Analysis of Growth Hormone Gene Polymorphism and Its Association with Growth Traits in Kenguri Sheep

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Abstract— *The present study investigated polymorphisms of the growth hormone (GH) gene and their association with morphometric traits in Kenguri sheep, an important meat-type breed of Karnataka, India. A total of 60 adult sheep (16 males and 44 females) were sampled for genomic DNA isolation using a modified high-salt method. The GH gene fragment (422 bp) spanning exon 2–3 was amplified through polymerase chain reaction (PCR) and genotyped by PCR–restriction fragment length polymorphism (PCR-RFLP) using the HaeIII enzyme. Two genotypes, AA and AB, were detected, while BB was absent. The frequencies of AA and AB genotypes were 0.417 and 0.583, respectively, with allele frequencies of 0.708 (A) and 0.292 (B). Chi-square analysis indicated significant deviation from Hardy–Weinberg equilibrium ($\chi^2=10.29$, $p<0.05$). Morphometric traits including body weight, body length, height at wither, and chest girth were recorded. Males showed significantly ($p<0.05$) higher body weight and chest girth than females, while other traits were comparable. Within genotypes, no significant association ($p>0.05$) was found between GH polymorphisms and body measurements in either sex. The findings confirm the presence of GH gene variability in Kenguri sheep, with predominance of heterozygotes, although no strong phenotypic association was evident. This suggests that while the GH gene harbours genetic variability, additional markers or larger populations may be required to elucidate its role in growth performance. The study provides baseline molecular information that could support marker-assisted selection (MAS) strategies for sheep improvement programs in India.*

Keywords— Kenguri sheep, growth hormone gene, PCR-RFLP, polymorphism, morphometric traits, marker-assisted selection.

I. INTRODUCTION

India harbours one of the world's richest livestock genetic resources, with 65 million sheep distributed across diverse agro-climatic regions [1]. Sheep play a vital role in rural livelihood through meat, wool, and skin production. In recent decades, the demand for mutton has increased steadily, underscoring the need for scientific breeding strategies that improve productivity while conserving indigenous breeds. Kenguri sheep, primarily reared in the Koppal and Raichur districts of Karnataka, are a medium-to-large sized mutton-type breed characterized by their adaptability, disease resistance, and increasing popularity in stall-feeding systems [2]. Despite their potential, genetic improvement efforts in Kenguri sheep remain limited. Molecular markers, particularly those associated with growth, offer an opportunity to accelerate breed improvement.

The growth hormone (GH) gene, synthesized by somatotroph cells of the pituitary gland, is a crucial regulator of postnatal growth, lactation, reproduction, and metabolism [3,4]. It influences body weight, height, and carcass quality both directly and through the insulin-like growth factor (IGF) pathway [5]. Due to its central role, GH has been widely investigated as a candidate gene for marker-assisted selection (MAS) in livestock [6].

Polymorphisms in GH have been reported in several sheep breeds worldwide, including Indian populations such as Nellore, Patanwadi, and Vembur [7–9]. However, the extent of GH variability in Kenguri sheep and its association with growth traits has not been systematically studied. So this study was designed to detect polymorphisms of the GH gene in Kenguri sheep using PCR-RFLP, estimate genotype and allele frequencies and test Hardy–Weinberg equilibrium and assess associations between GH polymorphism and morphometric traits (body weight, body length, chest girth, and height at wither).

II. MATERIALS AND METHODS

2.1 Experimental Animals:

A total of 60 adult Kenguri sheep (16 males and 44 females) were randomly sampled from flocks maintained at Veterinary College, Bidar, and in their breeding tract.

2.2 DNA extraction and quantification:

Blood samples (10 ml) were collected aseptically from the jugular vein into sterile vacutainers containing EDTA as anticoagulant. Samples were transported on ice and stored at 4 °C until DNA extraction. Genomic DNA was extracted using the phenol–chloroform method (Andersson *et al.*, 1986). DNA quality was assessed on 0.8% agarose gel electrophoresis, and concentration and purity were determined spectrophotometrically (OD260/OD280 ratio).

2.3 PCR amplification of GH gene:

Primers amplifying a 422 bp fragment spanning exon 2–3 were used [4]. PCR conditions included: 35 cycles of denaturation at 95°C (30 s), annealing at 62°C (30 s), and extension at 72°C (30 s), followed by a final extension at 72°C (8 min).

TABLE 1
PRIMER SEQUENCES OF *FECB (BMPR1B)* AND *BMP15* GENES

Candidate gene	5'-Primer sequence-3'		Length (bp)	Product Size (bp)
FecB	Forward	CCAGAGGACAATAGCAAAGCAAA	23	190
	Reverse	CAAGATGTTTTCATGCCTCATCAACACGGTC	31	
BMP15	Forward	AGAGCCACTGTGGTTTACCG	20	434
	Reverse	GATGCAATACTGCCTGCTTG	20	

2.4 Restriction digestion and genotyping:

PCR products were digested with *HaeIII* enzyme, which recognizes the sequence GGCC. Products were separated on 2.5% agarose gel and visualized under UV. Genotypes were classified as:

- AA: 366 bp + 56 bp
- AB: 422 bp + 366 bp + 56 bp
- BB: absent

2.5 Morphometric traits:

Each animal was measured for body weight (kg), body length (cm), height at wither (cm), and chest girth (cm).

2.6 Statistical analysis:

- Genotypic and allelic frequencies estimated by direct count.
- Hardy–Weinberg equilibrium tested using χ^2 [11].
- Association between genotypes and morphometric traits tested using t-test [12].

III. RESULTS AND DISCUSSION

Genomic DNA isolated from 60 Kenguri sheep ranged between 220 and 637 µg/ml, with a mean concentration of 296.7 ± 15.9 µg/ml and OD ratios averaging 1.75, confirming its suitability for PCR amplification. A clear 422 bp amplicon of the GH gene was consistently obtained (Fig. 1). Following *HaeIII* digestion, two genotypes were identified: AA (366 and 56 bp) and AB (422, 366, and 56 bp). The BB genotype was absent in the population (Fig. 2). Among the 60 animals, 25 were AA and 35 were AB, corresponding to genotype frequencies of 0.417 and 0.583, respectively. Allele frequencies were 0.708 for A and 0.292 for B (Table 2).

TABLE 2
GENOTYPE AND ALLELE FREQUENCIES OF GH GENE IN KENGURI SHEEP

Genotype	Male (n=16)	Female (n=44)	Total (n=60)	Frequency
AA	9	16	25	0.417
AB	7	28	35	0.583
BB	0	0	0	0

FIGURE 1: PCR amplification of growth hormone gene (422bp) of Kenguri sheep

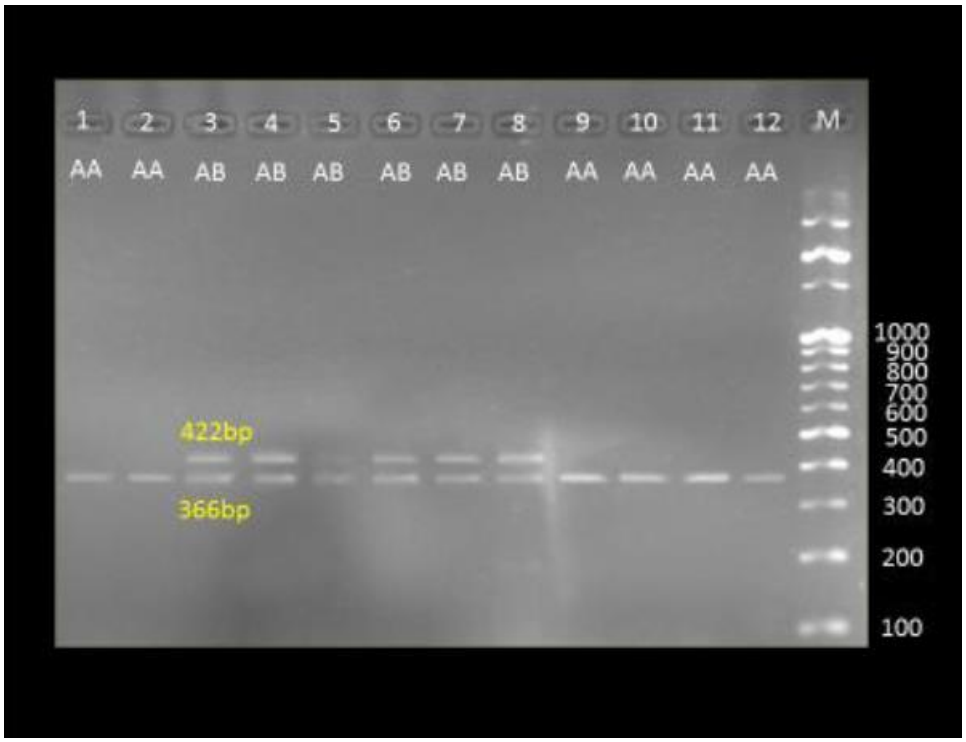

FIGURE 2: PCR-RFLP pattern of growth hormone gene (422bp) of Kenguri sheep

The predominance of the heterozygous AB genotype and the absence of BB mirror earlier findings in Indian breeds such as Vembur and Dumba [8,9]. This suggests restricted allelic diversity at this locus, which may be the result of historical selection pressures or drift. Chi-square testing showed significant deviation from Hardy–Weinberg equilibrium ($\chi^2 = 10.29$, $p<0.05$), indicating non-random mating or selection effects within the studied population. Similar disequilibrium has been reported in other Indian sheep breeds, including Marwari and Patanwadi [9].

Morphometric comparisons between sexes revealed that males were significantly heavier (37.46 ± 0.69 kg vs. 34.98 ± 0.59 kg) and broader-chested (83.18 ± 0.84 cm vs. 79.72 ± 0.72 cm) than females (Table 3). Height at wither and body length did not differ significantly between sexes. When analysed by genotype, however, no significant differences were observed for body weight, body length, height at wither, or chest girth in either males or females (Tables 4 and 5). For example, male AA animals averaged 38.31 kg in body weight compared to 36.38 kg for AB, but the difference was non-significant. Similarly, female AA animals averaged 34.76 kg against 35.10 kg in AB animals. These results demonstrate that GH gene polymorphism at the A781G locus is not strongly associated with morphometric traits in Kenguri sheep.

TABLE 3
MEAN MORPHOMETRIC TRAITS OF MALE AND FEMALE KENGURI SHEEP

Trait	Male (Mean ± SE)	Female (Mean ± SE)	p-value
Body weight (kg)	37.46 ± 0.69	34.98 ± 0.59	0.03*
Height at wither (cm)	71.25 ± 0.59	70.97 ± 0.38	0.41
Body length (cm)	69.18 ± 0.90	69.22 ± 0.50	0.47
Chest girth (cm)	83.18 ± 0.84	79.72 ± 0.72	0.02*

TABLE 4
BODY MEASUREMENTS OF FEMALE KENGURI SHEEP BY GENOTYPE

Trait	AA (n=16)	AB (n=28)	p-value
Body weight (kg)	34.76 ± 1.15	35.10 ± 0.69	0.62
Height at wither (cm)	71.00 ± 0.72	70.96 ± 0.44	0.88
Body length (cm)	69.56 ± 1.09	69.03 ± 0.51	0.57
Chest girth (cm)	79.37 ± 1.30	79.92 ± 0.88	0.49

TABLE 5
BODY MEASUREMENTS OF MALE KENGURI SHEEP BY GENOTYPE

Trait	AA (n=9)	AB (n=7)	p-value
Body weight (kg)	38.31 ± 1.18	36.38 ± 0.38	0.11
Height at wither (cm)	71.88 ± 0.80	70.42 ± 0.91	0.09
Body length (cm)	69.89 ± 0.79	68.28 ± 1.92	0.27
Chest girth (cm)	84.00 ± 1.26	82.14 ± 1.09	0.12

Comparable outcomes have been reported in Baluchi and Avikalin sheep, where GH polymorphisms showed little or no significant correlation with growth traits [13,14]. Nonetheless, studies in Indonesian sheep have shown that AA genotypes may be associated with higher weaning weights compared to BB [15], indicating breed- and environment-specific effects. The lack of association in Kenguri sheep could be due to small sample size, breed-specific genetic architecture, or the polygenic nature of growth traits.

While the present results do not support GH polymorphism as a direct marker for growth in Kenguri sheep, the presence of variability highlights its potential utility for diversity monitoring. Moreover, the predominance of heterozygotes suggests that the population retains genetic variability that could be exploited in future breeding programs. For effective marker-assisted selection, however, multi-gene panels including GH, IGF-1, MSTN, and LEP, along with larger population sizes, should be evaluated.

IV. CONCLUSION

This study documents the presence of GH gene polymorphism in Kenguri sheep, identifying two genotypes (AA and AB) with a predominance of heterozygotes and absence of BB. The population deviated significantly from Hardy–Weinberg equilibrium, suggesting non-random mating or selection effects. Although no significant associations were detected between GH polymorphism and morphometric traits, the findings provide baseline molecular information useful for breed conservation and genetic diversity studies. Future research with larger populations and multiple candidate genes is required to clarify the role of GH and related markers in growth performance and to establish effective marker-assisted selection strategies in Kenguri sheep.

CONFLICT OF INTEREST

Author declares no conflict of interest

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