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POLYMORPHISM OF GROWTH FACTOR 1 GENE IN NEW ZEALAND RABBIT BREED IN CU CHI FARM, VIETNAM

Phu Nam Anh Bui[1,✉], Van Hop Nguyen[2], Ba Chung Le[2] and Minh Tri Ngo[1]

[1]*Animal Genetics Group, Faculty of Biotechnology, Ho Chi Minh city Open University, 35-37 Ho Hao Hon street, Co Giang ward, District 1, Ho Chi Minh city, Vietnam*

[2]*Institute of Animal Sciences for Southern Vietnam, Organization, 12 Nguyen Chi Thanh street, Ward 2, District 10, Ho Chi Minh city, Vietnam*

[✉]*To whom correspondence should be addressed. Email: anh.bpn@ou.edu.vn*

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ABSTRACT

Rabbit husbandry in Vietnam is a growing sector of the livestock industry, offering significant potential for sustainable meat production and economic development, particularly in rural areas. Rabbits are well-suited to Vietnam's tropical climate due to their adaptability, high reproductive rates, and relatively low resource requirements compared to larger livestock. The New Zealand White breed and various local crossbreeds are the most commonly raised rabbits, valued for their resilience, efficient feed conversion, and quality meat. The Growth Hormone 1 (*GH1*) gene encodes the growth hormone (GH), a pivotal endocrine regulator essential for normal development and metabolic function across animal species. GH is synthesized in the anterior pituitary gland and plays a central role in growth, tissue repair, and metabolism. This study investigates the polymorphism of the Growth Hormone 1 (*GH1*) gene and its association with growth performance in New Zealand white rabbits reared at Cu Chi Farm, Vietnam. Fifty-six rabbits were genotyped for the *GH1* c.-78C>T polymorphism using the PCR-RFLP method, and their body weight at 70 days of age was measured. The genotypic frequencies were distributed as CC (28.57%), CT (44.64%), and TT (26.79%), with allele frequencies of 0.515 for C and 0.485 for T. Statistical analysis revealed that heterozygous CT rabbits exhibited significantly higher body weight (1885 ± 17.04 g) compared to CC (1785.45 ± 12.05 g) and TT (1752 ± 19.35 g) genotypes. The equal allele frequency observed may result from the selective advantage of the heterozygous genotype, maintaining a balanced genetic structure within the population. These findings suggest that *GH1* polymorphism influences growth traits and highlight its potential as a genetic marker for selective breeding programs aimed at improving growth efficiency in rabbits. Further studies are recommended to explore additional genetic markers and validate these results on a larger scale.

Keywords: *GH1* gene polymorphism, New Zealand White rabbits, rabbit growth traits, PCR-RFLP genotyping, selective breeding

INTRODUCTION

Rabbit husbandry is a multifaceted field encompassing the care, breeding, and management of rabbits for diverse purposes, including meat, fiber, laboratory research, and companionship. In meat and fur production, rabbits offer a sustainable source of high-quality protein and luxurious fiber with relatively low resource input compared to larger livestock (Cai *et al*., 2022; Wang *et al*., 2017; Migdał *et al*., 2023). Notably, breeds like the New Zealand White are essential in biomedical research due to their docile nature, manageable size, and physiological compatibility with various human medical studies, particularly in immunology, toxicology, and reproductive health. Additionally, the Angora rabbit contributes significantly to the global fiber industry, especially in China, despite rising welfare concerns that have led to regulatory responses in some regions. Modern rabbit husbandry integrates advancements in genetics, nutrition, and veterinary care, particularly for pet and show rabbits, where selective breeding aims to promote both health and desirable traits (Safaa *et al*., 2023). Globally, rabbit husbandry is gaining traction as a sustainable livestock practice, with an emphasis on improving animal welfare and minimizing environmental impact, positioning rabbits as a viable livestock alternative in resource-constrained settings.

The *GH1* gene encodes the growth hormone protein, a key regulator in growth, metabolism, and cellular repair in humans. Located on chromosome 17q23, *GH1* is a member of the growth hormone family, which also includes several other paralogs expressed primarily in the pituitary gland. The *GH1* protein, a 22-kDa polypeptide, functions by binding to the growth hormone receptor (GHR) on target cells, activating the JAK-STAT signaling pathway and stimulating downstream effects critical for longitudinal bone growth, muscle development, and lipid metabolism. Mutations or deletions in *GH1* are linked to growth hormone deficiency (GHD), characterized by short stature and metabolic abnormalities due to impaired GH secretion. Recombinant *GH1*-derived GH therapy has been a significant clinical intervention for GHD and other growth disorders, providing therapeutic benefits that underscore *GH1*'s essential role in growth and metabolic health. Recent research also explores *GH1*'s role in age-related and metabolic diseases, given its involvement in cellular senescence, insulin-like growth factor-1 (IGF-1) signaling, and modulation of body composition.

In animals, *GH1* protein is pivotal in regulating growth, development, and metabolic functions across livestock species, making it a key focus in animal husbandry. GH directly influences muscle growth, fat distribution, and bone development by interacting with the growth hormone receptor and activating downstream pathways, notably the IGF-1 pathway, which promotes tissue growth and repair (Zhang *et al*., 2011). Selective breeding programs often focus on *GH1* gene variants associated with enhanced growth traits to improve yield and production efficiency. Additionally, genetic modifications and biotechnological interventions targeting *GH1* are being explored to optimize animal growth without compromising health or welfare. Understanding the *GH1*-growth correlation is thus crucial for advancing sustainable livestock production, improving meat quality, and meeting global food demands in animal agriculture.

Polymorphisms in the *GH1* gene play a crucial role in determining growth, metabolic efficiency, and overall fitness across animal species (Akers, 2006; Procter *et al*., 1998). These genetic variations, often in the form of single nucleotide polymorphisms (SNPs), can lead to differences in growth hormone secretion, receptor binding, and subsequent activation of pathways that influence growth and body composition, such as the IGF-1 pathway (Esteban *et al*., 2007; Gao *et al*., 2010; Giordano *et al*., 2001; Li *et al*., 2021). In domesticated animals, particularly livestock and companion animals, certain *GH1* polymorphisms have been associated with desirable traits like enhanced growth rates, lean muscle accumulation, and feed efficiency (Mazurowski *et al*., 2015; Pal *et al*., 2014; Seevagan *et al*., 2015; Zhang *et al*., 2011). This makes *GH1* polymorphisms valuable markers in selective breeding programs aimed at improving productivity and animal performance. By understanding and leveraging *GH1* genetic diversity, breeders can enhance specific traits suited to production or environmental needs, while also exploring how these variations impact health, longevity, and adaptability, thus supporting both economic and welfare goals in animal breeding and management.

Table 1. Examples of *GH1* polymorphisms in various animals and their associated traits. This table shows how different *GH1* polymorphisms impact traits like milk yield, muscle growth, and body composition, making them valuable markers for selective breeding in animal husbandry.

Species	Polymorphism	Genetic Variation	Associated Traits	Notes	References
Cattle	AluI	L127V (Leucine to Valine)	Increased milk yield, feed efficiency	Common in dairy breeds; influences milk production and growth efficiency.	(Curi *et al*., 2010; Dario *et al.*, 2008)
	MspI	Promoter region SNP	Improved carcass quality, muscle mass	Seen in beef breeds; enhances muscle growth and feed utilization.	(Pal *et al*., 2014; Barendse *et al.*, 2006)
Sheep	F279Y	Phenylalanine to Tyrosine	Increased body weight, wool growth	Found in wool breeds; related to better growth rates and wool production.	(Dettori *et al*., 2015; Rashijane *et al*., 2022)
	PvuII	SNP in *GH1* gene	Enhanced post-weaning growth	Common in meat breeds; associated with improved weight gain after weaning.	(Radhika *et al*., 2016; Seevagan *et al*., 2015)

Goat	MspI	SNP affecting GH expression	Higher milk yield, altered fat composition	Valuable in dairy goat breeding; influences milk quantity and quality.	(Abbas *et al.*, 2022; Ilham *et al.,* 2016; Mahrous *et al.*, 2018)
	AluI	SNP affecting milk traits	Improved milk production traits	Similar to MspI in goats, affects milk yield and composition.	(Gitanjli *et al.*, 2020; Hua *et al.*, 2009)
Pig	HaeIII	SNP linked to backfat	Reduced backfat, leaner muscle growth	Important in pork production; impacts lean muscle growth and fat content.	(Zhang *et al.*, 2011)
	GH/MspI	SNP influencing GH levels	Faster growth, better feed efficiency	Associated with growth rate and feed efficiency in pork production.	(Babicz *et al.*, 2020)
Chicken	DdeI	SNP in *GH1* gene	Increased growth rate, body weight	Common in broilers; linked to faster growth and heavier body weight.	(Yan *et al.*, 2003)

Rabbit husbandry in Vietnam has grown in importance due to the species' adaptability, high reproductive rate, and relatively low resource requirements compared to other livestock. Vietnamese farmers primarily raise rabbits for meat production, which provides a valuable protein source in rural areas. Rabbit breeds such as the New Zealand White and local crossbreeds are favored for their resilience in tropical climates and relatively low susceptibility to common diseases when managed correctly. However, challenges such as limited veterinary services, low farmer awareness about advanced health management, and the threat of viral hemorrhagic disease (VHD) persist. Addressing these challenges through training programs and improved infrastructure could enhance rabbit production and contribute to food security in Vietnam's rural areas (CountryReport, 2003).

Researching *GH1* gene polymorphism in rabbits in Vietnam is crucial due to its potential impact on rabbit growth, reproduction, and meat quality, traits that are highly valuable in Vietnam's growing livestock industry. The *GH1* gene, known to influence growth hormone regulation, could reveal genetic variations that correlate with improved growth rates and feed efficiency, directly benefiting rabbit farmers in Vietnam by enhancing productivity (Nahácky *et al.*, 2018). Additionally, understanding these genetic polymorphisms can help in developing breeding programs tailored to the local environment and market demands, potentially increasing resilience to local diseases and improving overall adaptability. This research could ultimately lead to

optimized rabbit breeds that support sustainable and profitable meat production, meeting both local and export market demands.

MATERIALS AND METHODS

Sample collection and DNA extraction

In this study, we evaluated data from a total of 56 New Zealand White rabbits. The animals were housed in a heated facility equipped with nipple drinkers for water, a 14-hour light and 10-hour darkness cycle, and exhaust ventilation. Both water and feed were provided *ad libitum*. The diet consisted of a commercial pelleted feed containing 15% crude protein, 16.1% crude fiber, and 3.5% crude fat.

Before collecting saliva, ensure the animal's mouth is free of food or any other substances by having them rinse with water and refrain from eating or drinking for 30 minutes prior to sample collection. Open a 15 mL centrifuge tube containing 2.5 mL of PBS buffer, being careful not to touch the inside of the tube or cap. Follow the procedure that has been published by Goode *et al.* (2014). Secure the cap and gently mix by inverting until the solution is uniform. Store samples at room temperature for short-term storage or at 4 °C for long-term storage (over three months). DNA was extracted from FastPure® Blood/Cell/Tissue/Bacteria DNA Isolation Mini Kit, following the manufacturer's protocol. The DNA samples were then quantified using electrophoresis and a Nanodrop spectrophotometer.

PCR-RFLP conditions

Genotyping for *GH1* polymorphism c.-78C>T (Fontanesi *et al*., 2012) was conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. DNA fragments were amplified using 2 × Rapid Taq Master Mix (Vazyme, China). Approximately 80 ng of template DNA was added to the Master Mix, then nuclease-free water was used to reach a final volume of 15 µl. PCR was performed on a T100 thermocycler (BioRad, USA) with an initial denaturation at 95°C for 2 minutes, followed by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. PCR products were visualized on 1% agarose gel.

Subsequently, PCR samples (3 to 4 µg) were digested in 50-µL reactions, following the buffer and condition recommendations from the enzyme supplier (New England Biolab). Post-digestion, the DNA samples were separated on 0.7% or 1.5% agarose gels in 0.5× Tris-borate EDTA buffer and detected by fluorescence (using a GelDoc-IT imager). The enzyme *Hae*III digests a 231 bp amplicon, yielding 169, 62 bp for the C allele and 231 bp for the T allele. Each genotype was subsequently sequenced for verification.

Statistical analysis

The frequencies of *GH1* were determined based on the Hardy-Weinberg equilibrium (HWE) principle. A linear mixed model was used to evaluate the association between genotypes and their effects on body weight at 70 days after birth. The model can be described as follows:

$$Y_{ijk} = \mu + S_i + G_i + e_{ijk}$$

Y_{ijk}: observed trait values (body weight at 70 days after birth)

µ: overall mean

S_i: effect of sex

G_i: effect of genotype *GH1*

e_{ijk}: random residual effect

The results for the traits were presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS software (version 20; SPSS Inc., Chicago, IL, USA), with a student's t-test employed to determine significance. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

PCR-RFLP genotyping

DNA quality can be assessed effectively using gel electrophoresis, a widely used technique in molecular biology. In this process, DNA samples are loaded into a gel matrix, usually made of agarose, and subjected to an electric field, which causes the DNA fragments to migrate based on their size. High-quality DNA typically appears as a distinct, clear band, indicating that the DNA is intact and free from significant degradation. If the DNA is fragmented or degraded, it will appear as a smear rather than a sharp band, suggesting breaks in the DNA strands. Additionally, electrophoresis can reveal the presence of contaminants or RNA by showing extra bands. The sharper and more distinct the band, the higher the quality of the DNA, making this method an essential step in evaluating sample integrity before downstream applications like PCR or sequencing. Our data suggested that our DNA quality is suitable for PCR applications.

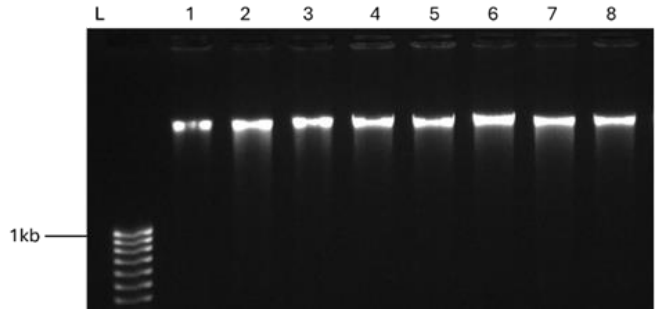

Figure 1. The quality of total DNA from eight samples was checked by electrophoresis. L: molecular marker

The quality of a PCR product with a target size of 261 bp can be evaluated using gel electrophoresis (Figure 2). A high-quality PCR product appears as a single, sharp band at the expected position corresponding to 261 bp on the gel, confirming the successful amplification of the intended fragment without nonspecific products.

PCR-RFLP analysis can differentiate alleles based on the digestion of a specific PCR amplicon. For a 231 bp amplicon subjected to digestion with the restriction enzyme *Hae*III, the enzyme cuts only when the C allele is present, generating two fragments of 169 bp and 62 bp. In contrast, the T allele lacks the recognition site for *Hae*III, leaving the amplicon undigested at its original size of 231 bp. When visualized on an agarose gel, a homozygous C allele sample will show two distinct bands at 169 bp and 62 bp, a homozygous T allele will display a single band at 231 bp, and a heterozygous CT sample will exhibit three bands: 231 bp, 169

bp, and 62 bp. This clear distinction in band patterns allows for accurate genotyping, provided the digestion reaction is complete and the gel resolution is sufficient. Three samples with three genotypes TT, CT and CC were subsequently sent for Sanger sequencing for verification.

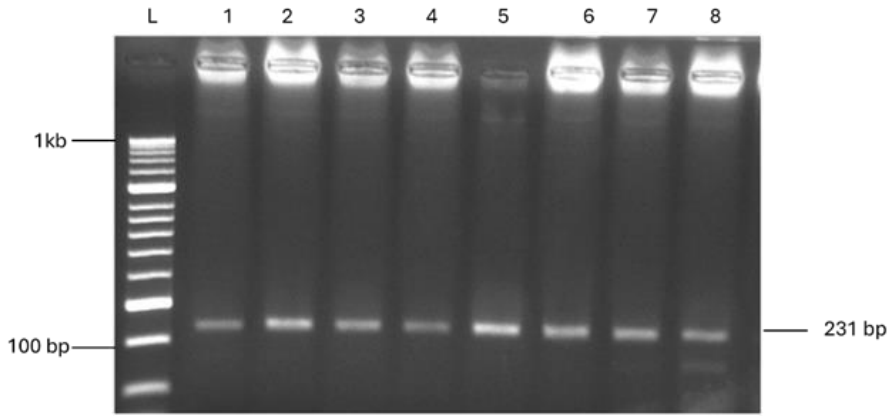

Figure 2. PCR amplification of 231 bp amplicon containing *GH1* polymorphism c.-78T>C. L: Molecular ladder

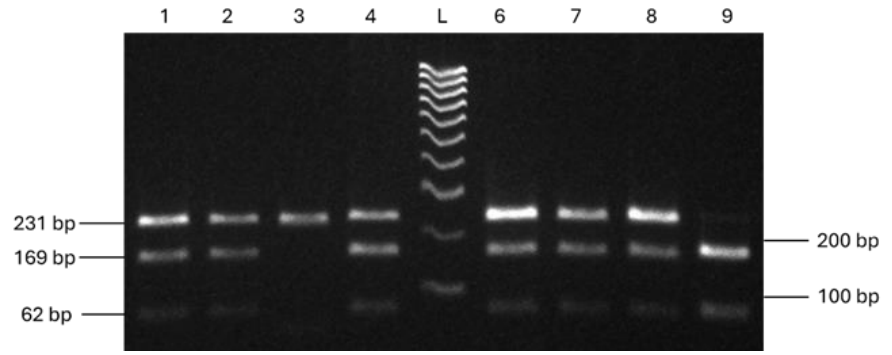

Figure 3. Genotyping *GH1* at locus c.-78C>T. Sample 3: genotype TT, sample 9: genotype CC, L: Molecular Ladder 100 bp

Association studies

In this study, we examined the relationship between *GH1* gene frequency and growth parameters in a population of 56 rabbits. The *GH1* gene, which encodes growth hormone, plays a central role in regulating body growth and metabolism in mammals. Variations in *GH1* gene frequency have been linked to differences in growth rate, body composition, and overall size in several animal species, suggesting that *GH1* may serve as a potential genetic marker for growth performance (Fontanesi *et al*., 2012).

To assess this relationship in our rabbit population, *GH1* gene frequencies were determined through genotyping methods, and growth metrics, including body weight at 70 days after birth. Statistical analyses were performed to evaluate correlations between specific *GH1* allele frequencies and observed growth rates.

The data in Table 2 illustrates the genotypic and allelic frequencies of the *GH1* gene,

showing a close alignment between observed and expected values, which suggests the population is in HWE. Specifically, the observed frequencies of the CC, CT, and TT genotypes are 16, 25, and 15, respectively, while the expected frequencies are 14, 28, and 14. This similarity is further supported by the p-value of 0.1, indicating no significant deviation from HWE. The allele frequencies for C and T are 0.515 and 0.485, respectively, reflecting a balanced distribution with no strong selective pressure favoring either allele. The consistency between observed and expected genotypic frequencies and the non-significant p-value suggests that the population is stable with respect to the *GH1* gene and not influenced by factors such as selection, mutation, or migration, though minor discrepancies could be attributed to random sampling variations.

Table 2. Genotypic and allelic frequencies of *GH1*

	Genotype			**Allele frequency**		**p**	**HWE**
	CC	CT	TT	C	T		
Observed values	16	25	15	0.515	0.485	0.1	+
Expected values	14	28	14				

Preliminary results indicate a positive association between certain *GH1* alleles and body weight at 70 days after birth, with rabbits carrying both C and T alleles exhibiting significantly higher weight at 70 days after birth compared to others (Table 3). It is noteworthy that both alleles exhibit equal frequency within this population. This balance may result from the heterozygous genotype providing a selective advantage over both homozygous genotypes, contributing to the maintenance of stable allele frequency. This equilibrium is likely influenced by strong selection pressure favoring body weight at 70 days after birth. This suggests that *GH1* allele frequency may influence growth variability within the population, potentially making *GH1* a candidate gene for selective breeding programs aimed at enhancing growth traits in rabbits. Our result is in accordance with data from Fontanesi *et al*. (2012), exhibiting the importance of heterozygosity of *GH1* in New Zealand rabbit populations.

Further investigation with a larger sample size and additional genetic markers could provide a more comprehensive understanding of the *GH1* gene's influence on growth and its potential utility in genetic selection for rabbit breeding programs. This study contributes to our understanding of genetic factors affecting growth and may inform breeding strategies for improved growth performance in rabbits.

Table 3. Association between *GH1* and body weight at 70 days after birth

	Genotype		
	CC	CT	TT
Body weight at 70 days of birth	1785.45±12.05^{a}	1885± 17.04^{b}	1752±19.35^{c}

CONCLUSION

This study highlights the influence of *GH1* gene polymorphism on the growth performance of New Zealand White rabbits in Cu Chi Farm, Vietnam. The observed equal allele frequency of the *GH1* polymorphism (c.-78C>T) suggests a potential advantage of the heterozygous genotype in promoting growth efficiency. Rabbits with the CT genotype exhibited significantly higher body weight at 70 days of age compared to those with CC or TT genotypes, emphasizing the role of *GH1* heterozygosity in growth traits. These findings align with previous research and underscore the value of *GH1* as a candidate gene for selective breeding programs aimed at optimizing growth performance in rabbits. Further studies with larger sample sizes and additional genetic markers are recommended to validate these results and enhance our understanding of the genetic mechanisms underlying growth traits. This research provides a foundation for the development of targeted breeding strategies to improve productivity and efficiency in rabbit husbandry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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