http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.741.747

Research Article
Mapping Growth Hormone Gene of Body Weight Krui Cattle in Pesisir Barat Regency Lampung, Indonesia

Akhmad Dakhlan, Kusuma Adhianto, Sulastri, Dian Kurniawati, Ratna Ermawati and Tri Doni Saputra

Department of Animal Husbandry, Faculty of Agriculture, Universitas Lampung, Indonesia

Abstract

Background and Objective: The growth hormone (GH) gene plays a role in meat growth and has been shown to increase the growth rate and carcass composition after being given GH. For this function, this gene is used as a strong candidate for genetic markers for meat growth traits. The research objective was to map the growth hormone (GH) gene of the bodyweight of Krui cattle in the Pesisir Barat Regency. **Materials and Methods:** This research used 30 blood samples of 30 Krui cattle. The method used was by taking quantitative data and blood samples from adult Krui cattle in Pesisir Barat Regency and then the blood samples were analyzed by DNA isolation method. PCR amplification used was a pair of GH-Forward primers: 5 'ATC CAC ACC CCC TCC ACA CAGT 3' and GH- reverse: 5 'CAT TTT CCA CCC TCC CCT ACA G 3', as well as digestion using the RFLP method at the Laboratory of Animal Breeding and Genetics of Universitas Gadjah Mada, Yogyakarta. Association between genotype and body weight was analyzed descriptively. **Results:** The results showed that Krui cattle had polymorphic genes with three genotypes found, namely: CC, CT and TT. Cattle with CT genotype had the largest average body weight or meat production compared to those with other genotypes. **Conclusion:** These results indicated that the GH gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes of body weight traits of Krui meat production in the Pesisir Barat Regency. Krui cattle with CT genotype can be developed further because it has high economic value with high average body weight and meat production.

Key words: Bodyweight, gene mapping, growth hormone, krui cattle, polymorphism, genetic approach, breeding

Citation: Dakhlan, A., K. Adhianto, Sulastri, D. Kurniawati, R. Ermawati and T.D. Saputra, 2022. Mapping growth hormone gene of body weight krui cattle in Pesisir Barat Regency Lampung, Indonesia. Pak. J. Biol. Sci., 25: 741-747.

Corresponding Author: Kusuma Adhianto, Department of Animal Husbandry, Faculty of Agriculture, Universitas Lampung, Indonesia
Tel: +62 81227972696

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Krui cattle is a local cattle in Pesisir Barat Regency, Lampung Province. Currently, Krui cattle by the Ministry of Agriculture of Republic Indonesia to be a local breed which is only available in Pesisir Barat Regency with No 693/KPTS/PK.040/M/11/2021. Krui cattle have a relatively small body size compared to other breeds of cattle in Indonesia such as Bali cattle and Madura cattle and it has characteristics that are different from the other local cattle in Indonesia. Krui cattle can survive in high ambient temperatures and low-quality feed[1]. The cattle are raised semi-intensively, during the day they are grazed until the afternoon and then the cattle are kept at night.

Krui cattle need to be preserved as germplasm originated from the Pesisir Barat Regency because the longer the existence of Krui cattle will be increasingly rare if not paid attention. The current belief is that the performance of Krui cattle always decreases from year to year due to the decreasing quality of the environment for the cattle and the possibility of ongoing inbreeding. This is due to the absence of a clear breeding program. To improve the performance of Krui cattle production, it is necessary to apply careful and continuous selection. Selection based on individual livestock records through a quantitative genetic approach can improve the genetic quality of livestock and their productivity. However, this method requires a very long time and accuracy in the recording. In addition, this conventional selection is less accurate and does not cover the genotypic aspects of livestock that reflect their potential genetic advantage.

The growth hormone (GH) gene plays a role in meat growth and has been shown to increase the growth rate and carcass composition after being given growth hormone[2]. For this function, this gene is used as a strong candidate for genetic markers for meat growth traits[3]. This trait is the most concerning aspect in improving the genetic quality of beef cattle. The mapping between the GH gene and growth traits in beef cattle can be seen by linking the mapping of GH genes to the growth traits of beef cattle.

Growth hormone (GH) plays a role in physiological processes in livestock, such as growth, carcass composition and milk production. Allele variations in the GH gene have been reported to be associated with variations in milk production[4], carcass characteristics such as carcass weight gain, meat value, live weight and others[5].

Factors that influence meat growth are genetic, physiological and environmental. In terms of genetic factors, in general, the differences between breeds of cattle are caused by genetic differences that regulate a trait. Variations in the characteristics of production in each cattle in the same breed are due to heredity. Differences in production capability between breeds of cattle reflect genetic differences in terms of differences in the frequency of genes that regulate the quantity and quality of production[6].

Gene mapping is the variation in the frequency of alleles at a locus within a gene. Much research has been done in trying to determine the relationship of gene mapping to production traits[7]. If the relationship can be found and the relationship is close enough and is characteristic of the entire population, then it can be used for selection as an indicator of productivity. One way to detect polymorphisms is through the PCR-RFLP method[8].

Selection based on genetic markers has been believed to increase the efficiency and acceleration of selection implementation so that genetic progress can be increased[9]. This study aimed to determine the association or relationship between the genetic markers of growth hormone genes and the bodyweight of Krui cattle in the Pesisir Barat Regency.

MATERIALS AND METHODS

Study site: This study was carried out for 6 months from April-September, 2021 at two different locations, namely the Pesisir Barat Regency for the data collected and the Laboratory of Animal Breeding and Genetics of Universitas Gadjah Mada, Yogyakarta, Indonesia to analyze the blood samples.

Data collection and blood sampling: Data collection was carried out from thirty Krui cows aged 3-4 years reared by farmers in the Pesisir Barat Regency. The data collected were body weight, body length, shoulder height, chest girth and blood sample. About 5 mL of blood from each cow was collected and then put into a 15 mL propylene tube that contains the anticoagulant EDTA. Blood samples were analyzed at the Laboratory of Animal Breeding and Genetics of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Molecular DNA analysis: DNA isolation from cow blood was using the SDS-PK extraction method, which is a modification of the method of Putra *et al.*[10] The results of the isolation were then carried out through DNA electrophoresis and the results were seen visually using Ultraviolet (UV) light. Amplification of specific DNA fragments was using the PCR (Polymerase Chain Reaction) method. The results were seen visually using UV light. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was carried out on the GH gene target. The results were seen visually using a UV trans-

illuminator. PCR products that are recognized by this enzyme will have three possible results of digestion, namely: Two bands showing the CC genotype, three bands showing the CT genotype and one band showing the TT genotype[11,12].

The allele and genotype frequencies are calculated by the following formula Putra *et al.*[10]

Allele frequency:

$$A = \frac{\Sigma \text{locus A}}{\Sigma(\text{locus A} + \text{locus B})}$$

$$B = \frac{\Sigma \text{locus B}}{\Sigma(\text{locus A} + \text{locus B})}$$

Genotype frequency:

$$AA = \frac{\Sigma \text{locus AA}}{\Sigma \text{individual in population}} \times 100$$

$$AB = \frac{\Sigma \text{locus AB}}{\Sigma \text{individual in population}} \times 100$$

$$BB = \frac{\Sigma \text{locus BB}}{\Sigma \text{individual in population}} \times 100$$

If the calculation of the allele frequency of GH genes found in the studied population did not exceed 0.99, then the GH gene was categorized as polymorphic. The mapping of the GH gene on the growth traits of meat was analyzed using descriptive methods.

Data analysis: The allele frequency was used to analyze the PCR-RFLP data. The allele frequency is calculated by the formula[10]:

$$p = \frac{2(CC) + (CT)}{2N}$$

$$q = \frac{2(ST) + (CT)}{2N}$$

Where:
p = Frequency of the C allele
q = Frequency of T and alleles
N = Number of cows tested data

The sequences were parallelized according to the GH gene sequence from the access code GenBank M57764 by alignment software (BioEdit and ClustalW).

RESULTS AND DISCUSSION

The mean, standard deviation and variation coefficient of body measurements of Krui cattle in the Pesisir Barat Regency were presented in Table 1.

Body weight: The average body weight of Krui cattle in Pesisir Barat Regency is 163.87±38.00 kg. Lamy *et al.*[13] stated that the factor affecting body weight and cattle production is nutritional adequacy, if livestock is deficient in vitamins and minerals, it will affect the metabolic process which results in inhibition of productivity and growth. According to Aidan *et al.*[6], two factors influence growth in beef cattle, namely internal factors (breed, age, genetics, gender and hormones) and external factors (feed, environmental temperature, disease, environmental stress and work/training).

Body length: The results showed that the average body length of Krui cattle in Pesisir Barat Regency was 100.93±5.93 cm. Body length is influenced by feed and maintenance management in cattle. If the livestock ratio during growth is deficient in nutrients, the bone formation will be less than perfect. Body length also has a relationship with body weight. According to Abadi *et al.*[14], body length shows spinal growth that includes the spine and lumbar. The direction of livestock growth begins with upward growth then extends and then circles on the chest.

Shoulder height: The results showed that the average shoulder height of Krui cows in the Pesisir Barat Regency was 100.03±15.03 cm. Shoulder height is one of the body measurements that can be used as supporting data in determining livestock performance. The relationship between shoulder height and weight will get tighter over time[14]. Body measurements such as chest girth, body length and shoulder height can provide indications of body weight with sufficient

Table 1: Mean of body weight and body measurements of Krui cattle in the observation area

	Population
Quantitative variable	(Pesisir Barat Regency) (n = 30)
Body weight (kg)	163.87±38.00
Body length (cm)	100.93±5.93
Shoulder height (cm)	100.03±15.03
Hip height (cm)	103.52±13.52
Chest width (cm)	30.62±7.62
Chest girth (cm)	125.97±11.97
Chest length (cm)	54.83±13.83
Hip circumference (cm)	137.31±18.31
Head width (cm)	42.52±2.52
Head length (cm)	18.10±2.1

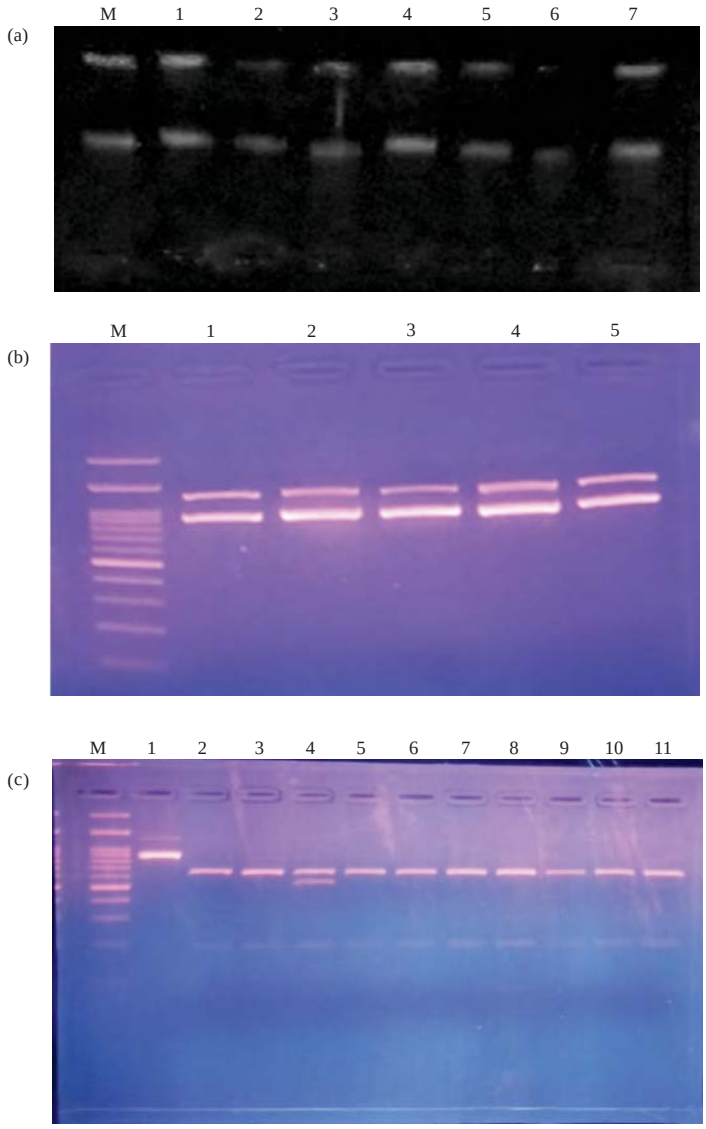

Fig. 1(a-c): Results of visualization of DNA products, (a) Isolation of DNA, (b) PCR and (c) PCR-RFLP

accuracy. The correlation between chest circumference, body length and shoulder height with live weight is very high compared to other body measurements[15].

Chest girth: The results showed that the mean chest circumference of Krui cattle in Pesisir Barat Regency was 125.97±11.97 cm. The average Krui cattle chest girth is thought to be influenced by environmental factors, including maintenance management and geographical conditions. According to Abadi *et al.*[14] chest girth has the closest relationship with body weight, the relationship between body weight and chest girth lasts more until an older age than the close relationship between body weight and other body measurements. Chest girth is the best parameter for estimating body weight at all age levels. The increase in body weight will be followed by an increase in chest girth[16].

DNA analysis

DNA isolation: DNA isolation from cow blood using the SDS-PK extraction method, which is a modification of the method of Putra *et al.*[10]. The results of electrophoresis of genome DNA from cow blood samples were visualized using 0.8% agarose gel with ethidium bromide dye which fluoresces in ultraviolet light. The DNA results look like in Fig. 1a.

The results of electrophoresis showed a thick and bright visible band. The DNA image that appears on the electrophoresis results is an illustration of the success rate of the extracted DNA. The bright band indicates that DNA

has been isolated, where the thicker the band, the more DNA is obtained. According to Putra *et al.*[10] DNA extraction from eukaryote organisms (humans, animals and plants) is carried out through the process of destroying cell walls, removing proteins and ribonucleic acid (RNA), DNA deposition and harvesting.

Polymerase Chain Reaction (PCR): Amplification of specific DNA fragments (growth genes) using the PCR (Polymerase Chain Reaction) method is an in vitro DNA synthesis technique that can rapidly multiply DNA fragments of a certain size enzymatically through the mechanism of temperature change[10]. The primers used in this study were according to Putra *et al.*[10] used a pair of primers, GH-forward and GH-reverse. GH-Forward: 5'ATCCACACCCCCTCCACACAGT3' and GH-Reverse: 5'CATTTTCCACCCTCCCCTACAG3'. The PCR program includes predenaturation: 97°C 1 min 30 sec, denaturation: 94°C 1 min, 65°C 1 min annealing, 1 min 72°C extension and 72°C 1 min final extension, 34 times.

Mapping detection of growth hormone (GH) genes in cattle in this study was carried out using PCR techniques, namely amplification of specific DNA fragments along 891 bp. The results of electrophoresis of PCR products using 3% agarose gel were shown in Fig. 1b.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP): The detection of growth hormone (GH) gene polymorphisms in Krui cattle in this study was carried out using the PCR-RFLP technique, namely the amplification of specific DNA fragments along 891 bp which was then followed by cutting (digestion) at a certain point using the Msp1 enzyme.

Digestion electrophoresis used 10% polyacrylamide gel because 10% polyacrylamide gel has a higher resolution capacity than agarose gel and can separate DNA in a narrow size range so that 52 bp fragments can be seen. The results of electrophoresis of PCR-RFLP products using 10% polyacrylamide gel were shown in Fig. 1c.

Digestion of PCR products along 891 bp with restriction enzyme Msp1 in this study produced two alleles, namely C and T alleles. The CC genotype was characterized by cutting DNA at the 194, 63, 110 and 524 bp positions. The CT allele was characterized by cutting DNA at the 194, 63, 110, 524 and 634 bp positions. The TT allele was characterized by cutting DNA at the following positions: 194, 63 and 634 bp. The PCR-RFLP results in this study showed that there were CC, CT and TT genotypes in Krui cattle.

Table 2: Allele genotype frequencies of GH genes in Krui cattle (N = 30)

Genotype frequency		Allele frequency	
TT	0.90	T	0.93
CT	0.07	C	0.07
CC	0.03		

TT: Thymine-thymine genotype, CT: Cytosine-thymine genotype, CC: Cytosine-cytosine genotype, T: Thymine allele and C: Cytosine allele

Allele and genotype frequency: Allele frequencies and genotypes of GH genes in this research of Krui cattle were presented in Table 2. Krui cattle population was polymorphic because the frequency of the common allele (T allele) is not more than 0.99. The frequency of the T allele of the GH gene in Krui cattle was 0.93 and the C allele was 0.067. The results of allele frequency calculations show polymorphism, which is stated by Putra *et al.*[10] that if the common allele frequency of the GH gene found is not more than 0.99, the cow population studied is polymorphic.

Genotype frequency of CC in GH-MSp1 gene in Bali cattle was 0.00, CT genotype was 0.00 and TT genotype was 1.00. The frequency of the C allele of the GH-MSp1 gene in Bali cattle was 0.00 and the T allele was 1.00. Genotype frequency of CC in GH-MSp1 gene in Pesisir cattle was 0.05, CT genotype was 0.30 and TT genotype was 0.65. The frequency of the C allele of the GH-MSp1 gene in Pesisir cattle was 0.20 and the T allele was 0.80[10]. Genotype frequency of CC in the GH-MSp1 gene in Madura cattle was 0.23, CT genotype was 0.22 and TT genotype was 0.55. The frequency of the C allele of the GH-MSp1 gene in Madura cattle was 0.74 and the T allele was 0.26. Genotype frequency of CC in GH-MSp1 gene in Ongole cattle was 0.43, CT genotype was 0.50 and TT genotype was 0.07. The frequency of the C allele of the GH-MSp1 gene in Ongole cattle was 0.74 and the T allele was 0.26[17].

Genotype association with body weight or Krui beef production: The association between GH gene genotype and the average body weight of the research cattle was Krui cattle with TT genotype had 182.92 kg, CT genotype had 241.50 kg and CC genotype had 160.00 kg. The results showed that Krui cattle with the CT genotype had the greatest average body weight or meat production compared to those with other genotypes. Thus these results showed that the GH MspI gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes on body weight or Krui cattle production in Pesisir Barat Regency. Several other studies related to the polymorphism of the GH MspI gene reported that the heterozygous genotype (CT) had a bodyweight greater than the homozygous genotype (CC/TT) in Chinese cattle[18].

The results of this study were in line with the results of research by Hartati *et al.*[19] on the mapping of the GH gene in the analysis of the relationship between allele types and growth traits in Ongole Grade cattle (PO cattle), it was found that the CT genotype of GH gene had a significant effect on meat growth, where cattle with CT genotype were individuals who were superior in meat growth. Thus, Krui cattle with CT genotype can be developed further because they have high economic value with high average body weight and meat production.

CONCLUSION

The results showed that in Krui cattle there were three kinds of genotypes, namely, CC, CT and TT. The CT genotype has the largest average body weight or meat production compared to cows with other genotypes. Thus these results showed that the GH gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes on body weight traits or Krui beef production in Pesisir Barat Regency. Thus, Krui cattle with CT genotype can be developed further because they have high economic value with high average body weight and meat production.

SIGNIFICANCE STATEMENT

This study found high mapping of growth hormone gene activity in 3 types of genotype that can be beneficial for a strong candidate for genetic markers for meat growth traits. This study will help the researchers to uncover critical areas of finding alternative methods for solving the self-sufficiency in meat quickly through genetic markers that many researchers are unable to explore. This finding reinforces that new theories on candidate genetic markers for meat growth traits can arrive in the near time.

REFERENCES

1. Prawira, H.Y., Muhtarudin and dan Rudy Sutrisna, 2015. Potential development of beef cattle farming in Tanjung Bintang District, South Lampung Regency. J. Ilmiah Peternakan Terpadu, 3: 250-255.
2. Rehfeldt, C., M.F.W.T. Pas, K. Wimmers, J.M. Brameld and P.M. Nissen *et al.*, 2011. Advances in research on the prenatal development of skeletal muscle in animals in relation to the quality of muscle-based food. I. Regulation of myogenesis and environmental impact. Animal, 5: 703-717.
3. Silveira, L.G.G., L.R. Furlan, R.A. Curi, A.L.J. Ferraz and M.M. de Alencar *et al.*, 2008. Growth hormone 1 gene (*GH1*) polymorphisms as possible markersof the production potential of beef cattle using the Brazilian Canchim breedas a model. Genet. Mol. Biol., 31: 874-879.
4. Rotwein, P., 2020. Regulation of gene expression by growth hormone. Mol. Cell. Endocrinol., Vol. 507. 10.1016/j.mce.2020. 110788.
5. Grochowska, R., P. Sørensen, L. Zwierzchowski, M. Snochowski and P. Løvendahl, 2001. Genetic variation in stimulated GH release and in IGF-I of young dairy cattle and their associations with the leucine/valine polymorphism in the GH gene. J. Anim. Sci., 79: 470-476.
6. Aidan, P., Moloney and M. McGee, 2017. Factors Influencing the Growth of Meat Animals. In: Lawrie's Meat Science, Toldra, F. (Ed.), Woodhead Publishing, United Kingdom, ISBN: 978-0-08-100694-8, pp: 19-47.
7. Williams, K.L., 2018. Gene mapping. Encycl. Bioinf. Comput. Biol., 2: 242-250.
8. Wang, S., X. Duan, T. Wang, X. Feng and P. Wang *et al.*, 2016. Detection of the rs10250202 polymorphism in protection of telomeres 1 gene through introducing a new restriction enzyme site for PCR–RFLP assay. SpringerPlus, Vol. 5. 10.1186/ s40064-016-2214-5.
9. Ge, W., M.E. Davis, H.C. Hines, K.M. Irvin and R.C.M. Simmen, 2001. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth trains in Angus cattle. J. Anim. Sci., 79: 1757-1762.
10. Putra, W.P.B., T. Hartatik and S. Sumadi, 2013. Growth hormone gene genotyping by MspI restriction enzyme and PCR-RFLP methods in aceh cattle breed at indrapuri district of aceh province. J. Indonesian Trop. Anim. Agric., 38: 207-211.
11. Rashijane, L.T., V.G. Mbazima and T.L. Tyasi, 2022. Polymorphism of growth hormone gene and its association with body measurement traits in boer goat does. S. Afr. J. Anim. Sci., 52: 44-49.
12. Kaplan, R.C., A.K. Petersen, M.H. Chen, A. Teumer and N.L. Glazer, 2011. A genome-wide association study identifies novel loci associated with circulating IGF-I and IGFBP-3. Hum. Mol. Genet., 20: 1241-1251.
13. Lamy, E., S. van Harten, E. Sales-Baptista, M.M.M. Guerra and A.M. de Almeida, 2012. Factors Influencing Livestock Productivity. In: Environmental Stress and Amelioration in Livestock Production, Sejian, V., S.M.K. Naqvi, T. Ezeji, J. Lakritz and R. Lal (Eds.), Springer, Berlin, Heidelberg, ISBN: 978-3-642-29205-7, pp: 19-51.
14. Abadi, M., L.O. Nafiu, A.S. Aku and R. Badaruddin, 2020. Correlation of body weight and age on the selling price of Bali cattle Ranomeeto District, Konawe Selatan Regency. Int. J. Sci. Res. Sci. Eng. Technol., 7: 26-32.

15. Tyasi, T.L., N.D. Mathye, L.W. Danguru, L.T. Rashijane and K. Mokoena *et al*., 2020. Correlation and path analysis of body weight and biometric traits of Nguni cattle breed. J. Adv. Vet. Anim. Res., 7: 148-155.
16. Nurgiartiningsih, V.M.A., A. Budiarto, Kusmartono and Suyadi, 2016. Evaluation of performance in female Madura cattle in Madura Island, Indonesia. Anim. Prod., 18: 125-130.
17. Vulkandari, S.D., T. Hartatik and dan Sumadi, 2013. Polimorfisme gen growth hormone (GH) pada sapi limura (growth hormone (GH) gene polymorphism of Limura cattle), Buletin Peternakan, 37: 67-73.
18. Zhang, B., G. Zhao, X. Lan, C. Lei, C. Zhang and H. Chen, 2011. Polymorphism in *GHRH* gene and its association with growth traits in Chinese native cattle. Res. Vet. Sci., 92: 243-246.
19. Hartati, H., A.A.R. Hapsari, B.D.P. Soewandi, S. Anwar, S.P. Rahmadani, A. Aryogi and D. Pamungkas, 2021. Association of GHR|AluI gene polymorphism with body weight parameters in Grati-PO cattle. J. Indonesian Trop. Anim. Agric., 46: 272-281.