 44 unproductive Kebumen OG cows from slaughter house in Kebumen. Slaughter characteristics contain of slaughter weight, hot carcass weight and dressing percentage. Three milliliters of blood samples were collected from *vena jugularis*. DNA were extracted from blood samples using salting out method. Genotyping of *INSIG1* gene (A4366G) used PCR-RFLP method with *Taq*I restriction enzyme. Genetic diversity data in this study were allele and genotype frequencies, heterozygosity, PIC and HWE. Association of genotypes of *INSIG1* gene with slaughter characteristics were analyzed using ANOVA univariate model.
Results: Polymorphic of *INSIG1* (A4366G) gene was found in the unproductive Kebumen OG cows. Three variants of genotypes (AA, AG, GG) with two alleles (A and G) were found with allele frequencies 0.795 for G allele. The population was in equilibrium genetic. Association of slaughter characteristics with genotypes were not significant (p>0.05).
Conclusions: Polymorphic of *INSIG1* gene (A4366G) was found in unproductive Kebumen OG cows population with dominant of G allele. The population was in genetic equilibrium. The association of slaughter characteristics with genotype of *INSIG1* gene (SNP A4366G) was not significant.

Keywords: *INSIG1* gene; SNP A4366G; slaughter weight; carcass weight; dressing percentage; unproductive Kebumen OG cows

INTRODUCTION

Ongole Grade cattle (OG cattle), well-known as *Peranakan Ongole*, is one of Indonesian local cattle breed widely spread in Java Island primarily found in Central Java and West Java Provinces [1]. This breed is the second largest local cattle population after Bali cattle which play an important role in national meat supply needs in Indonesia [2]. In its progress, OG cattle breed can developed into several lines such as Grati-OG line which developed by the Indonesian Beef Cattle Research Station (IBCRS), Ministry of Agriculture of Indonesia in Grati [2] and OG cattle originated from Kebumen Regency, called as Kebumen Ongole Grade line (Kebumen OG line) that has been established as a new line of OG breed cattle through a ministerial decree No. 358/Kpts/PK.040/6/2015 completed with phenotypic and morphometric characteristics data [3]. Genetic diversity analysis both using morphometric and genomic data showed that Kebumen OG line was separated from the OG cattle in other population regions [4, 5]. Surprisingly, Kebumen OG line were closely linked to Nellore cattle than to the OG cattle population [5]. In several previous studies, Kebumen OG line have significant larger measurement in body weight and body size than OG cattle in other population region or the Standard in Indonesia (SNI) for OG cattle breed [6, 4]. It showed that Kebumen OG line have the potential prospect of becoming superior beef cattle in Indonesia.

Culling of cows from beef or dairy cattle herds are common things that occurs in cattle breeding farm throughout the country. These rates are equivalent to the estimated optimal culling rates in dairy herd-levels ranging from 19 to 29% [7].

Cull cows have a high contribution to beef circulating supply in the market. In France, cull dairy and beef cows contribute more than 50% beef supply [8]. The cull cows will usually be fed with a finishing diet before slaughter to improve the quality and yield of the carcass [9, 8]. In Indonesia, there is no record of culling rate of cow herds.

However, the slaughter of cull cows is frequently found in some slaughterhouses. Therefore, the performance of cull cow (including Kebumen OG line) is important to study because it's contributes to the national beef supply.

Carcass weight is one of the main economic traits in beef production. Greater dressing percentage of the carcass based on carcass weight can improve the revenues of producers [10]. Carcass weight can be influenced by animal pre-slaughter weight because it has a strong correlation (r = 0.93, p <0.001) [11]. The positive correlation was also found in PO, SimxPO, and LimxPO cattle [12].

Insulin-induced gene-1 (*INSIG1*) gene is one of many genes that are considered important in influencing carcass characteristics. *Insulin-induced gene* (*INSIG*) consists of two isoforms, *INSIG1* and *INSIG2*, playing an important role in lipid metabolism (adipogenesis and or lipogenesis) [13]. The regulation of *INSIG1* and *INSIG2* differ in terms of their role in lipid metabolism. The *INSIG1* plays a central role in mediating cholesterol biosyntesis in rodent liver whereas *INSIG2* controls differentiation of precursor adipocytes [14].

Several previous studies have identified the *INSIG1* gene was one of the strongly associated genes with lipid metabolism in meat and milk production in ruminants. In beef cattle, the *INSIG1* gene has identified as differentially expressed gene on meta-analysis associated with body weight gain x feed intake interaction in beef steers [15]. The highest fold changes of *INSIG1* reached at 167 days in expression relative to 0 days in early weaning beef steers [16]. In candidate gene analysis, four SNPs were found in intronic region of *INSIG1* gene [4366(A>G), 4534(T>C), 5001(T>C), and 5235(G>A)] that are associated with growth and carcass traits in Qincuan beef cattle. However, only SNP of g.4366A>G significantly associated with slaughter weight (P<0,01) and carcass weight (P<0,05) [17].

The evidences indicated the importance of the *INSIG1* gene in the biological process of lipid metabolism and may subsequent on body weight and carcass traits in beef cattle. Therefore, this study was aimed to detect the polymorphism of *INSIG1* gene (A4366G) and its association with slaughter characteristics (slaughter weight, carcass weight and dressing percentage) in unproductive Kebumen Ongole Grade cows.

MATERIALS AND METHODS

Animals and DNA Extraction

A total of 44 blood samples of unproductive Kebumen OG cows (more than eight years old) were used in this study. Samples were collected from slaughter house in Kebumen district, Central Java Province which the samples have inspected of the age and reproductive status (via rectal) by veterinarian. Samples were collected from small farmer in south Kebumen region. We assumed that the samples were almost got the same management and environment factor (temperature, and humidity). The slaughter weight, hot carcass weight and dressing percentage of slaughter cows were collected as phenotypic traits. Three millilitres of blood were collected from *vena jugularis* and put into vacutainer containing anticoagulant K_3EDTA. Then, the blood samples were stored at refrigerator. DNA was extracted using salting out method [18] and was stored at -20°C for next analysis.

Amplification of *INSIG1* gene

Amplification in this study used Polymerase Chain Reaction (PCR) method with a pair of primer sequence, Forward: 5'-GTGGGACTGTGGATGACT-'3 and Reverse: 5'-GAGGAAGGCGATGGTGAT-'3 [17]. PCR reaction was performed in a 10 µl mixture consisting of 5 µl PCR Mastermix (My Taq™ Red Mix, Bioline), 1 µl each primer (10 pmol/µL), 2 µl nuclease-free water and 1 µl DNA template. The PCR mixture was amplified using Thermal Cycler Gradient (Techne Plus, UK). PCR condition was pre-denaturation 94°C for 5 minutes, 35 cycles of denaturation of 94°C for 30 seconds, annealing at 64°C for 30 seconds and extension at 72°C for 45 seconds then followed by a final extension at 72°C for 10 minutes. Amplicons of *INSIG1* gene were checked using electrophoresis on 1% agarose gel

(100 V, 1 hour) (Wide Mini-Sub Cell GT Biorad, California USA) and stained with ethidium bromide. DNA fragments were visualized under UV light using UV Transilluminator (MajorScience, USA).

Genotyping

Genotyping of *INSIG1* gene in this study using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. The SNP A4366G was recognized with *Taq*I (5′-T^CGA-‘3) restriction enzyme. Location of restriction can be seen at Figure 1. A 10 μl total volume of mixture containing 2 μl PCR product, 1 μl buffer, 0.3 μl enzyme (10 U/μl) (Thermo Fisher Scientific, USA) and 3.7 μl nuclease-free water was digested with *Taq*I enzyme. Digestion products were electrophoresed using agarose gel 2% and run on 100 Voltage for 1 hour (Wide Mini-Sub Cell GT Biorad, California USA). The gel was visualized under UV light using UV Transilluminator (MajorScience, USA).

Statistical analysis

Genetic population variation of *INSIG1* gene in unproductive Kebumen OG cows population were allele and genotype frequencies, observed heterozygosity (Ho), expected heterozygosity (He). According to Nei and Kumar [19], genotype and allele frequencies was calculated by following formula:

$$Xii = \frac{n_{ii}}{N} \times 100\%$$

$$Xi = \frac{\left(2n_{ii} + \sum_{i \neq j} n_{ij}\right)}{2N}$$

Note:

X_{ii} = ii[th] genotype frequency
X_i = i[th] allele frequency
n_{ii} = number sample of ii genotype
n_{ij} = number sample of ij genotype
N = total of samples

Then, the observed and expected heterozygosity was calculated following this formula [20].

$$Ho = \frac{\sum N_{ij}}{N}$$

$$Ho = \frac{2N}{2N-1}\left(1 - \sum_{i=1}^{n} p^2 i\right)$$

Where:

Ho = observed heterozygosity value

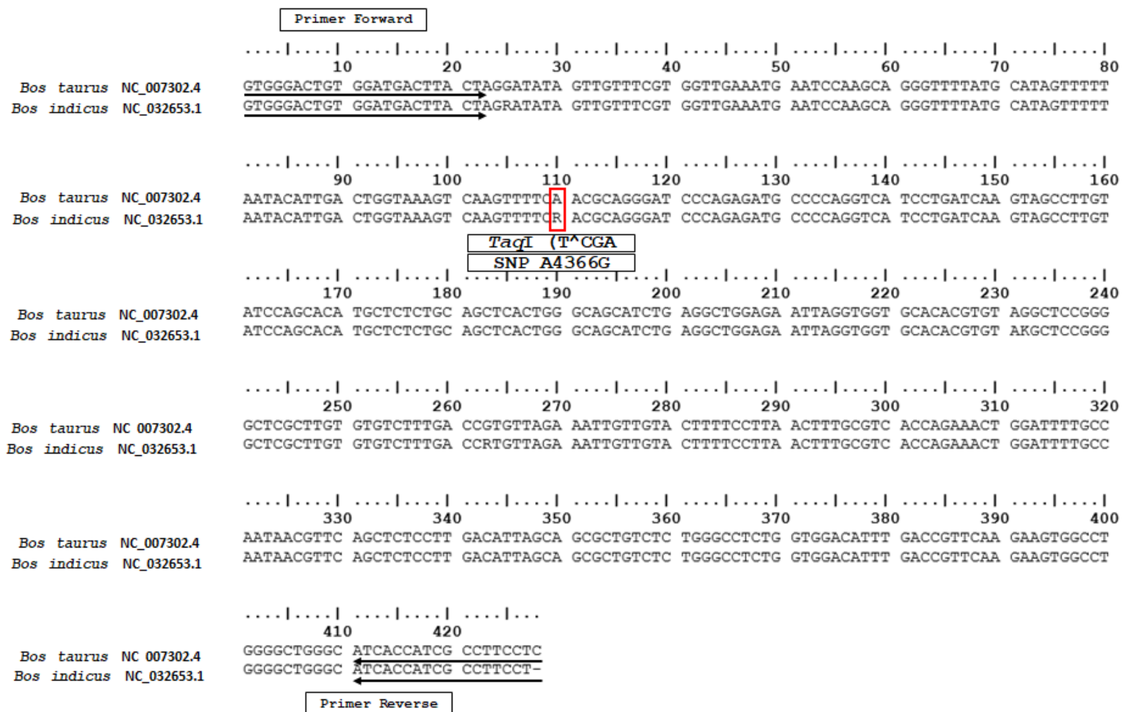

Figure 1. Location of SNP 4366 A>G and restriction site of *Taq*I enzyme (5′-T^CGA-‘3) at *INSIG1* gene based on GenBank Accesion No. NC_007302.4 (*Bos taurus*) and NC_032653.1_Nellore cattle (*Bos indicus*)

He = expected heterozygosity value
N_{ij} = number sample of diploid individuals with genotype A_iA_j and $i \neq j$
N = number of samples

Meanwhile, the genetic equilibrium was calculated according to Hardy Weinberg Equilibrium. Chi-Square test used this formula [21].

$$x^2 = \frac{(O - E)^2}{E}$$

Where:
χ^2 = chi-square test
O = observed value
E = expected value

The homogeneity test (Levene's test) of the data was calculated using SPSS program. If the result showed p>0.05, the population was in homogeneity while if P<0.05 showed heterogeneity. Association of *INSIG1* gene genotypes with s slaughter weight, hot carcass weight and dressing percentage of slaughter cows were investigated using ANOVA univariate animal model. Data were analysed using IBM SPSS Statistic software version 25.0 with following formula [17]:

$$Yijkl = \mu + Bfi + Mj + Gk + eijkl$$

Where:
Yijkl= traits observed
μ = overall population mean
Bfi = fixed effect of the ith breed and farm
Mj = fixed effect of the jth of slaughter
Gk = fixed effect of the kth single SNP marker genotype
eijkl = random error

RESULTS

Slaughter characteristics of unproductive Kebumen Ongole Grade

Slaughter characteristics in this study were body weight live before slaughtering (pre-slaughter weight), carcass weight and dressing percentage of slaughter cows. Kebumen Ongole Grade cows were culled at slaughter house in Kebumen Regency with the average age of cows was more than 8 years old.

Amplification and genotyping of *INSIG1* (A4366G) gene

A 428 bp of DNA fragment was successfully amplified using PCR method in Kebumen OG cattle (Figure 2). The amplicon was located at between intron 2 and exon 3, (GenBank accession No. NC_007302.4) while the SNP 4366 (A>G) was located at intron 2 which changed Adenine to Guanine. The SNP was recognized by *Taq*I (5′-T^CGA-'3) restriction enzyme. There were three genotype patterns: AA (a fragment of 428 bp), AG (428, 318 and 110 bp) and GG (318 and 110 bp). In this study was found all of genotype patterns (AA, AG and GG) of *INSIG1* genotype (Figure 3).

Genetic population of *INSIG1* (A4366G) gene

In the present study, the SNP of A4366G of *INSIG1* gene in unproductive Kebumen Ongole Grade cows was found to be polymorphic (Table 2). Genotype GG and allele G was found dominant. This population was in Hardy-Weinberg Equilibrium (HWE) (p>0.05).

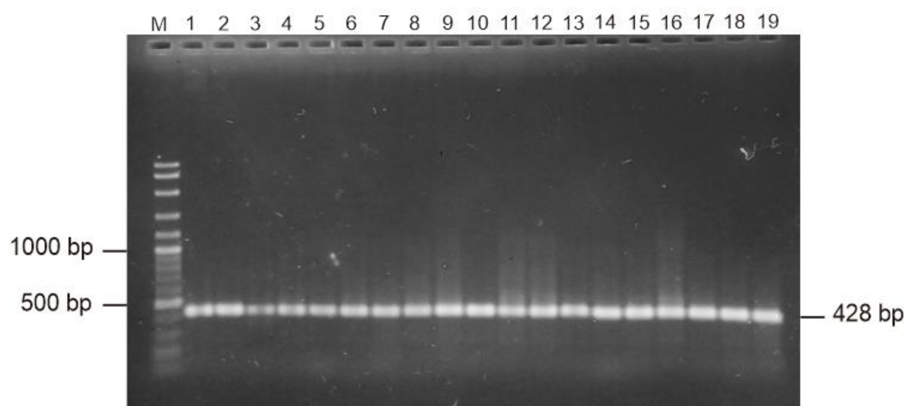

Figure 2. Visualization of PCR product of *INSIG1* gene (428 bp). M: Ladder 100 bp; 1-19: PCR product

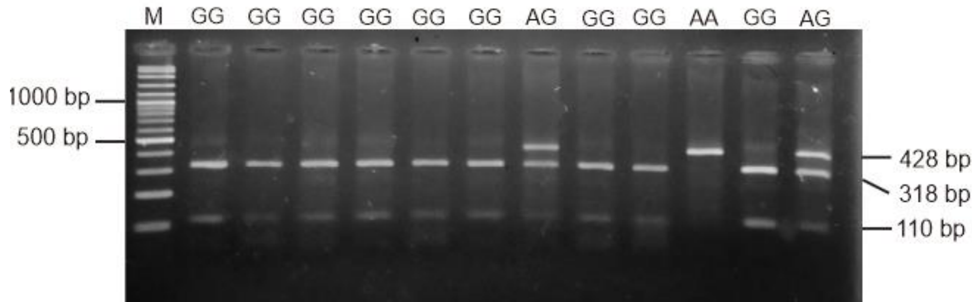

Figure 3. Visualization of RFLP's product of *INSIG1* gene. M: Ladder 100 bp; GG (318, 110 bp), AG (428, 318, 110 bp) and AA (428 bp) genotypes

Association of *INSIG1* (A4366G) gene with slaughter characteristics of unproductive Kebumen Ongole Grade cows

The homogeneity analysed in this study using Levene's test. According to Table 3, the Levene's test value was 0.096 for slaughter weight, 0.076 for hot carcass weight and 0.082 for dressing percentage that showed the population was in homogeneity (p>0.05). The association of slaughter weight, hot carcass weight and dressing percentage of slaughter cows with genotype of *INSIG1* gene (SNP A4366G) was not significant in this study with value 0.853; 0.807 and 0.805, respectively (p>0.05).

DISCUSSION

Meat productivity is an economically important trait in livestock production which growth process depend on genetic and environmental factors. Carcass composition is influenced by genetic, age, sex, nutritional and other environmental factors [22]. The parameter of slaughter characteristics in this study such as slaughter weight, carcass weight and dressing percentage of unproductive Kebumen OG cows. The average of carcass weight of Kebumen OG cows in this study reached 194.83 ± 36.71 kg. Meanwhile, carcass weight of Ongole Grade cows (> 4.5 years old) in Kebumen was 281.58 ± 15.69 kg [12] and 125.07 ± 21.47 kg for Ongole Grade cattle in Mersi Abattoir Purwokerto [23]. The dressing of carcass reached 47.66 ± 1.25% and have the same result with the previous studies approximately 47 – 48% [23, 12, 24].

Growth performance was influenced by genetic and environmental factors (P = G + E). *Insulin-induced gene-1* (*INSIG1*) gene is one of many genes that are considered play a role in influencing carcass characteristics [13]. Based on genetic population parameters in Kebumen OG cows population, the polymorphic of *INSIG1* gene (SNP A4366G) was found with three variant genotypes (AA, AG, and GG) and two variant alleles (A and G) (Table 1).

Table 1. Slaughter characteristics of unproductive Kebumen OG cows

Paramaeters	N	Min	Max	Mean±SD
Slaughter Weight (kg)	44	266	615.0	408.61 ± 75.04
Hot Carcass Weight (kg)	44	129	302.2	194.83 ± 36.71
Dressing percentage (%)	44	44.7	049.2	47.66 ± 1.25

Table 2. Genetic variations of *INSIG1* gene (4366 (A>G) in unproductive Kebumen OG cows

Samples	N	Genotypes frequencies			Allele frequencies		Ho	He	HWE ($\chi2$ Calculated)
		AA (2)	AG (14)	GG (28)	A	G			
Kebumen Ongole Grade	44	0.045	0.318	0.636	0.205	0.795	0.329	0.325	0.022 (Sig)

$\chi2$ table = 3.841; if $\chi2$ Calculated > $\chi2$ table was in NS (p>0.05) or genetic disequilibrium; if $\chi2$ Calculated < $\chi2$ table was in Sig (p<0.05) or genetic equilibrium

Table 3. Association of *INSIG1* gene (A4366G) with slaughter characteristics in unproductive Kebumen OG cows

Parameters	Genotypes		Levene's test	P values
	AA (N=2)	GG (N=28)		
Slaughter Weight (kg)	457.50 ± 92.63	407.82 ± 83.93	0.096	0.853
Hot Carcass Weight (kg)	217.00 ± 49.36	194.77 ± 41.38	0.076	0.807
Dressing percentage (%)	47.66 ± 1.25	47.71 ± 1.28	0.082	0.805

The previous study has the same results with Qinchuan cattle. There were three genotype variants i.e AA, AG and GG (0.1733; 0.4860; and 0.3407, respectively) with two alleles A and G (0.4163 and 0.5837) [17]. The G allele was the predominant in Kebumen Ongole Grade with a frequency of 0.795, which is higher than Qinchuan cattle (0.5837) [17]. It might be due to different both of sub species, Ongole Grade cattle was *Bos indicus* but Qinchuan cattle was *Bos taurus*. Based on admixture analysis (GeneSeek HD 77K BeadChip, which contained 76,999 SNPs), the Qinchuan cattle from China is dominant ancestral origin from *Bos taurus* with a considerable heritage of *Bos indicus* [25]. Meanwhile, Kebumen Ongole Grade directly linked with Nellore cattle (*Bos indicus*) [5].

For genetic diversity, the heterozygosity value (observed and expected) in this study reached 0.329 and 0.325, respectively. Whereas, heterozygosity value of *INSIG1* gene (A4366G) in Qinchuan cattle was higher than our study (0.5140 and 0.04860 for observed and expected heterozygosity, respectively) [17]. Measuring of heterozygosity as a genetic marker in animal livestock is much easier (genotyped individual) and may be a useful indicator when predicting the ability of purebreds to produce good crossbreds [26].

About genetic equilibrium, the unproductive Kebumen OG cows population was in Hardy Weinberg Equilibrium (HWE). The Chi-square calculated value (0.022) less than chi-square table (0.3841) (Table 2). HWE is a situation where genotypic frequency of two alleles in a gene locus remain constant from one generation to generation without evolutionary influences. Natural or artificial selection, mutation, assortative mating, migration, inbreeding, and random sampling are factors affecting HWE [27].

Based on the analysis, the association between genotype of *INSIG1* gene (AA, AG and GG) with slaughter characteristics (slaughter weight, carcass weight and dressing percentage) was not significant (p>0.05). However, the previous study [17] reported that nucleotide mutation Adenine to Guanine at 4366 nt of *INSIG1* gene was associated with hip width, slaughter and carcass weight in Qinchuan cattle. The other study, investigation of *INSIG1* gene have done by Sun et al. [28] in Nanyang cattle using three molecular technique i.e PCR-RFLP, PCR-SSCP and direct sequencing. Ten novel SNPs were detected which four mutations located in coding region and another one located in intron. There are seven common haplotypes which GACT haplotype was most prevalent haplotype in the coding regions. But, the association between the haplotypes with growth traits (6, 12, 18 and 24 month old) were not significant (p>0.05).

Kebumen Ongole Grade cattle is one of local cattle and have the great potential to be developed for accomplish of national protein needs. Genetic potential is need to investigate or identified for breeding program strategy. However, in the present study, the A4366G SNP of the *INSIG1* gene was not associated with slaughter characteristics in unproductive Kebumen Ongole Grade cows. Therefore, it is necessary to look for other regions of the *INSIG1* gene.

CONCLUSION

The SNP of A4366G of *INSIG1* gene in unproductive Kebumen Ongole Grade cows was found to be polymorphic with the dominant G allele. However, the association of slaughter weight, hot carcass weight, and carcass dressing percentage with genotype of *INSIG1* gene (A4366G) was not significant.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

AUTHOR'S CONTRIBUTIONS

SDV, SA, PS, I, ASW and MC have the same contribution as main contributors while ETM, MFN, and IR were as member contributors in this paper. SDV and SA designed and performed the experiments. IR gave an access for collecting sample in slaughter house. SDV, MC and PS collected the phenotypic data and blood samples. SDV, I and ASW extracted DNA from the samples and were responsible for PCR-RFLP analysis. SDV and SA analysed the data. All authors wrote, read and approved the final manuscript.

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