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POLYMORPHISM OF *POU1F1* GENES AND ITS RELATIONSHIP WITH GROWTH TRAITS OF BACH THAO GOAT BREED

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ABSTRACT: This study aimed to evaluate the polymorphism of the *POU1F1* gene and its association with body weight and weight gain in Bach Thao goats. A total of 110 animals (64 males and 46 females) were used, and ear tissue samples were collected for DNA extraction and PCR-RFLP analysis to determine *POU1F1* genotypes. Allele and genotype frequencies were subsequently calculated. Three genotypes (TT, TC, and CC) were identified, with the T allele and TT genotype showing the highest frequencies. Body weight at birth, and at 3, 6, and 9 months of age, as well as average daily gain (ADG during the corresponding growth period were recorded. Goats with the TT genotype had significant higher body weight at birth, and at 3, 6, and 9 months of age, together with higher ADG during the corresponding period, than those with the CC genotypes ($P < 0.05$).. These finding indicates that *POU1F1* polymorphism is associated with growth performance in Bach Thao goats and may have potential as a molecular marker for marker-assisted selection to improve growth traits in this breed.

Keywords: *POU1F1*, Bach Thao goat, Weight, Gene polymorphism, PCR–RFLP.

INTRODUCTION

The Bach Thao goat, an indigenous goat breed of Vietnam with good adaptability to local production conditions, characterized by its ability to tolerate relatively low-quality diets, maintain growth under adverse climatic conditions, and adapt well to the local natural environment. In addition to its adaptability, Bach Thao goats are valued for their meat quality, high lean meat proportion, nutritional value, and relatively low cholesterol content, making them a potentially important genetic resource for sustainable goat production (Van Binh et al., 2017; Thuan et al., 2021). Therefore, improving growth performance while maintaining the adaptive characteristics of this breed is an important consideration for increasing production efficiency.

Growth performance is influenced by genetic and environmental factors, and identifying genes associated with growth traits can support genetic improvement. The *POU1F1* gene, which encodes the pituitary-specific transcription factor PIT-1, plays an important role in pituitary development and the regulation of growth, milk production, and thyroid hormone secretion (Cohen et al., 1996; Herman et al., 2012). Genetic variation in *POU1F1* may therefore contribute to phenotypic differences in growth and production traits. In goats, Işık and Bilgen (2019) reported associations between *POU1F1* variants and birth weight and growth parameters in Saanen goats. However, the effects of genetic polymorphisms may vary with breed, genetic background, and environmental conditions, indicating that findings from one goat population may not be directly applicable to others. Further investigation of *POU1F1* polymorphisms in locally adapted breeds, such as Bach Thao goats, is therefore warranted.

Several studies have reported associations between *POU1F1* polymorphisms and economically important traits in goats (Aboelenin et al., 2022; Pawar et al., 2024). These polymorphisms have been associated with milk yield and quality (Gündüz and Biçer, 2023), growth characteristics (Lan et al., 2007b), and body-size measurements in Jining gray goats (Feng et al., 2012). Li et al. (2016) further reported associations between polymorphisms in the *POU1F1-PROP1-PITX1-SIX3* gene pathway and growth traits in Guangzhou dairy and Hainan black goats. Similarly, Shaikh (2022) found that SNP rs670830003 significantly affected weaning weight and body weight gain, with heterozygous (TG) goats showing the highest values. More recently, Ayele et al. (2024) identified polymorphisms in the *GH, IGF-I,* and *PIT-I* genes associated with growth traits in Saanen, Alpine, and Boer goats. Collectively, these findings support the potential role of *POU1F1* and related growth-regulatory genes as candidate markers for improving growth performance in goats.

Despite these findings, information on the association between *POU1F1* polymorphisms and growth performance remains limited in indigenous and locally adapted goat breeds, including Bach Thao goats. In developing countries, limited systematic genetic selection highlights the need to identify genetic variants associated with economically

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important traits to support more effective breeding programs. Genetic markers associated with body weight and weight gain could facilitate the early identification of animals with superior growth potential and contribute to genetic improvement.

Based on the biological role of PIT-1 and previous evidence linking *POU1F1* polymorphisms with growth traits in goats, we hypothesized that *POU1F1* genetic variation is associated with body weight and weight gain in Bach Thao goats. Therefore, the objective of this study was to identify the polymorphism of *POU1F1* gene and its association with body weight and weight gain in Bach Thao goats.

MATERIALS AND METHODS

Ethical considerations

All animals in our study were conducted in accordance with the laws, guidelines of the relevant organizations and were approved by the Scientific Council of Tay Nguyen University under Decision No. B2024-TTN-03 and Contract No. 55/HĐ-ĐHTN of Tay Nguyen University dated 16 January 2024.

Animals and data collection

A total of 110 Bach Thao goats (64 males and 46 females), an indigenous goat breed of Vietnam, were included in the study. They were born to does aged 2–3 years, corresponding to parity 3–4, selected from five farms belonging to the Dak Lak Goat Cooperative in Dak Lak Province, Vietnam, and reared at their respective farms until 3 months of age. Thereafter, all goats were transferred to a single farm and reared together until the end of the experiment. From approximately 3 to 9 months of age, all experimental goats were maintained under the same housing and management conditions and offered the same total mixed ration (TMR) containing 13% crude protein. Each animal was identified by ear tag. Ear tissues were collected to analysis genotypes of *POU1F1* gene. The study parameters were body weight at birth (BWB) at 3 (BW3), 6 (BW6), 9 months of age (BW9), and average daily gain (ADG) at different growth stages. BWB was recorded within 24 hours from birth. BW3, BW6 and BW9 were determined using an electronic scale at 3, 6, and 9 months of age, respectively. ADG was calculated for the periods, including from birth to 3 months (ADG03), 3 to 6 months (ADG36), and 6 to 9 months (ADG69), and from birth to 9 months of age (ADG09).

DNA extraction

Ear tissue were collected from each goat using sterile ear forceps and preserved in 95% ethanol solution at 4°C until DNA extraction. Total DNA was extracted from ear tissue samples using a modified phenol-chloroform method as described by Green and Sambrook (2012), with minor modifications to accommodate laboratory conditions. Tissue samples were digested with proteinase K, and the resulting DNA was purified by phenol-chloroform extraction followed by ethanol precipitation. DNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), with samples showing an A260/A280 ratio between 1.8 and 2.0 and a concentration higher than 50 ng/µL considered acceptable for downstream analysis. DNA integrity was further evaluated by electrophoresis on a 1% agarose gel, in which good-quality DNA showed a single, clear band with no smearing, indicating that the DNA was intact and not degraded. Samples meeting both purity and integrity criteria were diluted to working concentrations and stored at -20°C until PCR reaction.

Genotype identification

The *POU1F1* gene was amplified using primers designed by Lan et al. (2007b): downstream 5′-AGGCTGTGAGGAAGTGAGAG-3′ and reverse 5′-GGAGGAGATGAGGAGGAAAG-3′, with an expected product size of approximately 450 bp. Genotyping was carried out by PCR–RFLP with *Alu*I according to Lan et al. (2007a). Briefly, 20 µL of PCR product was digested with 10 U of *AluI* enzyme at 37°C for 5 hours. The digestion DNA fragments were separated by electrophoresis on a 2.0% agarose gel and visualized under UV light. Based on the characteristic electrophoretic banding patterns, the goats were classified into three genotypes (TT, TC, and CC).

Statistical analysis

The Hardy-Weinberg equilibrium of *POU1F1* gene was performed by comparing the expected and observed genotype counts using chi-squared test (χ^2). Allele frequency was defined as the number of copies of a given allele divided by the total number of allele copies in the sampled population. For the two alleles T and C, the frequencies were calculated as follows:

$$p(T)=\frac{2N_{TT}+N_{TC}}{2N} \quad ; \quad q(C)=\frac{2N_{CC}+N_{TC}}{2N}$$

where N_{TT}, N_{TC}, and N_{CC} are the numbers of individuals with the TT, TC, and CC genotypes, respectively; $N = N_{TT} + N_{TC} + N_{CC}$ is the total number of individuals analyzed; and $p(T) + q(C) = 1$.

Genotype frequencies were calculated as follows:

$$f(TT)=\frac{N_{TT}}{N};\quad f(TC)=\frac{N_{TC}}{N};\quad f(CC)=\frac{N_{CC}}{N}$$

The effect of *POU1F1* genotype on body weights and average daily gain was analyzed using the statistical model below: $y_{ijk} = \mu + G_i + S_j + \varepsilon_{ijk}$

where: y_{ijk} = study parameters (body weights and average daily gain), μ = overall mean, G_i = effect of genotype i (i=3: TT, TC and CC), S_j = effect of sex j (j=2: male and female), ε_{ijk} = residual error. The Tukey test was used for the pairwise comparison. A generalized linear model (GLM) was applied using Minitab 16.0 software, with statistical significance determined at $P < 0.05$. The statistical parameters in the tables are number of observation (n), arithmetic mean (mean) ± standard deviation (SD.

RESULT

Polymorphisms of *POU1F1*

Frequency results were obtained based on the *POU1F1* gene band pattern of exon 6 using the restriction enzyme *AluI*, as shown in Figure 1. PCR amplification of DNA extracted from goat ear tissue samples produced a single band specific to the gene location. The designed primers annealed at 37°C and generated a 450 bp amplification fragment using a specific primer combination, cut with *AluI* of the *POU1F1* gene in all samples PCR-RFLP *Alu*I of the *POU1F1* gene. PCR-enhanced fragments cut with *AluI* showed three different restriction digestion patterns, indicating the presence of *POU1F1* gene variation in Bach Thao goats. PCR-RFLP *AluI* showed three genotypes, 'TT' (340 and 110 bp), 'CC' (216, 124 and 110 bp) and 'TC' (340, 216, 124 and 110 bp) (Figure 1).

Genetic variation, genotype and allele frequencies, and genetic diversity of genes belonging to the *POU1F1* pathway. Three expected genotypes (TT, TC, and CC) at *POU1F1* were detected in Bach Thao goat population (Table 1). The TT genotype was the most frequent (0.745), followed by TC (0.145) and CC (0.109). The frequencies of the T and C alleles were 0.818 and 0.182, respectively, with the CC genotype being the least frequent among the studied goats. The observed genotype distribution at the *POU1F1* locus deviated significantly from Hardy–Weinberg equilibrium at SNP6 ($\chi^2 = 28.69$, $P < 0.001$).

The effect of the *POU1F1* gene polymorphism on body weight at different growth of Bach Thao goats is presented in Table 2. Significant differences in body weight between *POU1F1* genotypes were observed at birth, 3 months, and 6 months of age ($P < 0.05$). Goats with the TT genotype had the highest body weight at all evaluated stages, followed by the TC genotype, while the CC genotype showed the lowest body weight values.

At birth, goats with the TT genotype had significantly higher body weight than those with the CC genotype, while individuals with the TC genotype showed intermediate values. A similar trend was observed at 3 and 6 months of age, suggesting a consistent effect of the *POU1F1* gene polymorphism on early growth performance. At 9 months of age, goats with the TT genotype showed significantly higher body weight than those with the CC genotype ($P < 0.05$).

The ADG of Bach Thao goats according to *POU1F1* genotype is presented in Table 3. Significant differences in ADG among genotypes were observed during the period from birth to 3 months and from 3 to 6 months of age ($P < 0.05$). Goats with the TT genotype showed the highest ADG during both periods, whereas those with the CC genotype exhibited the lowest values. Goat with the TC genotype generally showed intermediate ADG values.

During 6 to 9 month, there were no significant differences in ADG among *POU1F1* genotypes ($P > 0.05$), although goats with the TT genotype still tended to have higher ADG compared to those with other genotypes. These results suggest that the impact of the *POU1F1* polymorphism on growth performance is more pronounced during the early growth period.

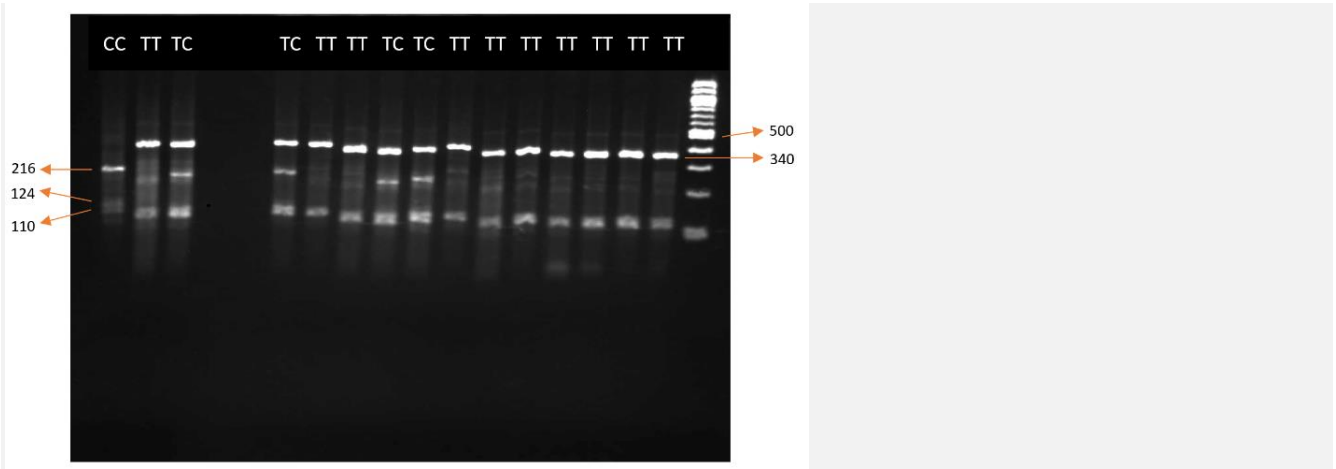

Figure 1 - Electrophoretic image of *POU1F1* products on a 2% agarose gel with AluI restriction enzyme. Genotype TT (340 and 110 bp); Genotype TC (340, 216, 124 and 110 bp); Genotype CC (216, 124 and 110 bp).

Table 1 - Genotypic and allelic frequencies of *POU1F1* gene

Item	Genotype				Allele		X^2
	TT	TC	CC	Total	T	C	
Observed count	82	16	12	110			
Expected count	73.63	32.73	3.64				
Observed frequency	0.745	0.145	0.109	1	0.818	0.182	28.69
Expected frequency	0.669	0.297	0.034				

Table 2 - Body weight (kg) of Bach Thao goats at different ages according to *POU1F1* genotype

Trait	TT			TC			CC			P-value
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
BWB	82	2.54^{a}	0.46	16	2.43^{ab}	0.34	12	2.20^{b}	0.49	0.046
BW3	79	12.42^{a}	1.05	16	11.96^{ab}	1.23	12	11.47^{b}	1.53	0.017
BW6	77	25.49^{a}	1.94	16	24.96^{ab}	1.50	12	23.82^{b}	2.71	0.024
BW9	67	33.94^{a}	2.61	13	32.39^{ab}	3.89	10	31.31^{b}	2.61	0.004

The means in the same row with different superscripts are significant different (P<0.05)

Table 3 - Average daily gain of Bach Thao goats according to genotype *POU1F1* (g/day)

Trait	TT			TC			CC			P-value
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
ADG03	79	109.80^{a}	11.94	16	105.96^{a}	12.71	12	103.01^{b}	115.16	0.031
ADG36	77	145.50^{a}	12.33	16	144.39^{a}	10.97	12	137.27^{b}	17.78	0.022
ADG69	67	95.70	4	13	85.30	27.9	10	91.33	22.80	0.118
ADG09	67	116.3^{a}	7.83	13	108.2^{b}	14.11	10	109.5^{b}	8.73	0.001

The means in the same row with different superscripts are significant different (P<0.05)

DISCUSSION

The genotype distribution of the *POU1F1* gene in the Bach Thao goat population showed a significant departure from Hardy–Weinberg equilibrium at SNP6 ($P < 0.05$). Similar deviations from Hardy–Weinberg equilibrium have been reported for *POU1F1* polymorphisms in other goat populations. Ma et al. (2017) reported significant deviations at SNP3, SNP5, and SNP6 in Guanzhong goats and at SNP2 and SNP5 in Hainan black goats. Polymorphisms in the *POU1F1* gene have also been identified in indigenous Chinese goat breeds (Lan et al., 2007a), Sarda goats (Daga et al., 2013), and Osmanabadi goats (Pawar et al., 2024), indicating that genetic variation at this locus occurs across diverse goat populations.

The observed departure from Hardy–Weinberg equilibrium in the present study should be interpreted cautiously because several factors can contribute to deviations from the expected genotype distribution. These include non-random mating, population substructure, genetic drift, selection, and other demographic processes. Although the relatively high frequency of the TT genotype, which was also associated with superior growth performance in the present study, may be compatible with selection at or near the *POU1F1* locus, the present data are insufficient to establish directional selection as the cause of the observed deviation. In particular, information on pedigree structure, genetic relatedness, population stratification, and the history of sire use was not available. Therefore, further studies incorporating population genetic analyses, pedigree information, and a larger and more genetically structured sample are required to determine the underlying causes of the Hardy–Weinberg disequilibrium.

Despite this limitation, the association between *POU1F1* genotype and growth performance observed in the present study provides evidence supporting the potential role of this locus as a candidate molecular marker for growth-related traits in Bach Thao goats. However, the potential application of *POU1F1* in marker-assisted selection should be validated in independent populations and under different production environments before being incorporated into practical breeding programs.

The present study showed significant associations between *POU1F1* genotype and several growth traits in Bach Thao goats. Goats carrying the TT genotype generally showed higher body weights at birth, 3, 6, and 9 months of age, as well as higher ADG during the birth-to-3-month and 3-to-6-month periods, compared with goats carrying the TC and CC genotypes. The significant effects observed during the early growth stages are consistent with the biological role of *POU1F1*, which encodes the pituitary-specific transcription factor *PIT-1* and regulates the expression of growth-related hormones, including growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone β (TSHβ) (Cohen et al., 1997; Hu et al., 2026).

Previous studies have also reported associations between *POU1F1* polymorphisms and growth-related traits in goats. Lan et al., (2007a, b) reported associations between *POU1F1* polymorphisms and weight-related traits in Chinese goats, while Nguyen et al. (2023) observed higher body weights in Nản goats carrying the D1D1 genotype. Similarly, Dorjay and Abraham (2021) reported significant associations between *POU1F1* polymorphism and body weight in indigenous goat populations. These findings, together with the present results, support the potential relevance of *POU1F1* variation to growth performance in goats.

The association observed in the present study should not, however, be interpreted as evidence that the SNP6 variant itself is necessarily the causal mutation. The *POU1F1* gene is part of a broader regulatory network involving genes such as *PROP1, PITX1,* and *SIX3*, which have also been implicated in growth-related processes (Ma et al., 2017). Therefore, the observed genotype–phenotype association may reflect linkage disequilibrium between the investigated *POU1F1* variant and other functional variants within or near this regulatory pathway. Previous reports of associations involving the *POU1F1-PROP1-PITX1-SIX3* pathway (Ma et al., 2017) and variants in *GH, IGF-I,* and *PIT1* (Ayele et al., 2024) further indicate that growth is controlled by a complex genetic network rather than by a single locus. Because linkage disequilibrium and functional interactions among these loci were not evaluated in the present study, the biological mechanism underlying the association between the *POU1F1* SNP6 genotypes and growth performance remains to be determined.

The stronger genotype effects observed during early growth, particularly for ADG from birth to 3 months and from 3 to 6 months, may also reflect the greater contribution of genetically regulated endocrine processes during early development. In contrast, the absence of significant genotype differences in ADG from 6 to 9 months suggests that environmental and management-related factors may increasingly contribute to variation in growth as animals mature. Growth performance is influenced by multiple non-genetic factors, including nutrition, feed availability, health status, housing, environmental conditions, and management practices. Although the goats were transferred to a common farm at 3 months of age and subsequently maintained under the same management conditions, these factors were not included as separate covariates in the statistical models. Consequently, their potential contributions to the observed variation cannot be completely excluded. The observed association between *POU1F1* genotype and growth should therefore be interpreted as a genotype–phenotype association rather than as an isolated genetic effect independent of environmental influences. In addition, the relatively small number of goats carrying the CC genotype may have limited the statistical power and precision of comparisons involving this less frequent genotype. Also, the present study was conducted in a relatively small population from a single geographical region, and the genotype effect was evaluated within this specific population. Therefore, the results, particularly those involving the CC genotype, should be interpreted cautiously and validated in larger, independent Bach Thao goat populations. Future studies incorporating larger sample sizes, pedigree information, population structure, additional growth-related genes, and genome-wide or targeted linkage disequilibrium analyses would help clarify whether the *POU1F1* SNP6 variant itself contributes functionally to growth or serves primarily as a marker linked to causal genetic variation.

Overall, the present findings provide breed-specific evidence of an association between *POU1F1* polymorphism and growth performance in Bach Thao goats. The consistent performance of TT goats across several growth traits suggests that *POU1F1* may have potential as a candidate molecular marker for growth-related traits. However, further validation in larger and genetically diverse populations is necessary before this locus can be recommended for routine marker-assisted selection in Bach Thao goat breeding programs.

CONCLUSION

In conclusion, three *POU1F1* genotypes (TT, TC, and CC) were identified in Bach Thao goats, with the TT genotype being the most frequent. The *POU1F1* polymorphism was significantly associated with body weight and average daily gain at several growth stages, with TT goats consistently showing superior growth performance compared with TC and CC goats. These finding suggest that the *POU1F1* gene polymorphism is a potential candidate for use as a molecular marker in breeding to improve the growth performance of Bach Thao goats.

DECLARATIONS

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Author's contribution statement
All authors had equal involvement in the design, experimentation, analysis, and manuscript writing.

Ethical approval
All experimental procedures involving animals were performed in accordance with the guiding principles for the use of animals in scientific research and with relevant animal welfare regulations under supervision of the Scientific Council of Tay Nguyen University.

Availability of data and materials
Available and can be provided upon journal request.

The sustainable development goals mentioned
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Competing interests
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