POLYMORPHISM OF *GROWTH HORMONE 1* GENE IN CO AND BACH THAO BREED GOATS: IS THE RECESSIVE HOMOZYGOUS BB GENOTYPE LETHAL?

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ABSTRACT

Goat is one of the most popular species in Vietnamese animal husbandry. However, phenotypic selection in goats is still popular in breeding programs. Thanks to the introduction of marker-assisted selection, genetics is currently playing a vital role in choosing the elite animals for breeding. One of the molecular markers that has been widely applied in animal husbandry is the *GH1* gene (growth hormone 1), which encodes for the GH protein. GH has been known for its essential role in the growth and development of animals. In goats, GH1 polymorphism is significantly associated with performance traits. In this study, we aim to investigate the GH1 frequency in two indigenous goat breeds in Vietnam: the Co and Bach Thao breeds. Our results showed that there were only two genotypes AA and AB detected in Co and Bach Thao breeds. In the Co goat breed, the frequency of AA and AB genotypes was 0.1 and 0.9, respectively. The frequency of A and B alleles was 0.55 and 0.45, respectively. In the Bach Thao goat breed, the frequency of AA and AB genotypes was 0.16 and 0.84, respectively. The frequency of the A and B alleles was 0.58 and 0.42, respectively. Chi-square values obtained from the Co and Bach Thao goat breeds revealed that there was a significant deviation from the Hardy-Weinberg Equilibrium in both breeds (33.47 and 32.31, respectively, with $P<0.001$). The lack of BB genotype led us to hypothesize that there was overdominance in three genotypes of GH1 and that the BB homozygous genotype is lethal. We suggested that phenotypic observation would be recommended to measure the superiority of the AB genotype goat compared to the AA genotype goats. The results from this study would be beneficial to goat breeders in improving the goat breeding program in Vietnam.

Keywords: Goat, GH1, Bach Thao, Co, PCR-RFLP, Marker-Assisted Selection, polymorphisms.

INTRODUCTION

Humans have been farming goats for centuries because of their need for meat, milk, and fiber for clothes. As a result, goat is widely acknowledged for its indispensable contributions in terms of socioeconomics, culture, and religious beliefs in different societies. Such traditional pastoralism is still playing a vital role in sustainable development and providing stable income for communities residing in the midlands, northern highlands, and central coastal provinces of Vietnam (Thuy *et al*., 2017). Currently, eight goat breeds are being bred for either meat or milk production in Vietnam, two of which are indigenous breeds: Co and Bach Thao goat. Co goat is distributed nationwide yet more common in the midland regions and is reared for meat consumption, while Bach Thao goat can be found in central coastal provinces and is raised for meat and milk (CountryReport, 2003).

In animal husbandry, growth is an important trait that determines the economic benefits for the breeders. Many factors, including genetics, nutrition, environment, and sex, are documented to influence the growth trait (Mahrous *et al*., 2018; Gitanjli *et al*., 2020). Growth hormone (GH) is a peptide hormone that belongs to the somatotropin/prolactin family and is secreted from the anterior lobe of the pituitary gland (Procter *et al*., 1998; Giordano *et al*., 2001). GH affects various biological aspects of the humans and animals, such as growth, development, lactation, and reproduction (Wagner *et al*., 2005; Akers, 2006; Esteban *et al*., 2007; Gao *et al*., 2010; Li *et al*., 2021).

Mountains of evidence showed the pivotal role of GH in selecting elite animals with agriculturally desirable characteristics. Therefore, by applying marker-assisted selection, animals with superior quality could be selected more accurately and rapidly. GH1 is one of the major molecular markers that has been widely implemented by animal breeders. In cattle, positive correlations have been found between GH1 and performance traits such as intramuscular fat, and milk quality (Barendse *et al*., 2006; Dario *et al*., 2008; Curi *et al*., 2010; Pal *et al*., 2014; Dettori *et al*., 2015). While GH1 significantly affects body size and disease resistance in pigs and chickens (Liu *et al*., 2001; Cheng *et al*., 2016; Babicz *et al*., 2020), respectively, associations have been found between GH1 and weight in rabbits and ducks (Fontanesi *et al*., 2012; Mazurowski *et al*., 2015).

In goats, the *GH1* gene is found to be located on the short arm of chromosome 19, containing five exons and four introns (An *et al*., 2011). There have been many studies on polymorphisms in the goat *GH1* gene in different goat breeds, such as Boer, Sihori, and Barbari (Hua *et al*., 2009; Gitanjli *et al*., 2020). *GH1* polymorphisms have been associated with agriculturally desirable traits including body weight, body height and length, lactation, litter size, and metabolism in different breeds including Chinese (An *et al*., 2011), Polish (Majewska *et al*., 2020), Indonesia (Ilham *et al*., 2016), Egypt (Mahrous *et al*., 2018), India (Radhika *et al*., 2016), Iraq (Abbas *et al*., 2022), South Africa (Rashijane *et al*., 2022). In this study, we conducted a PCR-RFLP method to detect polymorphisms in the *GH1* gene in Co and Bach Thao goats. To the best of our knowledge, this is the first study in Vietnam to investigate the genetic distribution of the GH1 gene in indigenous goat breeds.

MATERIALS AND METHODS

Animals

In this study, a total of 112 goats were selected from two populations located in Ninh Thuan province (Co, n = 50; Bach Thao, n = 62). All the chosen goats were healthy and unrelated. Blood samples were aseptically collected from the jugular veins of the animals as previously described (Tinh *et al.*, 2021).

DNA extraction

The genomic DNA was extracted using Kit GeneJet Whole Blood Genomic DNA Purification (Thermo Fisher Scientific) according to the manufacturer's manuals. The integrity of the extracted DNA was analyzed by electrophoresis. The concentration of the DNA was determined on a Nanodrop 2000 spectrophotometer (Thermo Scientific). Samples with a 260/280 ratio between 1.8 and 2.0 were diluted to a final concentration of 50 ng/µL and used for PCR amplification.

PCR amplification

The polymerase chain reaction was performed in a 12 µL test tube including 6 µL of 2X MasterMix (Salagene, Vietnam), 1 µL of primers (0.5 µL each), and 5 µL of distilled water. The information on *GH1* primers is listed in Table 1.

The reaction conditions for PCR were as follows: pre-incubation at 94°C for 5 minutes; 35 cycles including denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, extension at 72°C for 30 seconds; 1 cycle of final extension at 72°C for 7 minutes. The PCR reaction was carried out in the thermal cycler Gene Amp PCR System (Thermo Fisher Scientific). After thermal cycling, 5 µL of the amplified products were analyzed by horizontal electrophoresis on a 1% agarose gel. The electrophoresis conditions were 150V for 30 minutes using 0.5X Tris/Borate/EDTA (TBE) buffer along with a 100 bp molecular weight marker to determine the size of the amplified fragments. Gel images were captured by GelDoc-It2 (UVP, USA).

Restriction digestion

In the digestion step, *Hae*III, whose recognition site was 5′-GGCC-3′, was utilized to digest the PCR products. Specifically, 6 µL of PCR fragment was added to a 6 µL digestion mix entailing 1 µL of *Hae*III, 1 µL of rCutSmart buffer, and 4 µL of distilled water. Then, the samples were incubated in the incubator for 3 hours at 37°C. The PCR-RFLP result was eventually analyzed with 3% agarose gel electrophoresis.

Table 1. Sequence, melting temperature, and expected product length of *GH1* primer pairs.

Primers	Sequence (5'-3')	Tm ($^{\circ}$C)	Expected product length
Forward	CTCTGCCTGCCCTGGACT	61.01	422 bp
Reverse	GGAGAAGCAGAAGGCAACC	58.44	

Allelic and genotypic frequency calculation

The allele and genotype frequencies of the *GH1* gene were calculated and the Chi-square test was used to examine whether the populations of the two goat breeds are in the Hardy-Weinberg equilibrium for the *GH1* gene.

RESULTS AND DISCUSSION

After successful amplification of a 422 bp amplicon corresponding to exon 2 and exon 3 of the GH1 gene, restriction digestion of the PCR product was followed (Figure 1). The nucleotide substitution of denine to guanine at position 718 in cDNA, resulting in an amino acid change from serine to glycine at residue 35. Due to this single nucleotide polymorphism (A to G), the restriction site recognized by the *Hae*III enzyme was abolished, thus generating two bands for the AA genotype (366 bp and 56 bp), three bands for the AB genotype (42 2bp, 366 bp and 56 bp) and one band for the BB genotype (422 bp).

In our study, only two genotypes AA and AB were found in Co and Bach Thao breeds. The frequency of AA and AB genotypes was 0.13 and 0.87, respectively. The frequency of A and B alleles was 0.71 and 0.29, respectively (Table 2). Chi-square value revealed that there was a significant deviation from the Hardy-Weinberg Equilibrium in both breeds (32.89 with P < 0.001) (Table 3). The PCR products harboring AA and AB genotypes from the Co and Bach Thao breeds were sequenced for verification. Such a departure from the equilibrium can be explained by the lack of BB samples.

Our hypothesis from this study is that the B allele is recessive and lethal in both the Co and Bach Thao breeds. Our explanation could be supported by various studies that indicated the lack of BB genotype in other goat breeds. Additionally, there is a clear trend in the selection of the B allele in favor of the A allele. In 2008, Hua *et al.* reported that in 154 Boer goats, the genotypes AA and AB were 0.17 and 0.83, respectively (Hua *et al.*, 2009). In 2011, the data from 685 purebred and crossbred Chinese goats displayed the frequency of AA and AB genotypes was 0.37 and 0.65, respectively (An *et al.*, 2011). In 2011, Zhang *et al.* examined two breeds in 534 goats and documented that the frequency of AA and AB genotypes was 0.21 and 0.79, respectively (Zhang *et al.*, 2011). In 2016, a study conducted in Indonesia on the Kacang goat breed also indicated that the frequency of AA and AB genotypes in 168 goats was 0.0.95 and 0.094, respectively (Ilham *et al.*, 2016). In 2022, Rashijane *et al.* showed that the frequency of AA and AB genotypes in the Boer goat breed was 0.79 and 0.21, respectively (Rashijane *et al.*, 2022). In 2022, a study performed in Iraq exhibited that the frequency of AA and AB in five local goat breeds was 0.114 and 0.888, respectively (Abbas *et al.*, 2022). In these previous results, AB goats have better performance compared to AA goats, suggesting the overdominance phenomenon of GH1 in goat populations.

We proposed that there was a balancing selection, or overdominance in the *GH1* gene of goats. Specifically, the AB goats have better performance than the AA goats in agriculturally desirable traits, such as growth, development, and reproduction. There is clear evidence of the higher frequency of the B allele compared to the A

allele, confirming the benefits of the B allele in the breeding program. However, since BB goats are lethal, we could not detect any individual goat with the BB genotype. The homozygous BB animals might be associated with early embryonic lethality since the GH gene is found to get involved in the developmental stages of an organism (Kiapekou *et al*., 2005; Silva *et al*., 2009). Hence, the BB homozygotes fail to develop into full-term bodies, and eventually can not be observed.

In other animals, overdominance phenomena were also described (Bui *et al*., 2023). In pigs, accumulation of the recessive lethal alleles of documented genes, including *TADA2A*, *URB1*, *PORL1B, PKNP* and *BBS9,* resulted in embryonic and piglet lethality, respectively (Derks *et al*., 2019; Derks *et al*., 2018). Nevertheless, heterozygotes carrying one copy of the mutant allele of those genes could produce animals with better reproductive traits. In cattle, heterozygotic animals with the mutation in the *RNASEH2B* gene encoding

for the non-catalytic subunit of RNase H2, an endonuclease that specifically degrades the RNA of RNA, showed a significant effect on milk yield and composition (Kadri *et al*., 2014). In alpacas, the grey color phenotype is also autosomal dominant and homozygous recessive individuals are likely to be lethal before birth (Jones *et al*., 2019).

We hypothesize that in Co and Bach Thao goats, the BB genotype is lethal and the AB genotype is overdominant compared to the AA genotype. Our hypothesis was previously proposed by Seevagan, who suggested that the recessive lethality of *GH1* is also present in sheep since the authors could not detect the recessive homozygous genotypes (Seevagan *et al*., 2015). Due to the limitations of our study, we would like to recommend that phenotypic observations should be measured to confirm the superiority of AB goats to AA goats in the future. This will help breeders select the elite goats for breeding programs by applying *GH1* as the molecular marker.
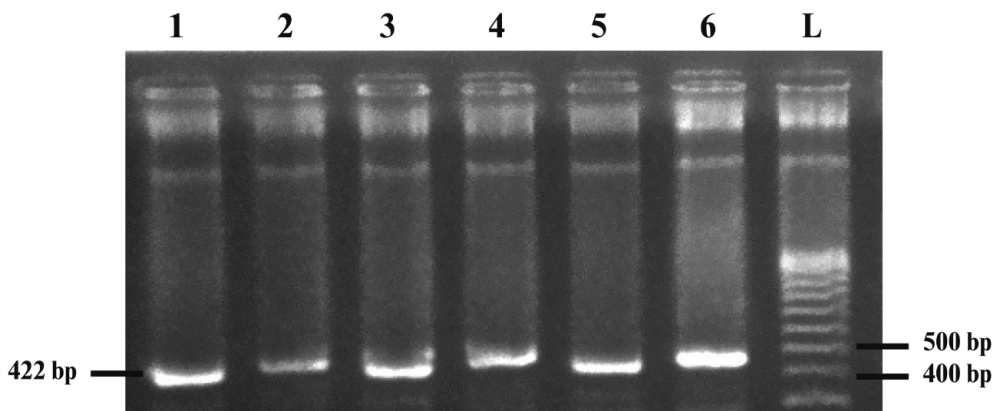

Figure 1. Gel electrophoresis of *Capra GH1* gene PCR product. Lane 1-6: goat DNA samples (422 bp); L: DNA ladder; (-): negative control.
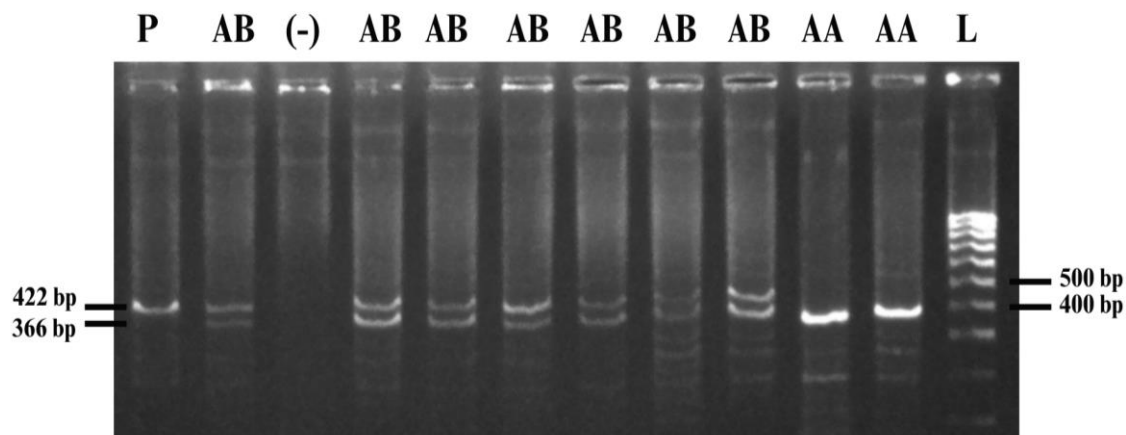
Figure 2. Digested products of *Capra GH1* gene, with AA (366 bp) and AB (422 and 366 bp) genotypes. P: undigested PCR product; (-): negative control; (L): DNA ladder.

Table 2. Genotypic, allelic frequencies, and Chi-square value of *Capra GH1* gene.

Breed	Population	Genotypic frequency n (%)			Allelic frequency		Chi-square test
		AA	AB	BB	A	B	
Co	50	5 (10)	45 (90)	0.0	0.55	0.45	33.47 (P<0.001)
Bach Thao	62	0.16	0.84	0.0	0.58	0.42	32.31 (P<0.001)

Table 3. Availability (A) observed and expected homozygosity and heterozygosity in GH1 locus of Boer and Bach Thao goat breeds.

Population	A	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity
Co	1.000	0.1	0.9	0.505	0.495
Bach Thao	1.000	0.161	0.839	0.513	0.487

CONCLUSION

In this study, we have successfully amplified the amplicon of *GH1* comprised of exon 2 and exon 3. By applying PCR-RFLP, we have detected the presence of two genotypes AA and AB of the *GH1* gene in the Co and Bach Thao goat populations. We hypothesized that the lack of the BB genotype was due to the lethality of the B allele. In the future, it is desirable to make phenotypic observations to compare the overdominance effect of the B allele in Co and Bach Thao goats.

Animal Sciences for Southern Vietnam for their contribution to sample preparation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Abbas, A. M., J. M. S. J., & Mohammed, A. B. (2022). GROWTH HORMONE GENE POLYMORPHISM IN DOMESTIC AND WILD GOAT BREEDS IN KURDISTAN REGION OF IRAQ USING PCR-RFLP AND SNP MARKERS. *Bulgarian Journal of Veterinary Medicine*.

Akers, R. M. (2006). Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *J Dairy Sci*, *89*(4), 1222-1234. https://doi.org/10.3168/jds.S0022-0302(06)72191-9.

An, X., Wang, L., Hou, J., Li, G., Song, Y., Wang, J., Yang, M., Cui, Y., & Cao, B. (2011). Novel polymorphisms of goat growth hormone and growth hormone receptor genes and their effects on growth traits. *Mol Biol Rep*, *38*(6), 4037-4043. https://doi.org/10.1007/s11033-010-0522-3.

Babicz, M., Pastwa, M., Kozubska-Sobocińska, A., Danielak-Czech, B., Skrzypczak, E., & Kropiwiec-Domańska, K. (2020). Association analysis of GH1 and CRP loci polymorphisms with reproductive traits in native Pulawska gilts and sows. *Canadian Journal of Animal Science*, *100*(4), 650-656. https://doi.org/10.1139/cjas-2019-0087.

Barendse, W., Bunch, R. J., Harrison, B. E., & Thomas, M. B. (2006). The growth hormone 1 GH1:c.457C>G mutation is associated with intramuscular and rump fat distribution in a large sample of Australian feedlot cattle. *Anim Genet*, *37*(3), 211-214. https://doi.org/10.1111/j.1365-2052.2006.01432.x.

Bui, A. P. N., Tam, T. L. H., Phuong, P. T., & Linh, T. T. (2023). Overdominance in livestock breeding: examples and current status. *Advancements in Life Sciences*, *10*(4), 525-529.

Cheng, Y., Liu, S., Su, D., Lu, C., Zhang, X., Wu, Q., Li, S., Fu, H., Yu, H., & Hao, L. (2016). Distribution and linkage disequilibrium analysis of polymorphisms of GH1 gene in different populations of pigs associated with body size. *J Genet*, *95*(1), 79-87. https://doi.org/10.1007/s12041-015-0611-0.

CountryReport. (2003). *The Vietnam National Country report on Animal Genetic Resources, NIAH, Hanoi, Vietnam*.

Curi, R. A., Chardulo, L. A. L., Giusti, J., Silveira, A. C., Martins, C. L., & de Oliveira, H. N. (2010). Assessment of GH1, CAPN1 and CAST polymorphisms as markers of carcass and meat traits in Bos indicus and Bos taurus–Bos indicus cross beef cattle. *Meat Science*, *86*(4), 915-920. https://doi.org/https://doi.org/10.1016/j.meatsci.2010.07.016.

Dario, C., Carnicella, D., Ciotola, F., Peretti, V., & Bufano, G. (2008). Polymorphism of Growth Hormone GH1-AluI in Jersey Cows and Its Effect on Milk Yield and Composition. *Asian-Australas J Anim Sci*, *21*(1), 1-5. https://doi.org/10.5713/ajas.2008.60586.

Derks, M. F. L., Gjuvsland, A. B., Bosse, M., Lopes, M. S., van Son, M., Harlizius, B., Tan, B. F., Hamland, H., Grindflek, E., Groenen, M. A. M., & Megens, H. J. (2019). Loss of function mutations in essential genes cause embryonic lethality in pigs. *PLoS Genet*, *15*(3), e1008055. https://doi.org/10.1371/journal.pgen.1008055.

Derks, M. F. L., Lopes, M. S., Bosse, M., Madsen, O., Dibbits, B., Harlizius, B., Groenen, M. A. M., & Megens, H. J. (2018). Balancing selection on a recessive lethal deletion with pleiotropic effects on two neighboring genes in the porcine genome. *PLoS Genet*, *14*(9), e1007661. https://doi.org/10.1371/journal.pgen.1007661.

Dettori, M. L., Pazzola, M., Pira, E., Paschino, P., & Vacca, G. M. (2015). The sheep growth hormone gene polymorphism and its effects on milk traits. *Journal of Dairy Research*, *82*(2), 169-176. https://doi.org/10.1017/S0022029915000047.

Esteban, C., Audí, L., Carrascosa, A., Fernández-Cancio, M., Pérez-Arroyo, A., Ulied, A., Andaluz, P., Arjona, R., Albisu, M., Clemente, M., Gussinyé, M., & Yeste, D. (2007). Human growth hormone (GH1) gene polymorphism map in a normal-statured adult population. *Clin Endocrinol (Oxf)*, *66*(2), 258-268. https://doi.org/10.1111/j.1365-2265.2006.02718.x.

Fontanesi, L., Dall'Olio, S., Spaccapaniccia, E., Scotti, E., Fornasini, D., Frabetti, A., & Russo, V. (2012). A single nucleotide polymorphism in the rabbit growth hormone (GH1) gene is associated with market weight in a commercial rabbit population. *Livestock Science*, *147*(1), 84-88. https://doi.org/https://doi.org/10.1016/j.livsci.2012.04.006.

Gao, C.-M., Gong, J.-P., Wu, J.-Z., Cao, H.-X., Ding, J.-H., Zhou, J.-N., Liu, Y.-T., Li, S.-P., Cao, J., Matsuo, K., Takezaki, T., & Tajima, K. (2010). Relationship between growth hormone 1 genetic polymorphism and susceptibility to colorectal cancer. *Journal of Human Genetics*, *55*(3), 163-166. https://doi.org/10.1038/jhg.2010.3.

Giordano, M., Lessi, M., Paracchini, R., Petri, A., Ozerkan, E., Cavallo, L., Wasniewska, M., Aimaretti, G., Momigliano-Richiardi, P., & Bona, G. (2001). Molecular Analysis of the Growth Hormone Gene (GH1) in Isolated Growth Hormone Deficiency. *2*(1), 45-54. https://doi.org/doi:10.1515/IJDHD.2001.2.1.45 (International Journal on Disability and Human Development).

Gitanjli, G., Sankhyan, V., Thakur, Y. P., & Dogra, P. K. (2020). Effect of growth hormone gene polymorphism on growth traits in migratory Gaddi goats of Western Himalayas,

India. *Trop Anim Health Prod*, *52*(4), 2091-2099. https://doi.org/10.1007/s11250-020-02227-4.

Hua, G. H., Chen, S. L., Yu, J. N., Cai, K. L., Wu, C. J., Li, Q. L., Zhang, C. Y., Liang, A. X., Han, L., Geng, L. Y., Shen, Z., Xu, D. Q., & Yang, L. G. (2009). Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. *Meat Science*, *81*(2), 391-395. https://doi.org/https://doi.org/10.1016/j.meatsci.2008.08.015.

Ilham, F., Rachman, S. D. A. B., Dagong, M. I. A., Rahim, L., & Yulianty. (2016). Genetic polymorphisms of growth hormone (GH) gene in Kacang goat population based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) identification. *Livestock Research for Rural Development*, *28*(9).

Jones, M., Sergeant, C., Richardson, M., Groth, D., Brooks, S., & Munyard, K. (2019). A non-synonymous SNP in exon 3 of the KIT gene is responsible for the classic grey phenotype in alpacas (Vicugna pacos). *Anim Genet*, *50*(5), 493-500. https://doi.org/10.1111/age.12814.

Kadri, N. K., Sahana, G., Charlier, C., Iso-Touru, T., Guldbrandtsen, B., Karim, L., Nielsen, U. S., Panitz, F., Aamand, G. P., Schulman, N., Georges, M., Vilkki, J., Lund, M. S., & Druet, T. (2014). A 660-Kb Deletion with Antagonistic Effects on Fertility and Milk Production Segregates at High Frequency in Nordic Red Cattle: Additional Evidence for the Common Occurrence of Balancing Selection in Livestock. *PLOS Genetics*, *10*(1), e1004049. https://doi.org/10.1371/journal.pgen.1004049.

Li, Q., Xu, Z., Zhang, M., Zhao, Z., Sun, B., Yang, L., Lu, W., Luo, F., & Sun, C. (2021). Mutations in GH1 gene and isolated growth hormone deficiency (IGHD): A familial case of IGHD type I and systematic review. *Growth Horm IGF Res*, *60-61*, 101423. https://doi.org/10.1016/j.ghir.2021.101423.

Liu, H. C., Kung, H. J., Fulton, J. E., Morgan, R. W., & Cheng, H. H. (2001). Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. *Proc Natl Acad Sci U S A*, *98*(16), 9203-9208. https://doi.org/10.1073/pnas.161466898.

Mahrous, K. F., Abdel-Aziem, S. H., Abdel-Hafez, M. A. M., Abdel-Mordy, M., & Rushdi, H. E. (2018). Polymorphism of growth hormone gene in three goat breeds in Egypt. *Bulletin of the National Research Centre*, *42*(1), 35. https://doi.org/10.1186/s42269-018-0035-0.

Majewska, K. A., Kedzia, A., Kontowicz, P., Prauzinska, M., Szydlowski, J., Switonski, M., & Nowacka-Woszuk, J. (2020). Polymorphism of the growth hormone gene GH1 in Polish children and adolescents with short stature. *Endocrine*, *69*(1), 157-164. https://doi.org/10.1007/s12020-020-02305-5.

Mazurowski, A., Frieske, A., Kokoszynski, D., Mroczkowski, S., Bernacki, Z., & Wilkanowska, A. (2015). Examination of Growth Hormone (GH) Gene Polymorphism and its Association with Body Weight and Selected Body Dimensions in Ducks. *Folia Biol (Krakow)*, *63*(1), 43-50. https://doi.org/10.3409/fb63_1.43.

Pal, A., Chakravarty, A. K., & Chatterjee, P. N. (2014). Polymorphism of growth hormone gene and its association with seminal and sexual behavioral traits in crossbred cattle. *Theriogenology*, *81*(3), 474-480. https://doi.org/https://doi.org/10.1016/j.theriogenology.2013.11.002.

Procter, A. M., Phillips Iii, J. A., & Cooper, D. N. (1998). The molecular genetics of growth hormone deficiency. *Human Genetics*, *103*(3), 255-272. https://doi.org/10.1007/s004390050815.

Radhika, G., K.C, R., & T.V, A. (2016). Polymorphism of exon 2 and 3 of growth hormone gene and presence of a rare genotype in native goat breed of Kerala, India. *Small Ruminant Research*, *145*, 81-84. https://doi.org/https://doi.org/10.1016/j.smallrumres.2016.10.024.

Rashijane, L. T., Mbazima, V. G., & Tyasi, T. L. (2022). Polymorphism of growth hormone gene and its association with body measurement traits in Boer goat does. *South African Journal of Animal Science*, *5*(1).

Seevagan, M., Jeichitra, V., Rajendran, R., & Tirumurugaan, K. G. (2015). Detection of lethal SNP (A781G) in growth hormone (GH) gene of Indian sheep. *Small Ruminant Research*, *126*, 13-15. https://doi.org/https://doi.org/10.1016/j.smallrumres.2015.03.003.

Thuy, L. T., Binh, D. V., Binh, N. T., Minh, L. Q., Thuy, T. T. T., Ton, N. D., Ba, N. V., Han, J.-L., & Periasamy, K. (2017). Evaluation of genetic diversity and structure of Vietnamese goat populations using multi locus microsatellite markers. Small Ruminant Research, 148, 43-50. https://doi.org/10.1016/j.smallrumres.2016.12.029.

Tinh, N. H., Hop, N. V., Anh, N. T. L., & Bui, A. P. N. (2021). Polymorphisms of Candidate Genes Associated with Growth and Carcass Traits in Canadian Duroc Pigs. *Indian Journal of Animal Research*. https://doi.org/10.18805/IJAR.B-1388.

Wagner, K., Hemminki, K., Israelsson, E., Grzybowska, E., Klaes, R., Chen, B., Butkiewicz, D., Pamula, J., Pekala, W., & Försti, A. (2005). Association of polymorphisms and haplotypes in the human growth hormone 1 (GH1) gene with breast cancer. *Endocrine-Related Cancer Endocr Relat Cancer*, *12*(4), 917-928. https://doi.org/10.1677/erc.1.01073.

Zhang, C., Liu, Y., Huang, K., Zeng, W., Xu, D., Wen, Q., & Yang, L. (2011). The association of two single nucleotide polymorphisms (SNPs) in growth hormone (GH) gene with litter size and superovulation response in goat-breeds. *Genet Mol Biol*, *34*(1), 49-55. https://doi.org/10.1590/s1415-47572010005000110.