

Study Polymorphism in Gh1 Gene and Relationship With Growth Performance and Feed Conversion Efficiency in Local Meat-Type Sheep in Karbala Governorate

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Abstract. Growth rate and feed efficiency are major determinants of profitability in meat-type sheep. This study investigated polymorphisms in exon 2 of the ovine GH1 gene and their associations with growth performance and feed conversion efficiency in 100. GH1 genotypes were determined by PCR-RFLP and confirmed by Sanger sequencing. The locus was polymorphic, with G and A allele frequencies of 0.64 and 0.36 and genotype frequencies of 0.42, 0.44, and 0.14 for GG, GA, and AA, respectively. The observed heterozygosity (0.44) was near to the expected heterozygosity (0.46), PIC was 0.35 and the population conformed to Hardy-Weinberg equilibrium ($P=0.67$). The study results showed that The initial body weight (BW 0) and final body weight (BW 90 $\pm$ 0) reached has been (18.4 $\pm$ 2.8) kg and (38.6 $\pm$ 4.2) kg, respectively, On days 30 and 60 body weights (BW-30 and BW-60), the weights were (24.8 $\pm$ 3.1) kg and (31.5 $\pm$ 3.6) kg, respectively. The average daily weight gain (ADG) over the entire period was (224 $\pm$ 38) g/day, indicating significant variation in growth performance between the animals. The total dry matter intake (DMI) after 90 days was (82.5 $\pm$ 12.4) kg, while the mean feed ratio (FCR) was (4.08 $\pm$ 0.64) kg feed/kg weight gain. GG lambs had the highest final body weight (40.2 $\pm$ 0.6 kg) and average daily gain (235 $\pm$ 5 g/day), followed by GA and AA lambs ($P<0.05$), but GG lambs had a more favourable feed conversion ratio (3.87 $\pm$ 0.08 kg feed/kg gain) than GA and AA lambs ($P<0.05$). These results indicate that GH1 variation, particularly the G allele, may be useful as a marker for improving growth performance and feed conversion efficiency in local meat-type sheep.

Keywords: Local Meat-Type Sheep; Growth Hormone; GH1 Gene; Polymorphism; Residual Feed Intake; Marker-Assisted Selection.

1. INTRODUCTION

The production of sheep meat, in an attempt to supply the increasing demand of protein in the worldwide market, is facing more challenges, and is equally trying to reduce the negative effects and production costs associated with it [1]. The growth rate and the feed conversion efficiency (FCE) are the most significant economical determinants of profitability in meat sheep, since feed constitutes the largest amount of input costs in intensive and semi-intensive businesses [2]. Feed efficiency can be improved by converting it into feed conversion ratio (FCR; feed intake per unit of weight gain) or residual feed intake (RFI; the difference between actual and predicted feed intake given maintenance and growth), which decreases feed utilization, lowers manure and methane emissions per unit of product and improves economic as well as environmental sustainability [3], [2].

In sheep the traits of growth and feed efficiency are moderately heritable [4], [5]. Studies of meta-analyses and genetic parameters have shown that the heritability's of RFI and FCR range between 0.11 and 0.49 in sheep, which means that significant genetic advances can be achieved through selection [5], [2]. Candidate gene and genome-wide association studies have revealed many loci influencing growth, carcass and feed efficiency phenotypes such as SHISA3 and RFC3 to FCR, and genes that influence muscle development, lipid metabolism and energy balance [6],[2]. Nevertheless, in spite of such a polygenic background, there remain, to be used as useful markers of marker-assisted selection (MAS) and as an ingredient of low-density SNP panels to implement genomic selection, major or moderate-effect loci [7].

One of the axes that support postnatal growth, skeletal growth, muscle accretion and fat metabolism is known as growth hormone (GH) system, the GH in sheep is coded by GH1 gene (also known as GH), which is predominantly expressed in the pituitary gland, but also by another copy GH2, which is predominantly expressed in the placenta [8]. GH stimulates the production of insulin-like growth factor-1 (IGF-1) and has an effect on nutrient partitioning between lean and fat tissues [9]. The GH1 locus variation is a viable cause of genetic variation in growth and carcass phenotypes [10], There are multiple studies in sheep and other livestock species that have identified single nucleotide polymorphisms (SNPs) and other sequence variants in GH or GH1, some of which have been strongly linked to body weight, average daily weight gain (ADG), linear body measurements, carcass trait and milk production [8].

A study showed a relationship between growth hormone gene polymorphisms and meat Productivity in meat and GH or GH1 polymorphisms have been reported in Merino -cross or Dorper, Harnali and other breeds and crossbreds, commonly detected with PCR -restriction fragment length polymorphism (PCR -RFLP) of exon 2 (GH/HaeIII) or other polymorphic fragments [11]. Polymorphism of growth hormone (GH Particular genotypes have also been linked with better growth and body conformation, but the extent and orientation of effects have differed among populations and environments, there were populations of Merinos that had the G allele at the GH/HaeIII site as the main one and were associated with desirable

body measurements [11], whereas heterozygous genotypes were found to have higher growth rate or higher carcass yield productivity [12].

In comparison, a limited number of studies have clearly used GH1 genotyping with direct measures of feed efficiency indices as FCR or RFI under standardized feeding conditions [2]. The majority of GH1 association studies have been done using growth and body size as the primary outcome with a few under field conditions with minimal control over diet and intake records. Since GH plays a key role in energy metabolism, it is plausible to assume that alleles affecting fast growth also affect feed intake and feed efficiency but the connection is not always direct and may require nutrient partitioning and efficiency in metabolism [13], [2].

Thus, there is a necessity in integrative studies that not only quantify growth performance but also feed efficiency with reference to GH1 genotypes under controlled conditions but especially in meat-type sheep as they are managed in the production system where genetic and nutritional approaches are being discussed of improving FCE. This knowledge would illuminate the possibility of GH1 polymorphisms to be used in breeding programs to achieve enhanced biological efficiency and carcass production, The current research aims to:

- a- Identifying the genetic polymorphism of the GH1 gene in 100 samples from local meat-type sheep.
- b- Estimate the parameters of genetic diversity at the GH1 gene locus in the studied population, such as allele and genotype frequencies, observed and predicted heterozygosity, polymorphic information content (PIC), and Hardy-Weinberg equilibrium tests.
- c- Measure the effect of GH1 gene polymorphisms on growth performance (body weights at specific ages and mean daily weight gain) and feeding efficiency characteristics (dry matter intake, feed ratio, and residual feed consumption, if applicable) in a 90-day controlled feeding trial.
- d- Estimate the relationship between GH1 genotypes and growth patterns and feeding efficiency using linear or mixed models.
- e- Exploring how to incorporate beneficial GH1 variants into molecular marker-assisted selection and genomic selection schemes to help meat-type sheep grow and feed more efficiently in various production systems.

2. MATERIALS AND METHODS

2-1: Animals, Management and Experimental Design.

The experiment was conducted on 100 samples of local meat-type sheep, from private farms in Karbala Governorate, raised in a cooperative commercial flock under an intensive or semi-intensive management system. Molecular genetic analyses were performed in a specialized laboratory to determine the genetic structures and their distribution ratios in the second exon of the GH1 gene and their relationship to growth performance and feed conversion efficiency in local Meat-Type Sheep. A young age group of lambs was selected at the start of the experiment to minimize age-related variation in growth characteristics. Both

males and females, as well as single and multiple-born lambs, were included, and their sex and type of birth were entered as a constant factor in the statistical models according to the design of growth hormone association studies [9], [6].

The experiment included an adaptation phase (14 days) and a (90 day) growth and feeding experiment (Figure 1). The lambs became accustomed to the housing environment, feeding system, and treatment system during the process of adaptation. During the experimental period, the lambs were provided with a standard basal diet designed to meet or exceed the nutrient-based requirements of growing lambs based on the existing feeding guidelines. The diet included a concentrated feed and a roughage component (such as hay or silage), and was provided in unlimited quantities or at a regulated level allowing to be expressed at full growth potential, as is done in feed efficiency research [14], [15]. Use standard health management procedures to minimize the spread of diseases or parasites. Lambs were placed in small pens with a known number of lambs to measure the amount of feed intake individually or in small groups, and a detailed record was made of the amount of feed intake and remaining, although it is preferable to use pens in which the amount of feed intake can be realistically estimated, as is the case in some feeding efficiency studies [2], [15].

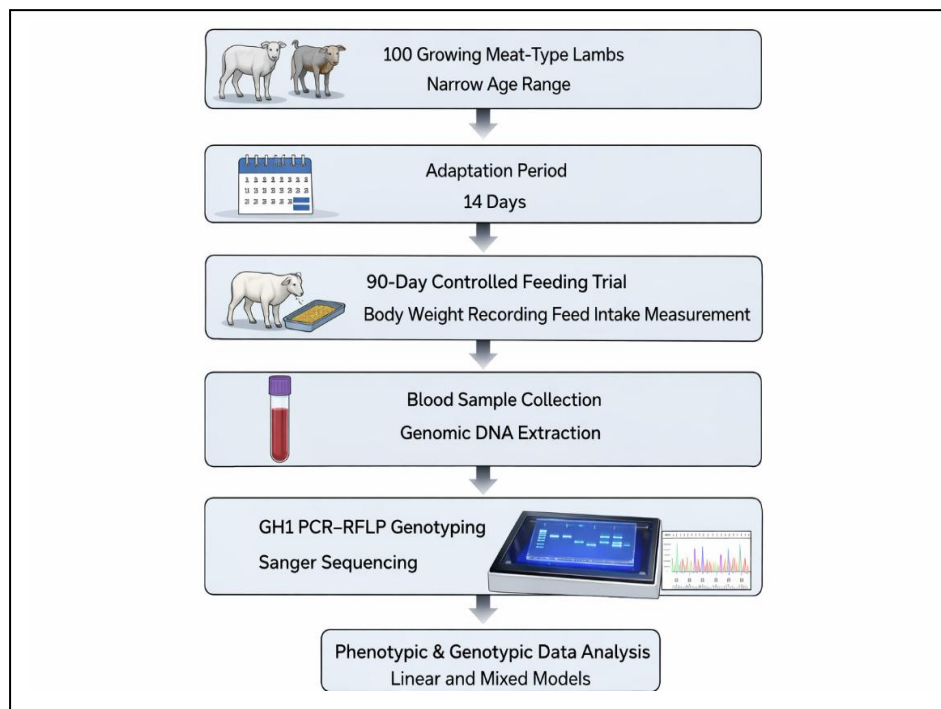


Figure 1 : Experiment diagram

2-2 : Diet Composition and Performance Recording

The basal diet were developed to maintain target ADG values of local meat-type sheep in intensive production. Standard laboratory analysis were on representative samples of the concentrate and forage components based on their dry matter, crude protein, ether extract, crude fibre or neutral and acid detergent fibre, ash and estimated metabolizable energy, according to similar protocols used by [14].

The amount of feed taken were documented in a daily basis, Individually fed lambs Individual feeds fed weighed and DM content feed to the lambs calculated as the difference between offered and refused feeds to the animals (daily dry mater intake, DMI). In the small pens, DMI were collected on the pen level with where necessary, a division among lambs based on the standard practices or automated feeding systems. At every 2 weeks interval and on day 90 of the 90 days of the trial, the weights of the lambs were be weighed before, during, and after the adaptation period. Weighing were performed in the morning following an overnight fast, or at a regular time in relation to feeding to reduce the variation in the gut-fill. The average daily gain (ADG, g/day) were calculated on the total period and the sub-periods (e.g. 0 -30, 30-60, 60-90 days).

Feed conversion ratio (FCR) for each lamb (or pen) over the trial calculated as:

$$FCR = \frac{\text{Total DMI over the period (kg)}}{\text{Total weight gain over the period (kg)}}$$

In cases where possible, residual were feed intake (RFI) approximated as the remainder of a multiple regression of DMI on metabolic body weight and ADG or other sources of energy expenditure (e.g. body weight change), using the procedures detailed in [2], [15]. The negative RFI values are associated with efficient animals (intake lower than it is supposed to be based on their size and growth), whereas positive ones are associated with inefficient animals [15].

2-3: Blood Samples Collection

At the right time (at the time of adaptation or at the beginning of the feeding experiment), Blood samples were drawn and collected from the jugular vein, one sample local meat-type sheep, totaling (15) ml, using a medical syringe, After cleaning and sterilizing the vein area with 70% ethyl alcohol, the blood was collected and placed in vacuum-sealed test tubes containing an anticoagulant Ethyl Diaminic Tetra Acetic - EDTA K3) [16], and to prevent blood clotting, the tube was moved immediately after collection for the purpose of mixing the blood with the anticoagulant for thrombosis [17], The samples were temporarily stored in a box containing ice at 4°C, or kept in a refrigerator at (20) °C for the long term until the DNA extraction process was carried out.

2-4 : DNA extraction

DNA was extracted from blood samples using a commercial genomic DNA extraction kit, a protocol commonly used for genomic DNA extraction in the small ruminant molecular genetics community [18] (Figure 2).

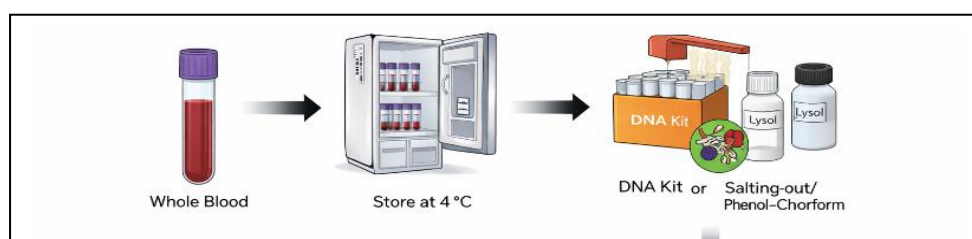


Figure 2 : The figure illustrates how genetic material is extracted from sheep blood. The blood is placed in special tubes with a preservative and then refrigerated until use. The cells are opened using either ready-made instruments or an old mixture of salt and solvent. The genetic material (DNA) is then purified to become independent. A device verifies its quantity by measuring light at specific wavelengths. Finally, the quality of the strands is tested by passing them through a jelly-like gel to examine their shape and size.

2-5 : Measuring the concentration and purity of DNA.

The concentration of extracted DNA was measured to determine sample quality using a Quantus Fluorometer, (1) microliter of extracted DNA was added to (199) microliters of diluted Quanta Fluor Dye and mixed well. The mixture was left for 5 minutes at room temperature, after which the concentration of genetic material was measured and was 15-20 ng/ μ l.

The purity of the DNA was measured using a spectrophotometrically device, as the use of this device made this process easy and fast, In addition to revealing the potential error rate in the sample, the standard reading for DNA is 1.8, while readings that differ from this ratio indicate contamination in the sample, meaning that the sample still contains protein or some other substances, the reading was taken at a wavelength of 260-280 nanometers.

2-6 : GH1 Genotyping and Polymorphism Detection.

2-6-1 : Target Region and Primers

The GH1 fragment that is analysed regarding polymorphism were a polymorphic fragment already described that contains the exon 2 GH/HaeIII site that has been widely utilised in association analysis of growth traits in sheep[11], [19]. The primer sequences can be based on the sequences described as corresponding to a 400-450 bp amplicon containing the HaeIII site [20], [21] (Table 1).

Table 1 : Primer sequence .

Primers	
Forward	5'- CTCTGCCTGCCCTGGACT -3'
Reverse	5'- GGAGAAGCAGAAGGCAACC -3'

A portion of the GH1 gene, approximately 422 base pairs long, was amplified by these primers and could then be further digested using HaeIII to reveal genotype-specific band patterns (Figure 3).

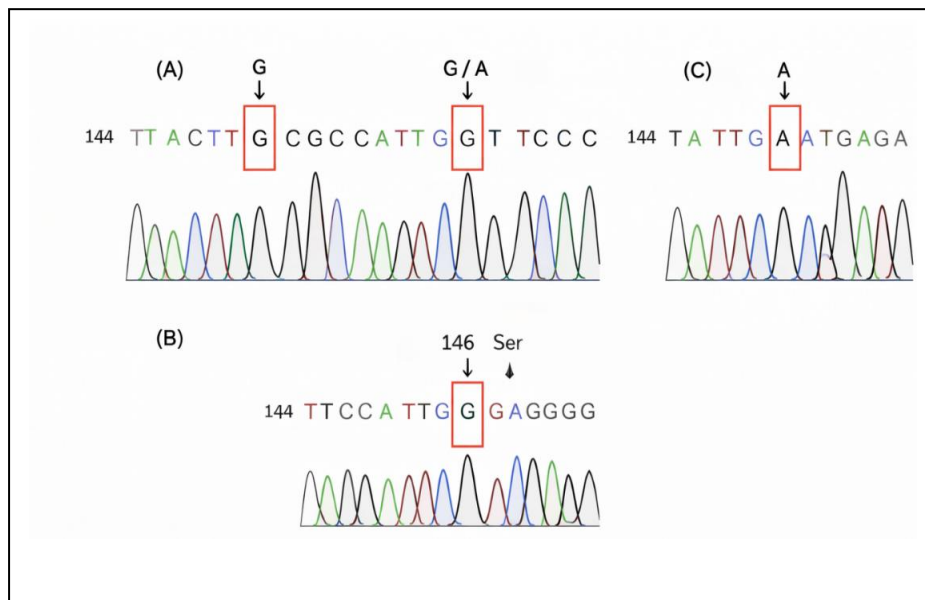


Figure 3. It shows typical Sanger traces for part of the sheep GH1 gene, specifically exon 2 near the GH/HaeIII variable spot. Part A displays a pure GG form - just one strong G signal right at the variant point. Instead, part B reveals a mix: both G and A signals pile up together, marking a GA blend. Then part C presents another pure type, only this time it's AA, marked by a lone A spike. Above each readout, you see which DNA swap occurred, along with any resulting shift in protein building block if relevant.

2-6-2 : PCR Amplification

The Polymerase chain reaction (PCR) was performed in a reaction volume between 20- 25 μ L, consisting of genomic DNA template, forward and reverse primers, dNTPs, MgCl₂, PCR buffer and Taq DNA polymerase. The optimal conditions for reaction cycle were determined according to protocol [11] (Table 2).

Table 2 : Thermal Cycling Protocol

Stages	Temperature o C	Time	Number of cycles
Initial Denaturation	94 – 95	3-5 Minute	35
Denaturation	94 – 95	30 Second	30
Annealing	60 – 65	30 Second	
Extension	72	30 - 45 Second	
Final Extension	72	5 - 7 Minute	1
Hold	10	10 Minute	

To identify the successful amplification of target DNA and the size of the expected products, PCR products was visualized on 1.52-percent agarose gels stained in ethidium bromide or a different DNA dye.

2-6-3 : PCR–RFLP and Sequencing

PCR-RFLP analysis was performed after a sample of each PCR product were digested using the appropriate amount of restriction enzyme HaeIII and buffer solution at 37°C for a sufficient time According to the manufacturer's instructions. Use 23% concentration agarose gel to separate the digestion products and detach any restriction fragments showing various genotypes patterns (Figure 4), in the case of the GH/HaeIII locus, common banding patterns can be used to indicate both homozygous (GG has a single fragment of around 366 bp; AA to an undigested band of around 422 bp) and heterozygotes (GA has all three bands), depending on the polymorphism described in the population [11]. In order to verify the nature of the polymorphism and confirm PCR RFLP genotyping, samples representing each pattern of RFLP thus observed was Sanger sequenced GH1 amplicon. This were done by aligning the sequences with GenBank reference ovine GH1 sequences and reported exon 2 and flanking region mutations in ovine GH [22], [19].

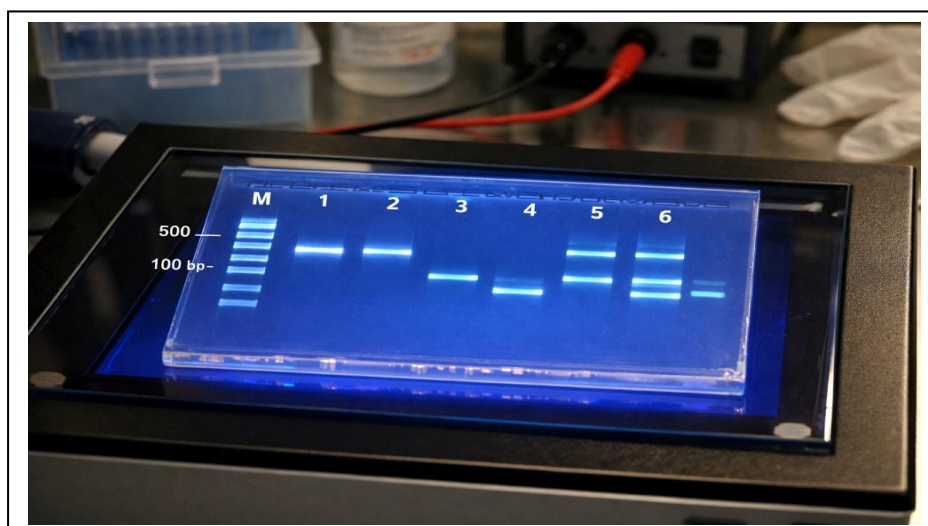


Figure 4. Agarose gel electrophoresis of GH1 PCR–RFLP products digested with HaeIII. Lane M: 100 bp DNA ladder. Lanes 1–2: AA genotype with an undigested fragment of approximately 422 bp. Lanes 3–4: GG genotype with a digested fragment of approximately 366 bp. Lanes 5–6: AG genotype showing both the 422 bp and 366 bp fragments.

2-7 : Genetic Diversity Analysis

The genetic diversity of GH1 locus was characterized by calculating allele frequencies, genotype frequencies, observed heterozygosity (H_o), expected heterozygosity (H_e), Hardy-Weinberg equilibrium (HWE), and polymorphic information content (PIC). The deviation from Hardy-Weinberg equilibrium was evaluated using chi-square tests or exact tests, as it is accepted in the literature of the sphere of studying gene diversity [23], [11]. The significance of the GH1 index in the studied sample was calculated using the PIC index, and values greater than 0.25 are considered in most cases to indicate moderate to high genetic polymorphism, The results were compared with the GH1 diversity in other studies on sheep breeds [24], [18].

2-8 : Association Analysis by Statistic Methods.

Depending on the nature of the data, some linear models or linear mixed models were used to test associations between GH1 genotypes and phenotypic characteristics. Where Y is a trait and X is a fixed covariate (animal BW 0, overall ADG) i.e. $Y = 1 + \text{Covariate } X$.

$$Y_{ijkl} = \mu + G_i + \text{Sex}_j + \text{BirthType}_k + e_{ijkl}$$

It has been applied, where Y_{ijkl} is the observed trait value, μ is the overall mean, G_i is the fixed effect of the GH1 genotype ($i = 1, 2, 3$), Sex_j is the fixed effect of sex, BirthType_k is the fixed effect of birth type (single, twin, etc.), and e_{ijkl} is the random residual error term. This structure is consistent with models used in GH genotype association studies in meat type and Merino derived sheep [12], [25].

For traits with repeated measures (e.g. body weight over time), mixed models including animal as a random effect were used, for example:

$$Y_{mijkl} = \mu + G_i + \text{Sex}_j + \text{BirthType}_k + \text{Time}_m + A_l + e_{mijkl}$$

Where "Time"_m is the fixed measurement time effect, A_l is the animal random effect and the rest are as stipulated above. Repeated measures were reflected on appropriate covariance structures (compound symmetry or autoregressive).

Statistical programs like SAS, R or other such tools were used to conduct the analyses. Each GH1 genotype class were estimated to have an least squares means (LS Means) and its standard error, as well as comparing genotypes to each other by using appropriate multiple comparison procedures (Tukey test). Statistical significance were proclaimed at $p < 0.05$ and tendencies at 0.05.

Additive and dominance effects of GH1 alleles were estimated where feasible using standard contrasts coding genotypes with the value of allele substitution as a contribution to the trait variation as is common with quantitative genetic analyses[23]. The digestion of DNA using the restriction enzyme HaeIII resulted in three band patterns corresponding to three genotypes at the GH1/HaeIII locus: GG.

3. RESULTS AND DISCUSSION.

3.1. RESULTS

3-1.1. GH1 polymorphism and genetic diversity

The results of the study showed that DNA amplification (PCR) of the 422-base-pair exon 2 segment of the GH1 gene was successful in all 100 lamb samples taken, confirming the success of the target amplification process. The digestion of DNA using the restriction enzyme HaeIII resulted in three band patterns corresponding to three genotypes at the GH1/HaeIII locus: GG (single band at 366 bp), GA (two bands at 422 and 366 bp), and AA (undigested band at 422 bp). DNA sequencing on a representative sample for each band pattern revealed the GA single nucleotide polymorphism (SNP) at position 127 in exon 2, which is consistent with the sheep GH1/HaeIII SNP.

3.1.2. Allele and genotype frequencies and diversity indices at the GH1 locus in local meat-type sheep.

Table (3) shows allele and genotype frequencies and diversity indices at GH1 locus, The table showed that the G allele was the most common, with a frequency of (0.64), while the frequency of the A allele was (0.36), The frequency of the genotypes was 0.42, 0.44 and 0.14 in GG, GA and AA respectively. The observed heterozygosity (0.44) was near to the expected heterozygosity (0.46) when there is Hardyweinberg equilibrium, This suggests that the population is genetically balanced as a result of selection or artificial insemination, or it may be attributed to genetic and environmental factors. chi-square test was used to check whether there is some active directional selection at this locus in the flock under study ($\chi^2 = 0.18$, $p = 0.67$), and The result indicated the absence of selection at this genetic locus. The polymorphic information content (PIC) was 0.35 which indicating moderate genetic diversity and therefore GH1 gene locus is informational value in this population.

Our results were similar to those of [11] in their study on Merino crossbreeds in terms of allele and genotype ratios, as well as the average PIC value. The G allele was the most common, with a frequency of 0.85, followed by the A allele at 0.15. The genotype frequencies were 0.70, 0.30, and 0.00 for GG, GA, and AA, respectively, and the PIC value was 0.22. Our results also closely matched those of [26] in their study on Dorper sheep, where the A allele was the most common, with a frequency of 0.85, followed by the B allele at 0.15. The genotype frequencies were 0.70, 0.30, and 0.00 for AA, AB, and BB, respectively, and the PIC value was 0.22. GH1 showed moderate levels of Polymorphism. This stability among populations justifies the potential use of GH1 as a genetic locus for genetically supported selection in local meat-type sheep.

Regarding the observed and expected heterozygosity ratio, our results differed from those of [26] in his study on Dorper sheep, where the observed heterozygosity ratio was 0.74 and the expected ratio was 1.56. Our results also differed from those of [11], where the observed and expected heterozygosity ratios were (0.30 and 0.25) respectively.

Table 3. Allele and genotype frequencies and diversity indices at the GH1 locus in local meat-type sheep (n = 100)

Parameter	Value
Number of Lambs (n)	100
Allele G frequency	0.64
Allele A frequency	0.36
Genotype GG frequency	0.42
Genotype GA frequency	0.44
Genotype AA frequency	0.14
Observed heterozygosity (Ho)	0.44
Expected heterozygosity (He)	0.46
Polymorphic information content (PIC)	0.35
χ^2 for Hardy–Weinberg equilibrium (p-value)	0.18 (p = 0.67)

3.1.3. Growth performance traits and their association with GH1 genotypes

Table (4) shows the overall average growth characteristics and feeding efficiency of 100 lambs. The initial body weight (BW 0) and final body weight (BW 90 $\pm$ 0) were (18.4 $\pm$ 2.8) kg and (38.6 $\pm$ 4.2) kg, respectively. On days 30 and 60 body weights (BW -30 and BW 60), the weights were (24.8 $\pm$ 3.1) kg and (31.5 $\pm$ 3.6) kg, respectively. The table also shows that the average daily weight gain (ADG) over the entire period was (224 $\pm$ 38) g/day, with a coefficient of variation of 17, indicating significant variation in growth performance between the animals. The results of the same table showed that the total dry matter intake (DMI) after 90 days was (82.5 $\pm$ 12.4) kg, while the mean feed ratio (FCR) was (4.08 $\pm$ 0.64) kg feed/kg weight gain. The estimated 92 lambs residual feed intake (RFI) was found to be on the negative side of feed intake (average values of 0.02 +0.58 kg/day with a range of -1.34 to +1.28 kg/day).

Table 4. Descriptive statistics of growth and feed efficiency traits in local meat-type sheep (n=100)

Trait	Mean $\pm$ SD	Min	Max	CV (%)
Initial body weight (BW ₀) kg	18.4 $\pm$ 2.8	13.2	25.1	15.2
Final body weight (BW ₉₀) kg	38.6 $\pm$ 4.2	28.5	48.3	10.9
BW at day 30 (BW ₃₀) kg	24.8 $\pm$ 3.1	18.4	32.0	12.5
BW at day 60 (BW ₆₀) kg	31.5 $\pm$ 3.6	23.7	40.2	11.4
ADG (0–90 days) g/day	224 $\pm$ 38	152	315	17.0
Total DMI (0–90 days) kg	82.5 $\pm$ 12.4	58.3	110.2	15.0
FCR (0–90 days) kg / kg	4.08 $\pm$ 0.64	2.85	5.92	15.7
RFI (n = 92) kg/day	-0.02 $\pm$ 0.58	-1.34	+1.28	—

3.1.4. Least-squares means ($\pm$ SE) of growth traits by GH1 genotype

Table (5) shows the least squared means of growth traits for the GH1 gene genotypes (Figure 5). The table shows no significant effect of baseline body weight on genotypes, indicating that the groups were similar in terms of body mass at the beginning of the experiment, There were, however, definite genotype influences observed in the feeding period. The study results showed a highly significant superiority ($P \leq 0.01$) in final body weight (BW

90 kg) for lambs with the GG genotype, reaching (40.2 ± 0.6 kg), followed by the GA and AA genotypes, with weights of (38.1 ± 0.5) and (36.3 ± 1.2 kg) respectively.

The results in the same table showed a significant difference ($P \leq 0.05$) in the average daily weight gain, which was similar. Lambs of the GG genotype gained weight faster (235 ± 5 g/day), lambs of the GA genotype gained weight at an average rate (220 ± 4 g/day), and lambs of the AA genotype gained weight at a slower rate (208 ± 9 g/day). The results also indicated significant differences ($P \leq 0.05$) in the Mean intermediate body weights on days 30 and 60 of rearing. The GG genotype was more prone to weight gain on days 30 and 60, with weights of (25.4 ± 0.5) and (32.7 ± 0.5) respectively, compared to the GA and AA genotypes. The difference in body weight at age 0 between the genotypes was found to be statistically significant.

Table 5. Least-squares means ($\pm$ SE) of growth traits by GH1 genotype (model: $Y = \mu + \text{Genotype} + \text{Sex} + \text{BirthType} + e$)

Trait	GG (n = 42)	GA (n = 44)	AA (n = 14)	p -value
BW ₀ (kg)	18.6 ± 0.4	18.3 ± 0.4	18.2 ± 0.7	N. S.
BW ₉₀ (kg)	40.2 ± 0.6^a	38.1 ± 0.5^b	36.3 ± 1.2^b	**
ADG (g/day)	235 ± 5^a	220 ± 4^b	208 ± 9^b	*
BW ₃₀ (kg)	25.4 ± 0.5^a	24.6 ± 0.4^{ab}	23.8 ± 0.9^b	*
BW ₆₀ (kg)	32.7 ± 0.5^a	31.2 ± 0.5^{ab}	30.1 ± 1.0^b	*

Superscripts (a, b) within a row indicate significant differences between genotypes at $p < 0.05$.

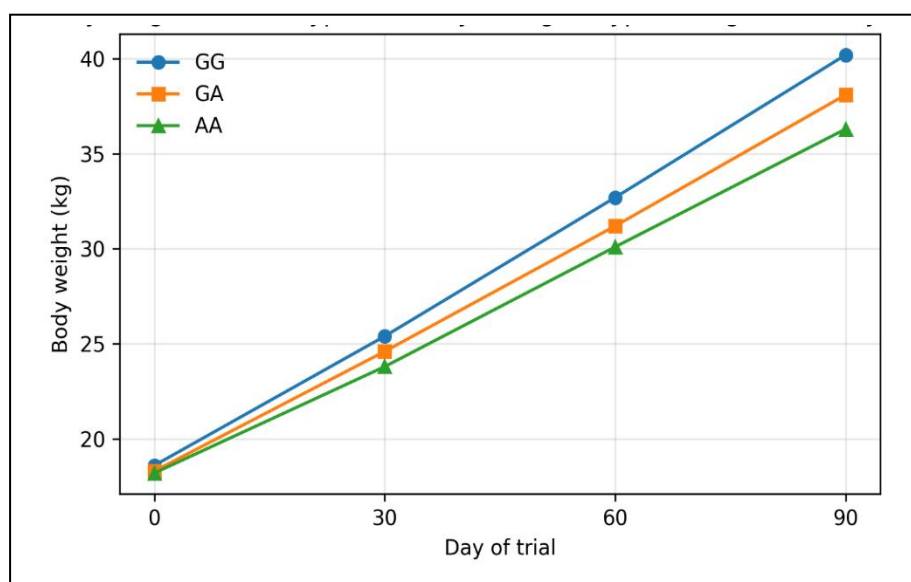


Figure 5 : growth curves by genotype

3.1.5. Feed efficiency traits and their association with GH1 genotypes

Table (6) contains the descriptive statistics of the feed efficiency characteristics of all lambs and based on genotype. The table results showed no significant effect of the three

genotypes on the total DMI during the ninety-day period in lambs, However, the superiority of the genotype was observed GG lambs (84.6 ± 1.8 kg) compared to GA (81.2 ± 1.5 kg) and AA (79.8 ± 3.2 kg) lambs, While the results of the same table showed a significant difference ($P \leq 0.05$) in the feed conversion ratio (FCR) between the genotypes (Figure 6), as lambs of the GG genotype recorded the best feed conversion ratio, which amounted to (3.87 ± 0.08 kg feed/kg weight gain), while lambs of the GA genotype were average (4.12 ± 0.07 kg feed/kg), and lambs of the AA genotype recorded the worst feed conversion ratio (4.35 ± 0.15 kg feed/kg) .

Genotype did not have any significant difference on residual feed intake, although the average values of RFI indicated a tendency whereby GG lambs exhibited a slight higher efficiency of residual feed intake (-0.08 ± 0.09 kg/day) than GA ($+0.01 \pm 0.08$ kg/day) and AA ($+0.05 \pm 0.17$ kg/day) lambs.

Table 6. Least-squares means ($\pm$ SE) of feed efficiency traits by GH1 genotype.

Trait	GG (n = 42)	GA (n = 44)	AA (n = 14)	p-value
Total DMI (kg)	84.6 ± 1.8	81.2 ± 1.5	79.8 ± 3.2	n. s.
FCR (kg/kg)	3.87 ± 0.08^a	4.12 ± 0.07^b	4.35 ± 0.15^b	$p < 0.05$
RFI (kg/day)	-0.08 ± 0.09	$+0.01 \pm 0.08$	$+0.05 \pm 0.17$	n. s.

Superscripts (a, b) within a row indicate significant differences between genotypes at $p < 0.05$.

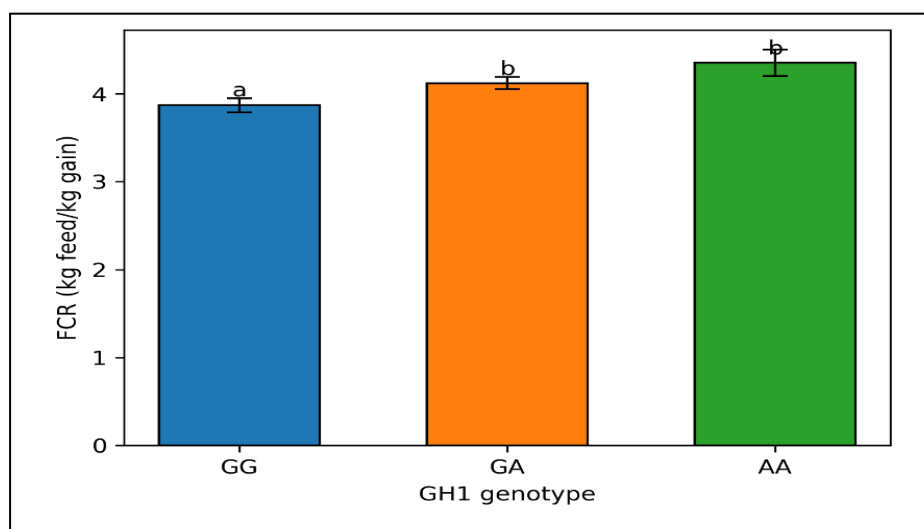


Figure 6. Feed conversion ratio (FCR) of local meat-type sheep across GH1 genotypes. Least-squares means ($\pm$ SE) of FCR (kg feed/kg gain) for GG, GA and AA lambs are shown. Lower FCR values indicate better feed efficiency, with GG lambs displaying the most favourable FCR

3.1.6. Implications for breeding programs and study limitations

The strong correlations between GH1 genotypes and growth and FCR imply that GH1 polymorphism is a good candidate marker that could be used in the local meat-type sheep breeding programs, By genotyping the lambs at the GH1/HaeIII locus at an early stage by

conducting a straightforward PCR-RFLP assay of the locus, breeders would selectively maintain the GG homozygotes as replacement stock which would result in a higher average growth rate and successive generations with improved feed-to-gain ratios. Since PCR-RFLP is technically simple and comparatively low-cost, GH1 genotyping can be performed even when resources are limited.

With more advanced breeding programs which already use genomic selection, the GH1 SNP can be included in low- or medium-density SNP panels with other growth, carcass and efficiency loci, and may increase the precision of the genomic prediction of these economically significant traits. Meanwhile, selection choices must explore potential correlated reactions in other features, e.g., mature body size, reproductive achievement and carcass structure, to promote a balanced progression in genes, and to save unwanted trade-offs.

3.2. DISCUSSION

The results of the current study show that GH1 genotype affects feed efficiency primarily via its effect on the growth rate as opposed to significant alterations in voluntary feed intake or basal metabolic needs. Lambs carrying the GG genotype grew faster and used the same total feed to gain more body tissue producing a lower FCR with no apparent statistically significant benefit in RFI. This trend is accredited to the effect of growth hormone in the stimulation of lean tissue accretion and in the delivery of nutrients to growth and indicates that GH1 in this population is more of a growth gene with secondary beneficial impacts on FCR. Conversely, those genes that have been directly effect in creating variation in RFI in other experiments can have more specific effects on metabolic efficiency per se.

The results of our current study confirm that polymorphisms in the GH1 gene influence postnatal growth variation in local meat-type sheep. The minimum difference in final body weight, 10 percent in GG relative to AA lambs and the 13 percent difference in the ADG are biologically significant differences, which would reflect into a few kilograms of added live weight in a normal fattening time. This kind of gain is economic under intensive and semi-intensive systems in which the rate of growth has a direct relationship with days to slaughter and the cost of the feed per Kg of weight gain. The additive nature of the G allele, which shows that GG lambs are excellent in comparison to GA and AA lambs is similar to what has been noted in other sheep populations where most favorable GH1 genotypes were found to have been associated with excellent growth and body conformation.

4. CONCLUSIONS

- 1- This study was able to describe polymorphisms of the ovine GH1 gene as well as describe the important relationships between the same with growth performance and feed conversion efficiency in a population of 100 local meat-type sheep.

- 2- The GH1 locus was proven to be polymorphic with moderate genetic variation (PIC = 0.35) with the G allele being the dominant one with a frequency of 0.64.
- 3- Sheep with the GG genotype had very high body weight, average daily gain, and favourable ratio of feed consumed over both GA and AA genotypes.
- 4- Effects of GH1 genotype GG on feed efficiency were mainly mediated by improvement in growth rate rather than by the alteration of residual feed intake which implies that GH1 acts more as a growth-related gene with secondary effects of feed conversion efficiency.
- 5- The additive gene effect that is evident with the G allele implies that the GG homozygotes would be optimally selected to maximize genetic returns in terms of growth and efficiency.

All these results lead to the conclusion that GH1 markers can be used in local meat sheep breeding programs as either a single marker in low-input systems or a part of low-density SNP array in genomic selection programs. By combining GH1 genotyping with other informative markers with sound phenotypic assessment, genetic enhancement of growth and feed efficiency can be hastened and can help improve the systems of providing sheep meat in a more sustainable and profitable manner.

DECLARATION OF COMPETING INTEREST

None

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