# Molecular characterization of candidate genes for body weight traits in Awassi sheep breed

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## Abstract

The objective of present study was to determine the genotypes of three loci (GH, CAST and MHC) that related to body weight traits in Awassi lambs. This experiment was conducted in commercial flock of Awassi sheep located at the Dhemat village –Erbil / Kurdistan region of Iraq. A total of 100, 92 and 87 lambs at birth, weaning and marketing age were used respectively, at lambing all relevant information were recorded and lambs were weighed at different ages. Also a total of 87 Blood samples at marketing ages were collected from lamb's for extracted the genomic DNA for each lamb, the purity and quantity of isolated DNA was measured using agarose electrophoresis and Nano Drop.

Most of Fixed effect have a significant effect on the lamb's body weight. The BLUP as a breeding value for body weight at BW, WW and MW ranged from -1.839 to. 2.161, -12.162 to. 10.837 and -18.283 to. 18.316 kg/lambs, respectively. According to the BLUP value for body weight at MW the lambs ranged in three groups (high production 10%, replacement group 70% and low production group 20%). The lambs at high production group significantly give higher body weight than the lambs in low production group. The DNA concentration and DNA purity was calculated were ranged between (4.82-10.61) ng/ µl and (1.20-1.28), respectively.

The PCR-RFLP results show that the CAST, MHC and GH loci have polymorphism among lambs. The superior genotype for (GH, Cast and MHC) genes investigated in this study for body weight traits is CDBCCD recorded for lambs in high production group (55.33 ± 1.84 kg/lamb), while the genotype of lambs in low production group (33.41 ± 3.36 kg/lamb) is BECDBE. The high rang for body weight BLUP value of lambs and different allele with genotype for each locus means the selection can play major roles in speeding genetic improvement for body weight and increasing incomes for breeders.

**Keywords:** Birth weight, Weaning weight, Marketing weight, BLUP, GH, Cast and MHC.

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## Introduction

The Awassi sheep, the most numerous small ruminant breed of Iraq (58.2%), are predominantly white with brown or red faces [1]. The head is big, with a pronounced convexity in males tending to mask its length. Males have long spiral horns and females are polled: although some females have short horns. The ears are semi-pendulous and the legs are usually covered with short white hair with occasional colored patches. The tail is fat, round, medium sized and short, reaching only to the hocks. The native sheep breeds in Iraq are fat tailed and carpet-wool type and are raised mainly for lamb and mutton production [2,3,4].

Body weight is the most important growth and development index in sheep production, and it influences meat and wool production, and reproduction of sheep, both directly and indirectly. During production, the birth, weaning, yearling, and adult weights are used to reflect the growth and development of sheep. The growth in body weight is influenced by a combination of the genetic background of the sheep and its feeding and management. Growth in weight is a highly heritable trait and is one of the main indexes of selection [5].

Selection of farm animals depends on the availability of information's in animals. Classic breeding methodology by estimating breeding values requires a lot of efforts, time and fund. Identifying major genes can speed up (at more precise and accurate) genetic improvement of economic traits of sheep breeds. Identification and characterization of breeds is required to identify the the genetic resources and also to prioritize breeds for conservation and development. Recently, the use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing role in animal genetics studies [6].

Calpastatin gene located on the chromosome No. five in sheep and has significant functions in base of skeletal muscles, degradation and meat tenderness after carnage [7]. Calpastatin is the most documented gene for selection of quantity and quality traits as double muscle and meat tenderness in animals [8]. The MHC of sheep is known as Ovar (‘Ovar’representing *Ovis aries*) and is located on chromosome No. twenty [9]. The major histocompatibility complex (MHC) plays a key role in immune response and has significant effects on reproductive traits in all mammals [10].

The ovine growth hormone gene is found on chromosome 11 (11q25), consisting of five exons, 1795 bp in size. The GH is pivotal for postnatal growth, tissue development, lactation, reproduction, and the

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metabolism of proteins, lipids, and carbohydrates [11]. The molecular study of body weight traits in sheep breeds stands at the forefront of genetic research, providing a roadmap to understand the intricate mechanisms shaping the physical characteristics of these animals [12].

Therefore, the objective of the present study is to identify the genetic polymorphism and best genotypes for lamb's growth using PCR-RFLP for three specific genes (CAST, MHC and GH).

## Material and Methods

### Experimental Animals and Locations

This study was undertaken in flock of Awassi sheep breed located at the Dhemat village –Erbil / KRG. In this study, 108 ewes were utilized. during September, 2024 to October, 2025. A total of 100 ewes lambed out of 108 ewes mated. At lambing ewes and lambs were identified with Neck collar. All animals were apparently healthy and were fed on pasture under open field conditions. Lambing date, birth weight, sex of lambs, and other relevant information were recorded. Body weights of 100 lambs at birth and 92 lambs at weaning weight (90 days) and 87 lambs at marketing weight (180 days), were also recorded.

### Blood collection and DNA extraction:

Blood samples were collected from a total of 87 lambs at marketing weight from jugular vein into 5 ml Vacutainer tubes containing he anticoagulant, ethylene diaminetetra-acetic acid (EDTA). These samples were stored at -20°C until DNA extraction. All research works were carried out in the Research center of Erbil International University, from November 2025 to March 2026. Genomic DNA was isolated from samples using extraction kit (Bio. Tech, koria). The quantity and quality of DNA was checked by Nanodrop spectrophotometer and gel electrophoresis.

### Loci Primers:

In the present study, a total of three specific loci (CAST, MHC and GH) primers which were obtained from (Macro gene) were used. The descriptions of primers sequencing, restriction site and PCR product size for all primer in present study are illustrated in (Table 1).

**Table 1. Primers, Restriction Enzymes and restriction site for Candidate genes.**

| Gene | Oligonucleotide | Amplicon size(bp) | RE | Cut Site | References |
|---|---|---|---|---|---|
| CAST | F 5'TGGGGCCCAATGACGCCATGATG-3´. | 622 | MspI | 5' ---C CGG---3'<br>3' ---GGC C---5 | [13] |
| | R 5'-GGTGGAGCAGCACTTTGATCACC- 3' | | | | |
| MHC | F 5'-TCTCTGCA GCACATTTCCTGG -3' | 279 | RsaI | 5' ---GT AC---3'<br>3' ---CA TG---5' | [14] |
| | R 5'- CTCGCCG CTGCACAGTGAAAC-3' | | | | |
| GH | F: 5-GGAGGCAGGAAGGGATGAA-3' | 934 | HaeIII | 5' ---GG CC---3'<br>3' ---CC GG---5' | [15] |
| | R: 5´-CCAAGGGAGGGAGAGACAGA-3´ | | | | |

**PCR Amplification:**

The PCR amplification system summarized was optimized to ensure reliable and reproducible amplification of the target genomic region. The use of a 2× Master Mix (AMPLIQON A/S Stenhuggervej 22) in a 50 µl reaction volume provides standardized concentrations of DNA polymerase, dNTPs, $MgCl_2$ , and buffer, reducing technical variation among reactions. This approach is commonly recommended for molecular genetic studies in livestock due to its consistency and efficiency Bioresearch PTC-200 Gradient thermocycler.

The forward and reverse primers at 20 pmol each fall within the optimal range for achieving high specificity and sufficient amplification yield, thereby minimizing non-specific products. Additionally, the inclusion of 50 ng/µl template DNA ensures adequate target availability, which is

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critical for accurate genotyping and downstream polymorphism analysis. The use of DNase-free water further safeguards reaction integrity by preventing contamination or DNA degradation.

The total reaction volume in this study was 50 µl. The reaction mixture contained 25 µl of Master Mix (2× concentration), which represents half of the total reaction volume. Both forward and reverse primers were added at a concentration of 20 pmol, with a volume of 3 µl for each. The reaction mixture also included 15 µl of DNase-free water, which was used to adjust the reaction mixture to the final desired volume while maintaining reagent purity. Finally, 4 µl of template DNA with a concentration of 50 ng/µl was added to the reaction mixture.

**PCR Program:**

The PCR amplification conditions optimized for the **CAST**, **MHC**, and **GH** genes, each targeting amplicons of different sizes (622, 279 and 934 bp), respectively. A common initial denaturation step at 95 °C for 5 minutes was applied to ensure complete DNA strand separation, followed by repeated denaturation at 95 °C for 38 seconds to maintain template accessibility throughout the amplification cycles.

Gene-specific annealing temperatures were employed to enhance primer specificity and amplification efficiency. The annealing temperature of **58 °C** for the **CAST** gene and **59 °C** for the **MHC** and GH genes reflects optimization based on primer melting temperatures and target sequence characteristics. Extension at 72 °C for 1 minute was sufficient for all targets, including the larger GH fragment, given the polymerase extension rate. A final extension step at 72 °C for 10 minutes ensured complete synthesis of all PCR products, followed by a holding step at 4 °C to preserve amplified DNA.

**DNA sequencing and RFLP detection:**

DNA sequencing, using only a forward primer was performed separately by ABI 3130X genetic analyzer (Applied Bio system). The PCR products of the eight samples were used as a source of DNA template for sequence-specific PCR amplification. The sequencing technology process was performed by the Macrogen company (Korean) using Sanger dideoxy sequencing.

PCR products were digested with the restriction endonucleases *HaeIII* for the GH gene with restriction sit (GG↓CC), *MspI* for Cast gene with restriction sit (C↓CGG) and *RsaI* for MHC gene with restriction sit (GT↓AC) to find out genotypes among high and low production groups of lambs. Distinguishing among them separately applied into NEBcutter, a program to cleave

DNA with restriction enzymes, available via a web server (http://tools.neb.com/NEBcutter).

**Statistical and Bioinformatics Analysis:**

The PROC GLM (General Linear Model) procedure [16] was used to analyze the data for body weight traits. Fixed effects studies were flock, age of dam, sex of lamb, and month of lambing and Season of birth were fitted in the following model:

$$Y_{ijklm} = \mu + A_i + S_j + C_k + P_l + \varepsilon_{ijklm}$$

where $Y_{ijklm}$ = Weights of lambs at different ages and average daily gain (ADG) of individual lambs; μ = Over all mean; Ai = Fixed effect of Age of ewes (i = 2,3,4 and 5 years old); Sj = Fixed effect of sex of lambs (j = 1, Male and 2, Female); $C_k$ = Fixed effect of month of lambing (k = l =Sep., 2= Oct, 3 = Nov., 4 =Dec., and 5= Jan.); Pl = Fixed effect of season of birth (Pl, 1= Autumn, 2= Winter); $E_{ijklm}$ = Random/error effect.

For genetics evaluation of lambs (High and low production) for various performance traits, Best Linear Unbiased Prediction (BLUP) procedure described by [16] was applied. The model used for this purpose was the Mixed Model (Fixed + Random effects) of [16] software. The individuals of the Awassi breed were assembled in three groups; high (9 lambs), medium (61 lambs) and low (17 lambs) production, according to the BLUP value for MW: where 10% of lambs for the 1st group (Top Production group), 70% for 2nd group and 20% for 3th group (low production group). The above Equation 1 was used to analyze the difference among lambs group after add the groups effect to the equation.

DNA sequences were analyzed by using the Chromas (V. 2.6.5 Technelysium Pty Ltd, https://technelysium.com.au/wp/chromas/).DNA sequence analysis and alignments were carried out using NCBI BLAST: Nucleotide sequence. For genotyping detecting the NEBcutter program was used.

**Genotypes' (AMOVA) effects for body weight traits**

$$\mathbf{Y_{ij} = \mu + L_i + E_{ij}}$$

Where:
$\mathbf{Y_{ij}}$ = Lambs weight traits.
**μ** = Over all mean.
$\mathbf{L_i}$ = all loci (GH, CAST and MHC) effects (i=1, CDBCCD High Production or i=2, BECDBE
$\mathbf{E_{ij}}$ = Experiment error.
Duncan's tests employed to compare all the traits means under study, as its suitable method for comparing least square means within this program.

**Results and Discussion**

**Phenotypic results**

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**Growth traits for body weight of Awassi lambs:**

The overall mean of birth weight (BW), weaning weight (WW) and marketing weight (MW) averaged 4.83 ± 0.86, 23.36 ± 4.21 and 42.81 ± 7.62 kg/ lamb, respectively (Table 2).

The present study demonstrated that several non-genetic factors significantly influenced body weight traits of local Awassi lambs. Ewes age had a significant effect ($p \leq 0.05$) on BW and WW, while its effect on MW was non-significant; lambs born to four-year-old ewes exhibited the highest BW (5.10 ± 0.80 kg/lamb) and WW (24.75 ± 3.77 kg/lamb) (Table 2), which may be attributed to the optimal reproductive efficiency and physical condition of ewes at this age, resulting in improved prenatal and early postnatal development. These findings are consistent with previous reports in Hamdani [17], Barki [18], and Baluchi sheep [19]. Month of birth significantly influenced BW, WW, and MW ($p \leq 0.05$), as lambs born in November recorded heavier BW (5.19 ± 0.94 kg/lamb) and MW (46.96 ± 8.82 kg/lamb), whereas those born in September achieved the highest WW (25.80 ± 4.75 kg/lamb); these variations are likely related to climatic conditions and management practices, in agreement with [20] and [21], although [22] reported non-significant effects of lambing month on BW in Boni sheep. In contrast, [23] reported a significant influence of lambing month on WW in Awassi sheep. Season of birth did not significantly affect BW or MW but had a significant effect on WW ($p \leq 0.05$), with autumn-born lambs being heavier (24.18 ± 4.32 kg/lamb) than winter-born lambs (21.82 ± 3.56 kg/lamb), supporting the findings of [24] and [22], who reported superior weaning weights for lambs born in favorable seasons. Furthermore, sex of lambs significantly affected BW, WW, and MW ($p \leq 0.05$), with males outperforming females (5.13 vs. 4.50 kg, 24.62 vs. 21.97 kg, and 47.00 vs. 38.53 kg, respectively), in agreement with [25] and [26]; these differences are commonly attributed to hormonal effects, particularly higher secretion of growth hormone and the influence of sex hormones in males [27] and [28].

**Growth traits of Awassi lambs:**

The overall means of average daily gain (ADG) of Awassi lambs at different growth stages are presented in (Table 3), with pre-weaning, post-weaning, and total ADG averaging (0.20 ± 0.04), (0.21 ± 0.05), and (0.21± 0.04 kg/lamb/day), respectively.

The present study showed that several non-genetic factors influenced ADG of Awassi lambs at different growth stages. Ewe age had no significant effect on pre-weaning, post-weaning, or total ADG, a finding consistent with [29], who reported non-significant effects of ewe age on pre-weaning daily gain

in Karakas, Ile de France × Akkaraman, and Turkish Awassi lambs, and with [21], who observed similar results for post-weaning gain in Karadi sheep (Table 3).

As in Table (3) the month of birth had a non-significant effect on post-weaning ADG but significantly influenced pre-weaning and total ADG ($p \leq 0.05$), with lambs born in September achieving the highest pre-weaning ADG (0.24 ± 0.02 kg/lamb/day), while those born in November showed the highest total ADG (0.23 ± 0.04 kg/lamb/day); similar significant effects of month of birth on ADG were reported by [21], [30], and [31]. Season of birth did not significantly affect post-weaning or total ADG but had a significant effect on pre-weaning ADG ($p \leq 0.05$), as autumn-born lambs exhibited higher gains (0.21 ± 0.04 kg/lamb/day) than winter-born lambs (0.18 ± 0.03 kg/lamb/day), in agreement with [32] and [4]. In addition, sex of lambs significantly affected pre-weaning, post-weaning, and total ADG ($p \leq 0.05$), with male lambs outperforming females by recording higher gains at all stages (0.21 ± 0.05, 0.25 ± 0.05, and 0.23 ± 0.04 kg/lamb/day, respectively); however, these results contrast with those reported by [33] in Rahmani and Chios sheep, [34] in Abera sheep, and **[**21] in Karadi lambs.

**Correlation Coefficients among Growth Traits:**

The correlation coefficients among body weight traits in Awassi lambs (Table 4) reveal strong and biologically meaningful relationships among growth traits from birth to marketing age, indicating that early-life weights are reliable predictors of subsequent growth performance.

The correlation between BW with WW concurred with the findings of [35] and [36]. The current study findings align with those of [19], revealing a significant correlation between birth weight with weaning weight, marketing weight, pre-weaning and post-weaning daily gain in Baluchi sheep. Overall, the findings in this study align with previous estimates from researchers such as [37], [38]and [19] across various sheep breeds.

**BLUP values for MW in Awassi lambs:**

To remove the effect of fixed factors mixed model was used to calculate the BLUP value which is one methods used to evaluation the animal upon his performances to obtained the realized value for each animals under study to ranking them according to his BLUP values for each trait to select the best one among all animals to speed up the production in next generation.

The results in **Table 5**. show the BLUP values for the total marketing weight of lamb per ewes. These values range from -18.283 kg/lambs (the lowest) to. 18.316kg/lambs (the highest). The top 10% (To be

parents for next generation) of lambs, identified as potential dams for ram lambs, had values between 12.316 and 18.316 kg/lambs. In contrast, the bottom 20% (Culling group), which were designated for culling, had values ranging from -5.683 to -18.283 kg/lambs.

**Table 2.** Least square means ± SD for body weights (kg /lamb) at different ages in Awassi lambs.

| Factors | Levels | Birth Weight | Weaning Weight | Marketing Weight |
|---|---|---|---|---|
| **Overall mean** | | 4.83 ± 0.86 | 23.36 ± 4.21 | 42.81 ± 7.62 |
| **Age of ewes (Year)** | | * | * | NS |
| | **2** | 4.74 ± 0.87$^{ab}$ | 22.60 ± 4.14$^{b}$ | 41.77 ± 7.65$^{a}$ |
| | **3** | 4.86 ± 0.90$^{ab}$ | 23.45 ± 4.04$^{ab}$ | 42.30 ± 8.36$^{a}$ |
| | **4** | 5.10 ± 0.80$^{a}$ | 24.75 ± 3.77$^{a}$ | 44.42 ± 7.57$^{a}$ |
| | **5** | 4.58 ± 0.82$^{b}$ | 22.30 ± 4.85$^{b}$ | 42.49 ± 6.77$^{a}$ |
| **Month of birth** | | * | * | * |
| | **9** | 4.92 ± 1.08$^{ab}$ | 27.05 ± 2.82$^{a}$ | 44.66 ± 8.50$^{ab}$ |
| | **10** | 4.49 ± 0.69$^{ab}$ | 22.01 ± 3.08$^{b}$ | 40.39 ± 7.06$^{b}$ |
| | **11** | 5.19 ± 0.94$^{a}$ | 25.80 ± 4.75$^{a}$ | 46.96 ± 8.82$^{a}$ |
| | **12** | 4.94 ± 0.90$^{b}$ | 22.36 ± 3.51$^{b}$ | 41.21 ± 6.08$^{b}$ |
| | **1** | 4.71 ± 0.66$^{ab}$ | 21.34 ± 3.64$^{b}$ | 41.39 ± 5.31$^{b}$ |
| **Season of birth** | | NS | * | NS |
| | **Autumn** | 4.85 ± 0.90$^{a}$ | 24.18 ± 4.32$^{a}$ | 43.60 ± 8.45$^{a}$ |
| | **Winter** | 4.81 ± 0.77$^{a}$ | 21.82 ± 3.56$^{b}$ | 41.31 ± 5.55$^{a}$ |
| **Sex of lambs** | | * | * | * |
| | **Female** | 4.50 ± 0.74$^{a}$ | 21.97 ± 3.02$^{a}$ | 38.53 ± 4.69$^{b}$ |
| | **Male** | 5.13 ± 0.85$^{b}$ | 24.62 ± 4.74$^{b}$ | 47.00 ± 7.64$^{a}$ |

*** It means there are significant at (P≤0.05), NS: means There are non-significant between the means, the different letters at same column means there are significant between means.**

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**Table 3.** Least square means ± SD (kg/ lamb/ day) for average daily gain, pre-weaning daily gains and post weaning daily gain in Awassi lambs.

| Factors | Levels | Daily gain | | |
|---|---|---|---|---|
| | | **Pre-weaning** | **Post weaning** | **Average** |
| **Overall mean** | | 0.20 ± 0.04 | 0.21 ± 0.05 | 0.21 ± 0.04 |
| **Age of ewes (Year)** | | **NS** | **NS** | **NS** |
| | **2** | 0.19 ± 0.04[a] | 0.21 ± 0.05[a] | 0.20 ± 0.04[a] |
| | **3** | 0.20 ± 0.03[a] | 0.21 ± 0.05[a] | 0.20 ± 0.04[a] |
| | **4** | 0.21 ± 0.04[a] | 0.21 ± 0.05[a] | 0.21 ± 0.04[a] |
| | **5** | 0.19 ± 0.05[a] | 0.23 ± 0.06[a] | 0.21 ± 0.03[a] |
| **Month of birth** | | * | **NS** | * |
| | **9** | 0.24 ± 0.02[a] | 0.19 ± 0.06[a] | 0.22 ± 0.04[ab] |
| | **10** | 0.19 ± 0.03[b] | 0.20 ± 0.05[a] | 0.19 ± 0.03[b] |
| | **11** | 0.22 ± 0.05[a] | 0.23 ± 0.05[a] | 0.23 ± 0.04[a] |
| | **12** | 0.19 ± 0.03[b] | 0.21 ± 0.03[a] | 0.20 ± 0.03[b] |
| | **1** | 0.18 ± 0.03[b] | 0.22 ± 0.06[a] | 0.20 ± 0.02[b] |
| **Season of birth** | | * | **NS** | **NS** |
| | **Autumn** | 0.21 ± 0.04[a] | 0.21 ± 0.05[a] | 0.21 ± 0.04[a] |
| | **Winter** | 0.18 ± 0.03[b] | 0.21 ± 0.05[a] | 0.20 ± 0.02[a] |
| **Sex of lambs** | | * | * | * |
| | **Female** | 0.19 ± 0.03[b] | 0.18 ± 0.03[a] | 0.18 ± 0.02[a] |
| | **Male** | 0.21 ± 0.05[a] | 0.25 ± 0.05[b] | 0.23 ± 0.04[b] |

*** It means there are significant at (P≤0.05), NS: means There are non-significant between the means, the different letters at same column means there are significant between means.**

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**Table 4.** The correlation coefficient among body weight traits in Awassi lambs.

| Traits / Traits | BW | WW | MW | DGB-W | DGW-M | ADG |
|---|---|---|---|---|---|---|
| **BW** | 1 | 0.41 *** | 0.48 *** | 0.22 * | 0.38 *** | 0.39 *** |
| **WW** | | 1 | 0.78 *** | 0.98 *** | 0.35 *** | 0.78 *** |
| **MW** | | | 1 | 0.74 *** | 0.85 *** | 0.99 *** |
| **DGB-W** | | | | 1 | 0.30 ** | 0.75 *** |
| **DGW-M** | | | | | 1 | 0.85 *** |
| **ADG** | | | | | | 1 |

*** It means there are significant at (P≤0.05). ** It means there are significant at (P≤0.01). *** It means there are significant at (P≤0.001).**

**Table 5.** BLUP values the for marketing weight (kg/lamb) in Awassi lambs.

| Lamb No. | BLUP | Group | Lamb No. | BLUP | Group | Lamb No. | BLUP | Group | Lamb No. | BLUP | Group |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 49 | 18.316 | | 13 | 5.716 | | 44 | -1.583 | | 57 | -6.683 | |
| 60 | 14.516 | | 14 | 5.616 | | 96 | -1.783 | | 5 | -6.983 | |
| 39 | 13.816 | | 38 | 5.416 | | 6 | -1.983 | | 26 | -7.483 | |
| 1 | 13.616 | | 81 | 4.716 | | 97 | -2.183 | | 25 | -7.583 | |
| 3 | 13.216 | 10% | 88 | 4.716 | | 73 | -2.683 | | 90 | -7.783 | |
| 41 | 12.516 | | 89 | 4.716 | | 95 | -2.783 | | 35 | -9.183 | |
| 46 | 12.516 | | 80 | 4.616 | | 59 | -3.183 | | 69 | -10.283 | |
| 40 | 12.416 | | 27 | 4.216 | | 31 | -3.283 | | 18 | -11.483 | |
| 51 | 12.316 | | 16 | 3.616 | 70% | 74 | -3.883 | | 15 | -11.583 | |
| 30 | 12.216 | | 92 | 3.516 | | 78 | -4.083 | | 19 | -11.783 | |
| 94 | 12.216 | | 91 | 3.416 | | 87 | -4.083 | | 36 | -12.283 | |
| 42 | 10.916 | | 10 | 2.416 | | 65 | -4.183 | | 50 | - | |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 18.283 | |
| 71 | 10.416 | | 34 | 2.016 | | 58 | -4.483 | | | | |
| 48 | 10.316 | | 66 | 2.016 | | 21 | -4.583 | | | | |
| 45 | 10.016 | | 20 | 1.516 | | 75 | -4.583 | | | | |
| 23 | 9.716 | | 93 | 0.716 | | 82 | -4.783 | | | | |
| 52 | 8.416 | | 47 | 0.316 | | 98 | -5.183 | | | | |
| 29 | 8.316 | | 2 | -0.183 | | 22 | -5.383 | | | | |
| 63 | 7.516 | | 32 | -0.183 | | 67 | -5.383 | | | | |
| 77 | 7.216 | | 100 | -0.183 | | 11 | -5.683 | | | | |
| 12 | 6.616 | | 33 | -0.283 | | 84 | -5.683 | | | | |
| 62 | 6.516 | | 79 | -0.783 | | 24 | -5.783 | | | | |
| 54 | 6.416 | | 7 | -0.883 | | 70 | -5.783 | 20% | | | |
| 37 | 6.316 | | 17 | -0.983 | | 61 | -6.183 | | | | |
| 68 | 5.816 | | 4 | -1.483 | | 99 | -6.183 | | | | |

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## Genotypic results

Limited molecular genetic information using RFLP markers is available on Iraqi Awassi sheep. Fig. (1) shows extracted genomic DNA of blood lambs in high group production (10% high BLUP values) and blood lambs in low group production (20 % low BLUP values which indicate that the quality of lamb's DNA were very good and the DNA concentration with purity was calculated by Nano drop were ranged between (4.82-10.61) ng/ µl and (1.20-1.28), respectively. These results show that DNA extracted can be used for future work and genetic analysis.

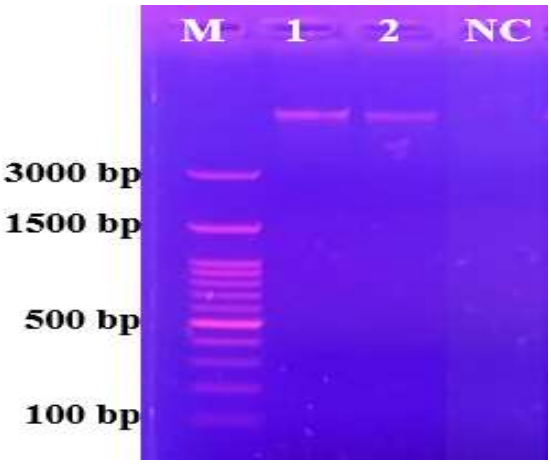


**Fig 1. DNA extraction image for high and low Awassi lamb's groups.**

NC: Negative control, M: DNA Ladder 100 *bp* and 1 ,2 lamb's DNA.

## Loci PCR Amplification:

The GH, CAST and MHC gene were amplification in order to detected these genes in Awassi lamb's samples by basic PCR technique. The results showed in Fig. 2 revealed the PCR product appeared as single band with size of 934, 622 and 279 *bp* for GH, CAST and MHC gene, respectively. The results showed high accuracy of optimum condition as appeared in agarose gel (1.5%) electrophoresis.

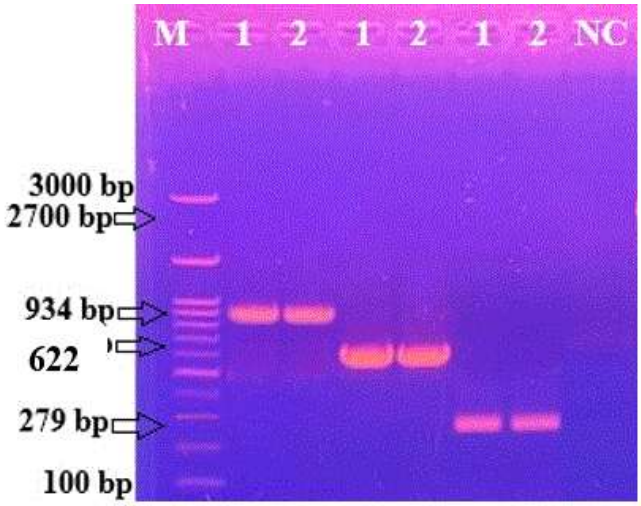


**Figure 2. PCR amplification of the partial genes from lambs include: GH gene with size 934 bp, CAST gene with size 622 bp and MHC gene with size 279 bp.**
M; DNA ladder (100 bp), NLine 2-6 Lambs DNA for high and low groups of each gene and NC: Negative control.

## GH, CAST and MHC genes sequencing

On chromosome No. 11, 5 and 20 of sheep located GH, CAST and MHC genes, respectively. These genes

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affected body weight, weight gain, meat quality and immune response and disease resistance in the sheep. The DNA sequencing of these genes (Fig. 3) in Awassi lambs with NCBI blast results show match among Awassi lambs GH, CAST and MHC locus with ten sheep breeds in world for same loci (Fig. 4, 5 and 6). All ten sheep breeds matched with Awassi lambs of these genes with above 90% of DNA base pairs. This results indicated that local Awassi sheep breed have a mutated these loci which have significant effect on body weight traits of sheep breeds in world (Fig.7).

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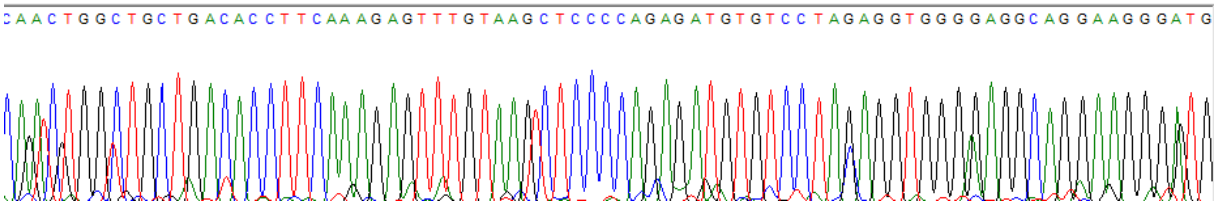
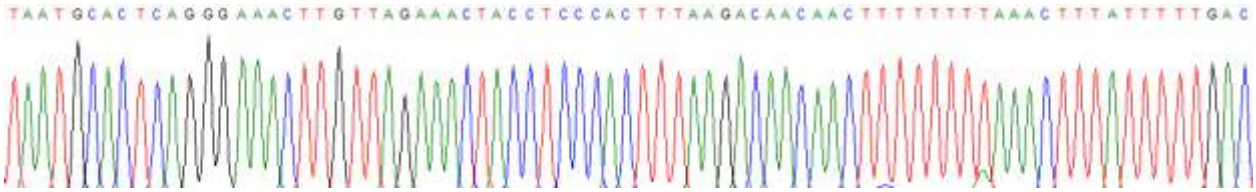
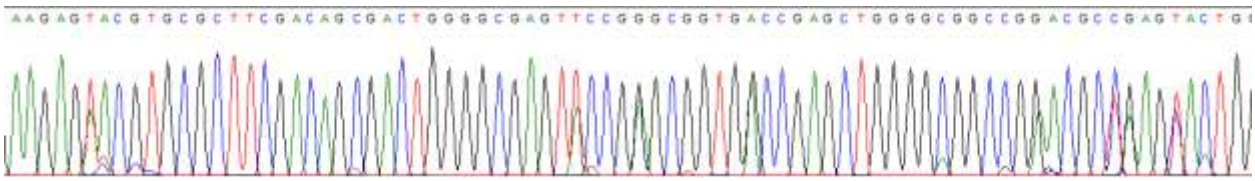


DNA sequence of GH in Awassi lambs.



DNA sequence of CAST in Awassi lambs.



DNA sequence of MHC in Awassi lambs.

Figure 3. The DNA sequence of GH, CAST and MHC in Awassi lambs.

```
>Ovis aries isolate GH2A2b growth hormone (GH) gene, Gh2 allele, complete cds
Sequence ID: DQ461665.1 Length: 3018
Range 1: 990 to 1108

Score:163 bits(88), Expect:5e-38,
Identities:109/119(92%),  Gaps:2/119(1%), Strand: Plus/Plus

Query  8     ATGGGA-GG-ACTGAGGACCTCAGTGGTATTTTATCCAAGTAAGGATGTGGTCAGGGGAG  65
              ||||||  || ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  990   ATGGGAGGGAACTGAGGACCTCAGTGGTATTTTATCCAAGTAAGGATGTGGTCAGGGGAG  1049

Query  66    TAGAAATGGGGGTGTGTGGGGTGGGGAGGGTCCCTCCCAACGCAGTGCTGGGAACCCCG  124
              ||||||||||||||||||||||||||||||||  ||    || ||||||  ||||||||||
Sbjct  1050  TAGAAATGGGGGTGTGTGGGGTGGGGAGGGTTCCGAATAAGGCAGTGAGGGGAACCCCG  1108
```

**Figure 4. Sequence alignment of Awassi lambs *GH* with Serra da Estrela sheep breed**

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```
>Ovis aries calpastatin (CAST) gene, partial cds
Sequence ID: AF016006.1 Length: 611
Range 1: 7 to 599

Score:970 bits(525), Expect:0.0,
Identities:572/594(96%),  Gaps:6/594(1%), Strand: Plus/Plus

Query  5    ATC-GACTT-AGCTGCAGTT-CCCTACAGCTGATGCAAAGAAAACTGAGAAAGAGGTATT  61
            ||| ||||| | |||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  7    ATCAGACTTCACCTGCAGTTCCCCTACAGCTGATGCAAAGAAAACTGAGAAAGAGGTATT  66

Query  62   GTTTTTAATGCACTCAGGGAAACTTGTTAGAAACTACCTCCCACTTTAAGACAACAACtt  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   GTTTTTAATGCACTCAGGGAAACTTGTTAGAAACTACCTCCCACTTTAAGACAACAACTT  126

Query  122  ttttttAAACTTTATTTTTGACTTCGCTGGGTCTTCATTGCTGTGTTCAGGCTTTCTCTA  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  TTTTTTAAACTTTATTTTTGACTTCGCTGGGTCTTCATTGCTGTGTTCAGGCTTTCTCTA  186

Query  182  GTTGGGGCAAGCGAGGGCTATTCTCTAGTTGCGATGTGCAGCCTTCTGCAGGTGGCTTCT  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  GTTGGGGCAAGCGAGGGCTATTCTCTAGTTGCGATGTGCAGCCTTCTGCAGGTGGCTTCT  246

Query  242  CTTGTTGCAGAGCCGGGGCTCTGGGTGCACAGGCTTCAGTTGTTGTGGCTTGAGAGCTCT  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  CTTGTTGCAGAGCCGGGGCTCTGGGTGCACAGGCTTCAGTTGTTGTGGCTTGAGAGCTCT  306

Query  302  AGAGCACAGGCTCAGTGGTCGTGGCGCACAGGCTTACTCCACGGCATGTGGGATCTTCCC  361
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  AGAGCACAGGCTCAGTGGTCGTGGCGCACAGGCTTACTCCACGGCATGTGGGATCTTCCC  366

Query  362  TGGCCAGGGAGCGAACCCGTGTCCCCTGCGTTGCAAGGCGGCCTCTTAACCACTGGCCAC  421
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  TGGCCAGGGAGCGAACCCGTGTCCCCTGCGTTGCAAGGCGGCCTCTTAACCACTGGCCAC  426

Query  422  CAGGGAAGCCCCAAGATGCCAAGGCTTTTTACTTCTGGTTCTTACCGTCTGGTTAATACA  481
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  CAGGGAAGCCCCAAGATGCCAAGGCTTTTTACTTCTGGTTCTTACCGTCTGGTTAATACA  486

Query  482  TTTCCTTCATCTGCCAGTCAAACCTTCTTTTGGATTTCCTTTTTTAAAAATCCACCGAAA  541
            |||||||||||||||||||||||||||||||| ||||| ||||| | ||||| || |||
Sbjct  487  TTTCCTTCATCTGCCAGTCAAACCTTCTTTTGTATTTCATTTTTCAGAAATCTACAGAAG  546

Query  542  AAGGTTTAAAAGCTCCATCCGCTGGGGTGAACA-AAGTGGGGCCTCC-CCCAAA  593
            | | ||||||||||  || |||||||||| || |||||  || ||| ||||||
Sbjct  547  AGGCTTTAAAAGCTCAGTCAGCTGGGGTGATCAGAAGTGCTGC-TCCACCCAAA  599
```

**Figure 5. Sequence alignment of Awassi lambs *CAST* with New Zealand sheep breed**

```
>Ovis aries isolate L6 MHC class II antigen (Ovar-DRB1) gene, Ovar-DRB1.2 allele, exon 2 and partial cds
Sequence ID: HQ215211.1 Length: 270
Range 1: 21 to 270

Score:401 bits(217), Expect:3e-110,
Identities:240/251(96%),  Gaps:2/251(0%), Strand: Plus/Plus

Query  1    AAGAGCGAGTGTC-TTTCCTCCACGGGACGGAGCGGGTGCGGTTCCCTGGACAGATACTT  59
            ||||||||||||| |||| || ||||||||||||||||||||| ||||||||||||||||
Sbjct  21   AAGAGCGAGTGTCATTTCTTCAACGGGACGGAGCGGGTGCGGTT-CCTGGACAGATACTT  79

Query  60   CTATAATGGAGAAGAGACCGTGCGCTTCGACAGCGACTGGGGCGAGTTCCGGGCGGTGGC  119
            ||||||||||||||||  ||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   CTATAATGGAGAAGAGTACGTGCGCTTCGACAGCGACTGGGGCGAGTTCCGGGCGGTGGC  139

Query  120  CGAGCTGGGGCGGCCGGACGCCAAGTACTGGAACAGCCAGAAGGACTTCCTGGAGCGGAC  179
            |||||||||||||||| |||||||||||||||||||||| ||||||||||||
Sbjct  140  CGAGCTGGGGCGGCCGGAAGCCAAGTACTGGAACAGCCAGAAGGAGTTCCTGGAGCGGAG  199

Query  180  GCGGACCGAGGTGGACACGTACTGCAGACACAACTACGGGGTCTTTGAGAGTTTCACTGT  239
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  200  GCGGACCGAGGTGGACACGTACTGCAGACACAACTACGGGGTCTTTGAGAGTTTCAGTGT  259

Query  240  GAAGCGGCGAG  250
            | |||||||||
Sbjct  260  GCAGCGGCGAG  270
```

**Figure 6. Sequence alignment of Awassi lambs MHC with Iranian sheep breed**

Job Title:GH HA
Program: BLASTN
Database: core_nt Core nucleotide BLAST database
Query #1: Query ID: lcl|Query_7691663 Length: 900

Sequences producing significant alignments:

| Description | Scientific Name | Common Name | Taxid | Max Score | Total Score | Query cover | E Value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovis aries isolate GH2A2b growth hormone (GH) gene, Gh2 allele... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 3018 | DQ461665.1 |
| Ovis aries isolate GH2ZA5 growth hormone (GH) gene, Gh2 allele... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2936 | DQ461622.1 |
| Ovis aries isolate GH2A3b growth hormone (GH) gene, Gh2 allele... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2924 | DQ461669.1 |
| Ovis aries isolate GH2A21b growth hormone (GH) gene, Gh2 allel... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 4804 | DQ461667.1 |
| Ovis aries genotype GG growth hormone (GH) gene, exons 2, 3 an... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 422 | KP120857.1 |
| Ovis aries isolate GH2A9b growth hormone (GH) gene, Gh2 allele... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2980 | DQ461681.1 |
| Ovis aries strain Awassi growth hormone (GH) gene, GH2-N allel... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 1736 | AH005493.2 |
| Ovis aries isolate GH2A8b growth hormone (GH) gene, Gh2 allele... | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2095 | DQ461679.1 |
| Ovis aries isolate GH2A7b growth hormone (GH) gene, complete cds | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2987 | DQ461677.1 |
| Ovis aries isolate GH2A17a growth hormone (GH) gene, complete cds | Ovis aries | sheep | 9940 | 163 | 163 | 13% | 5e-38 | 91.60 | 2958 | DQ461660.1 |

Job Title:CAST HA
Program: BLASTN
Database: core_nt Core nucleotide BLAST database
Query #1: Query ID: lcl|Query_6336455 Length: 594

Sequences producing significant alignments:

| Description | Scientific Name | Common Name | Taxid | Max Score | Total Score | Query cover | E Value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovis aries calpastatin (CAST) gene, partial cds | Ovis aries | sheep | 9940 | 970 | 970 | 99% | 0.0 | 96.30 | 611 | AF016006.1 |
| Ovis aries KHM1 CAST gene for calpastatin, partial cds | Ovis aries | sheep | 9940 | 966 | 966 | 97% | 0.0 | 96.89 | 621 | LC503748.1 |
| Ovis aries calpastatin (CAST) gene, CAST-G allele, partial cds | Ovis aries | sheep | 9940 | 966 | 966 | 97% | 0.0 | 96.89 | 620 | KX722533.1 |
| Ovis aries KHM6 CAST gene for calpastatin, partial cds | Ovis aries | sheep | 9940 | 961 | 961 | 97% | 0.0 | 96.72 | 621 | LC503753.1 |
| Ovis aries calpastatin (CAST) gene, CAST-A allele, partial cds | Ovis aries | sheep | 9940 | 961 | 961 | 97% | 0.0 | 96.72 | 620 | KX722534.1 |
| Ovis aries isolate 8A calpain inhibitor (Cast) gene, partial cds | Ovis aries | sheep | 9940 | 959 | 959 | 99% | 0.0 | 95.96 | 611 | JQ390049.1 |
| Ovis aries KHM7 CAST gene for calpastatin, partial cds | Ovis aries | sheep | 9940 | 955 | 955 | 97% | 0.0 | 96.55 | 621 | LC503754.1 |
| Ovis aries calpastatin (CAST) gene, partial cds | Ovis aries | sheep | 9940 | 953 | 953 | 99% | 0.0 | 95.79 | 611 | AF016008.1 |
| Ovis aries KHM155 CAST gene for calpastatin, partial cds | Ovis aries | sheep | 9940 | 950 | 950 | 97% | 0.0 | 96.37 | 621 | LC503762.1 |
| Ovis aries isolate 13A calpain inhibitor (Cast) gene, partial cds | Ovis aries | sheep | 9940 | 948 | 948 | 99% | 0.0 | 95.62 | 611 | JQ390050.1 |

Job Title:MHC-DRB1 HA
Program: BLASTN
Database: core_nt Core nucleotide BLAST database
Query #1: Query ID: lcl|Query_6475587 Length: 251

Sequences producing significant alignments:

| Description | Scientific Name | Common Name | Taxid | Max Score | Total Score | Query cover | E Value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovis aries isolate L6 MHC class II antigen (Ovar-DRB1) gene,... | Ovis aries | sheep | 9940 | 401 | 401 | 100% | 3e-110 | 95.62 | 270 | HQ215211.1 |
| Ovis aries MHC class II antigen (Ovar-DRB1) gene, Ovar-DRB1*25... | Ovis aries | sheep | 9940 | 401 | 401 | 100% | 3e-110 | 95.62 | 324 | JX236660.1 |
| Ovis aries MHC class II antigen (Ovar-DRB1) gene, Ovar-DRB1*TR... | Ovis aries | sheep | 9940 | 401 | 401 | 98% | 3e-110 | 95.97 | 286 | MH686554.1 |
| Ovis aries DNA for MHC class II DRB exon 2 | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 262 | Z92731.1 |
| Ovis aries isolate TAGEM19 MHC class II antigen (Ovar-DRB1)... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 268 | MT156740.1 |
| Ovis aries partial MHC class II DRB1 gene for MHC class II... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 270 | OU721368.1 |
| Ovis aries MHC class II antigen (Ovar-DRB1) gene, Ovar-DRB1*08... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 324 | JX236661.1 |
| Ovis aries mRNA for Cell surface protein (MHC class II DRB1... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 819 | LS990817.1 |
| Ovis aries isolate TAGEM8 MHC class II antigen (Ovar-DRB1) gen... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 268 | MT156729.1 |
| Ovis aries breed Chokla haplotype Chokla_10 MHC class II antig... | Ovis aries | sheep | 9940 | 396 | 396 | 100% | 1e-108 | 95.22 | 801 | KY780642.1 |

**Figure 7.** Sheep breeds and GenBank number of GH, CAST and MHC loci matched Awassi lambs.

**DNA Restriction Cite**

To detect lambs genotype two methods used, the 1st one is PCR-RFLP and the 2nd is DNA sequencing. In our study the DNA sequencing was theoretically treated with specific restriction enzyme for each gene to detect genotypes by used the NEBcutter (http://tools.neb.com/NEBcutter).

The result showed that the *HeaIII* restriction enzyme was cut GH locus of Awassi lambs in different cite in low (BE genotype) and high (CD genotype) production groups of lamb, the *MspI* restriction enzyme was cut CAST locus of Awassi lambs in different cite in low (BC genotype) and high (CD genotype) production groups of lamb, and the *RsaI* restriction enzyme was cut MHC locus of Awassi lambs in different cite in low (CD genotype) and high (BE genotype) production groups of lamb. Similar result was found by [39] in Salsk sheep breed by using RFLP-PCR for same genes in sheep breed.

**Molecular Markers and Quantitative Traits**

The effect of genetic markers on growth traits in Awassi lambs under investigation study were showed in table (6) and (7). The lambs with **CDBCCD** genotypes of GH, CAST and MHC-DRB1 in lambs in high group production had a significant effect on body weight at all ages and average daily gain compared with **lambs in low production group carrying BECDBE genotypes of above loci.**

As in the table (6) and (7) the lambs with **CDBCCD** (10%) genotype gave 1.4 kg/lamb (BW), 13.81 kg/lamb (WW), 21.92 kg/lamb (MW) higher body weight and higher daily gain (0.1 kg/day/lamb) at pre weaning, (0.133 kg/day/lamb) at past weaning weight and (0.117 kg/day/lamb) average daily gain compared with **BECDBE** genotype of lambs in low production group (20%). These results reveal that selection can play major role to increase body weight especially MW in Awassi sheep and the selected animal in

early stage of age with best genotype reduced the interval generation and improving the yearly genetic gain.

This result is similar with [40], who found in the CAST gene with MN genotype in Balkhi sheep give daily gain higher than the MM genotype at four months' age. Similar results were repoted by [41] for the CAST gene in Salsk sheep and by [9] in Iranian Makuie sheep. [42] Reported Relationship between calpastatin gene polymorphism and growth performance in Awassi sheep. [43] reported that CAST and MHC genes were polymorphism. Lambs with CC genotype for MHC and lambs with AB genotype for CAST locus gave higher body weight at all ages and higher average daily gain in Awassi sheep.

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**Table 6.** Least square means ± SD for effect of genotypes (GH, CAST and MHC) on body weights in Awassi lambs.

| Traits / Genotype | Birth Weight (Kg/Lamb) | Weaning Weight (Kg/Lamb) | Marketing Weight (Kg/Lamb) |
|---|---|---|---|
| **CDBCCD High Production** | $5.64 \pm 0.87^{a}$* | $33.55 \pm 6.43^{a}$* | $55.33 \pm 1.84^{a}$* |
| **BECDBE Low Production** | $4.24 \pm 0.62^{b}$ | $19.74 \pm 3.45^{b}$ | $33.41 \pm 3.36^{b}$ |

*** it means there are significant at (P≤0.05), the different letters at same column means there are significant between means.**

**Table 7.** Least square means ± SD for effect of genotypes (GH, CAST and MHC) on body weight gain in Awassi lambs.

| Traits / Genotype | Pre weaning daily gain (Kg/Lamb/day | Past weaning daily gain (Kg/Lamb/day) | Average daily gain (Kg/Lamb/day) |
|---|---|---|---|
| **CDBCCD High Production** | $0.267 \pm 0.031^{a}$* | $0.287 \pm 0.023^{a}$* | $0.277 \pm 0.011^{a}$* |
| **BECDBE Low Production** | $0.167 \pm 0.0357^{b}$ | $0.154 \pm 0.028^{b}$ | $0.160 \pm 0.019^{b}$ |

*** it means there are significant at (P≤0.05), the different letters at same column means there are significant between means.**

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## Conclusion

In conclusion, the present study demonstrated that growth performance of Awassi lambs is influenced by both non-genetic and genetic factors. Non-genetic factors such as ewe age, month and season of birth, and sex of lambs showed variable but important effects on body weight traits and average daily gain at different growth stages. Lambs born to mature ewes, during favorable months and seasons, and male lambs generally exhibited superior growth performance, highlighting the importance of appropriate flock management and breeding strategies to optimize productivity. The wide range between the BLUP values Lambs for growth traits indicate that selecting lambs according to their records at early ages will improve their later performance.

Moreover, the findings clearly revealed a significant influence of combined GH, CAST, and MHC genotypes on body weight and growth traits. Lambs carrying the favorable CDBCCD genotype consistently outperformed those with the BECDBE genotype in birth, weaning, and marketing weights, as well as in daily gain parameters. This indicates that advantageous allelic combinations contribute to improved growth efficiency from early life through marketing age. Therefore, integrating molecular genetic information with conventional management practices could serve as an effective approach for genetic improvement programs aimed at enhancing growth performance and overall productivity of Awassi lambs.

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