Fayoum Journal of Agricultural Research and Development
https://fjard.journals.ekb.eg

Article

Molecular Characterization of Callipyge (CLPG) and Calpastatin (CAST) Genes Related to Weight Gain and Meat Quality in Egyptian Ossimi Sheep

Mohamed I. Nassar and Yasser K. Badawi

Biotechnology Department, Animal Production Research Institute, Agricultural Research Center, Giza 12618, Egypt
*Correspondence: mnassar9977@gmail.com

Abstract: This study presented the polymorphisms of the Callipyge (CLPG) and Calpastatin (CAST) genes, assessing their effects on the growth of Egyptian Ossimi sheep. The lambs were categorized into three groups based on birth weight, with each group containing 40 lambs. Whole blood samples were collected from 27 lambs over three consecutive seasons, with three males selected per group each season. Significant differences were observed in average birth and weaning weight, total body gain, and daily body gain among the three Ossimi lamb groups. Genomic DNA was isolated to determine allele frequencies and genotypic polymorphisms in the CLPG and CAST genes using restriction fragment length polymorphism (RFLP). The CLPG gene was found only in the homozygous ++ genotype, while the heterozygous +L and homozygous LL genotypes were absent. For the CAST gene, the homozygous MM genotype was prevalent, with some heterozygous MN observed, while the NN genotype was not detected. The results indicate that the CLPG gene is monomorphic and unsuitable for marker-assisted selection due to its lack of association with body weight gain in lambs. However, the CAST gene is polymorphic and associated with increased body weight, suggesting its usefulness in breeding programs focused on selection and monitoring of polymorphisms.
Keywords: Ossimi sheep, PCR-RFLP, CLPG gene, CAST gene, MspI Restriction enzymes**.**

1. Introduction

Food security is one of the most important goals of sustainable development, and a lot of research is being done to find ways to provide a suitable food supply for the world's growing population. Protein insufficiency has frequently been identified as a major cause of child malnutrition, especially in developing countries, as it causes 50% of child deaths under the age of five (**Safdar *et al*., 2023**). An increase in livestock production is critical to overcome the rising demands for protein, as meat provides 40% of dietary protein. Currently, sheep breeders' interest is focused on both the quantitative and the qualitative of the meat. Classic selection of sheep, using information for pedigree data and phenotypes to predict the breeding values, has been very successful (**Sahu *et al*., 2017**). On the other hand, breeding values are more successful in prediction by utilizing information on variations in DNA sequences between animals. The best understanding of the relationship between genotype and phenotype required genome analysis of polymorphisms.

Skeletal muscles constitute more than 25 percent of a sheep's body weight (**Lee *et al*., 2001**). Three main factors that affect the size and rate of skeletal muscle growth include the rate of muscle protein formation, the quantity and size of skeletal muscle cells, and the rate of muscle protein degradation (**Khederzadeh, 2011**).

During the lambs' growth periods, muscle mass increases because muscle tissue synthesis proceeds more quickly than degradation due to an increase in calpastatin activity and a decrease in calpain activity (**Goll *et al*., 1998**). In contrast, the activity of calpain in slaughtered animals is higher than calpastatin, leading to a high rate of muscle disintegration and tenderizing of the meat.

Growth rate and meat quality are complicated traits that are controlled by a number of genes, such as callipyge (CLPG) and Calpastatin (CAST) genes. There are several methods for locating trait loci, including genome scans based on linkage mapping DNA markers and association testing employing candidate genes (**Andersson, 2001; Sahu *et al*., 2017**).

The CLPG gene was originally discovered in 1983 in a Dorset ram. It plays a crucial role in muscle development and is well-known for its significant effect on weight gain in sheep (**Cockett *et al.*, 2005**). This gene, located on the distal end of chromosome 18, CLPG is associated with a distinct type of postnatal muscular hypertrophy that is particularly localized to the pelvic region (**Jackson and Green, 1997**). This genetic mutation alters the normal growth pattern of sheep by increasing muscle mass. This mutation is an important factor in breeding programs to enhance meat production. The expression of the CLPG gene can result in substantial increases in muscle yield in certain sheep breeds.

The CAST is another important genetic factor in sheep and Located on the fifth chromosome, the polymorphism of this gene was studied in 1998 in Dorset Down sheep using the PCR-RFLP method. Calpastatin is crucial in regulating the cell's calpain activity. This gene is an exceptional marker in selection aimed at enhancing the quantity and quality of meat (**Casas *et al.*, 2006; Zhou *et al.*, 2007; Ardicli *et al.*, 2017**). Critical polymorphisms in the CAST gene are associated with varying levels of meat quality traits such as tenderness and juiciness (**Koohmaraie, 1992**). High calpastatin levels can inhibit calpain activity, leading to improved tenderness and quality of meat after slaughter (**Bozhilova-Sakova *et al.*, 2020**; **Talebi *et al.* 2022; Surov *et al.* 2023**).

Possibility of early detection of prospective breeding animals mainly based on the relationship between the qualitative traits and allelic variations of marker genes (CLPG and CAST) (**Kolosov *et al.*, 2021**). Knowing the variability in major genes among different breeds is an essential component of genetic evaluation. Breeding strategies using DNA markers improve overall productivity and meat quality of sheep breeds. There is little information on the relationship between these genes and body weight, although numerous research have studied these gene polymorphisms in different sheep breeds (Mahrous *et al*. 2015; Ramadevi *et al*. 2020; Alnajm *et al*. 2024; Sansyzbayeva *et al*. 2024; Daldaban *et al*. 2025). This study aimed to investigate the relationship between CAST-MspI and CLPG-MspI gene polymorphisms and early live weights from birth to weaning in Egyptian Ossimi sheep.

2. Materials and Methods

2.1. Experimental animals

The study was carried out between 2022 and 2023 at the Animal Production Research Station in Sids, located in the Bani-Suef Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. A total of 120 Ossimi lambs were observed across three consecutive seasons: January, September 2022, and May 2023. The lambs were divided into three groups based on their birth weight, each group consisting of 40 individuals. The first group represented the average birth weight flock (3.10 kg), while the second and third groups included lambs with weights adjusted to 3.10 kg + 10% and 3.10 kg - 10%, respectively. Production traits analyzed included birth weight, weaning weight (kg), total body gain (kg), and average daily weight gain (g), based on collected data. The lambs were weighed in the early morning following a 12-hour fasting period, with measurements rounded to the nearest 100 g. Additionally, polymorphism analysis was performed using blood samples collected from 27 lambs over the three seasons. These samples included three male lambs from each group per season.

2.2. Blood sampling

Three milliliters of blood were collected from the jugular veins of twenty-seven Ossimi lambs (three lambs from each group/season) were used to collect whole blood samples in vacationer glass tubes containing EDTA (1 mg/ml). Blood samples were transferred to the laboratory in an icebox and maintained at 4°C until further use. The protocols approved by Egypt's Biological Studies Animal Care and Use Committee were followed when the experiments were conducted. During the blood collection, all attempts were made to reduce discomfort.

2.3. DNA extraction

Genomic DNA was extracted from 150 µl of each blood sample using the Xanthogen method according to **Tillett and Neilan (2000).** The quantified DNA was kept at −20°C until use for further research.

2.4. PCR amplification of CLPG and CAST genes

Primer sequences were designed by Invitrogen Biotechnology Co. Ltd. (USA). A total volume of 25 µl used for PCR amplification, PCR reaction consists of 12.5 µl of Master Mix (OnePCR™), one µl of forward and revers primers for each gene, two µl of DNA (50 ng/µl), and 8.5 µl of sterile water. The PCR conditions included: pre-denaturation at 94°C for five min followed by 35 cycles

of DNA denaturation at 94°C for one min, annealing (CLPG, 58°C, and CAST, 60°C) for one min, and extension at 72°C for two min, then a final extension at 72°C for 10 min. All reactions were carried out using the MSLPCR13 thermal cycler (Applied Biosystems). The PCR products were analyzed through electrophoresis of 1.5% agarose gel prepared in 1× TAE buffer.

Table 1. Primer sequences used for PCR reactions in this study

Gene	Primer sequences	Annealing temperature	Product size (bp)
CLPG -F	5–TGA AAA CGT GAA CCC AGA AGC-3 ,	58° C	450
- R	5–GTC CTA AAT AGG TCC TCT CG-3	1 min	
CAST - F	5- GGG GCC CAA TGA CGC CAT CGA TC-3 ,	60° C	620
-R	5- GGT GAT CAG AAG TGC TGC TCC ACC-3	1 min	

2.5. Determining polymorphisms and genotypes analysis

The PCR products of CLPG and CAST genes (27 samples) individually, were digested using the Msp1 restriction enzyme (Fermentas, Germany, ER0541) according to the manufacturer's guidelines. A total volume of thirty-two µl including ten µl of PCR product, eighteen µl of nuclease free H_2O, two µl of 10× buffer, and two µl (five units) of Msp1enzyme. After gently mixing and rotating down for a few seconds, the final volume of the combination was incubated for 16 hours at 37°C in a water bath and the reaction was stopped for 10 minutes at 65°C. Electrophoresis with 2.5 % Agarose gel in 1× TAE buffer at 90 V for 2 h, and staining with ethidium bromide used to verify restriction digestion products. Analysis of PCR digestion products of CLPG and CAST genes was performed, and allele and genotype frequencies were determined.

2.6. Statistical analysis

The SPSS program, version 16.0 **(SPSS, 2007)** was used to analyze the average of birth and weaning weight (kg), total body gain (kg), and body gain (g/day) of Ossimi lambs. The data were shown as means ± standard error (SE) using Duncan's multiple range test at $P \leq 0.05$ (**Duncan, 1955**). Expected genotypic and allelic frequencies for the CLPG and CAST genes were calculated using a simple gene counting method (**Falconer and Mackey, 1996**).

3. Results and discussion

3.1. *Production traits of lamb*

Efficiency in the production of lamb meat is significantly influenced by production traits like birth and weaning weight, and daily body gain. The production traits of 120 Ossimi lambs, which are divided into three groups depending on average birth weight (kg), are presented in Table 2. The results indicated that there was a significant difference in the average of lambs' body weight among the three groups. As well as between average lambs' birth and weaning weight (kg), the average total body gain (kg), and average body gain (gday) when comparing the three groups. The highest values of lambs' birth weight (kg), weaning weight (kg), total body gain (kg), and average body gain (g/day) were observed in the 2nd group of Ossimi lambs (3.5, 21.15, 17.65, and 196), while the lowest values were found in the 3rd group (2.8, 18.1, 15.3, and 170), respectively, when compared with those in the 1st group. On the other hand, the 1st group represents the average flock weight (3.10, 19.39, 16.29, and 181), respectively. Body weight is an important economic trait that increases sheep productivity (**Mishra, 2014**). This study investigates the relationship between CLPG-MspI and CAST-MspI polymorphisms, which are thought to be potential predictors of early live weight in sheep. Several functional genes associated with growth traits have been identified as having a major influence on production quality and quantity (**Wang *et al.*, 2015; Zhang *et al.*, 2016; Jawasreh *et al.*, 2019; Pelmus *et al.*, 2020**).

Table 2. Mean production traits of Ossimi breed

Items \ Groups	1st Average	2nd Average + 10 %	3rd Average - 10 %	±SE
Avg. Lambs birth weight, kg	**3.10** [b]	**3.50** [a]	**2.80** [c]	**0.105**
Avg. Weaning weight, kg	**19.39** [b]	**21.15** [a]	**18.10** [c]	**0.385**
Total body gain, kg	**16.29** [b]	**17.65** [a]	**15.30** [c]	**0.284**
Avg. body gain, g/day*	**181** [b]	**196** [a]	**170** [c]	**3.167**

a, b, and c: Values within the same row with different superscripts show significant differences (P<0.05).*Avg. body gain, g/day = Total body gain, g /90 day.

3.2. *Amplification of CLPG and CAST genes*

A single fragment of approximately 450 and 620 bp of the CLPG and CAST genes nucleotide sequences was amplified from 27 lambs individually of the Ossimi sheep breed (Figures 1 and 2), respectively.

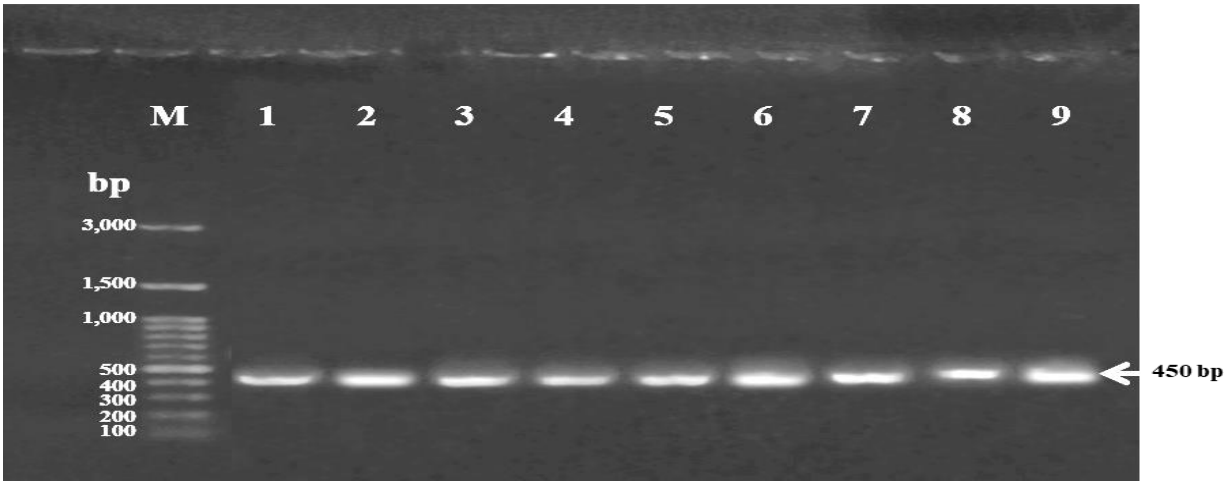

Fig. 1: Electrophoresis of PCR products (~**450** bp) of the **CLPG** gene from Ossimi breed. M: 100 bp DNA ladder, lanes 1-3 (1st group), lanes 7-9 (2nd group), and lanes 4-6 (3rd group).

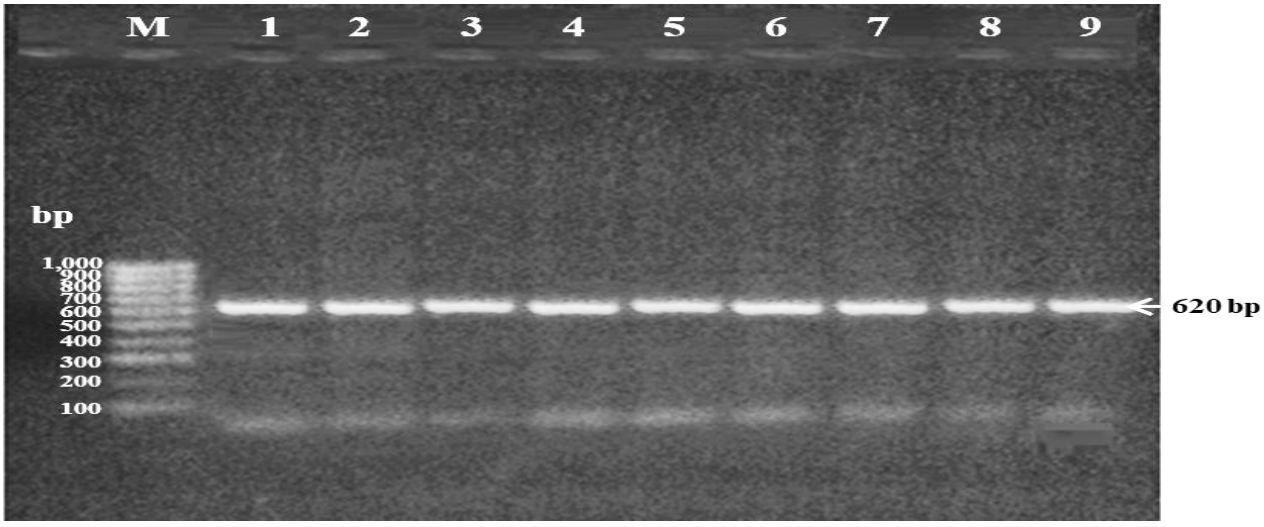

Fig. 2: Electrophoresis of PCR products (~620 bp) of the **CAST** gene from Ossimi breed. M: 100 bp DNA ladder, lanes 1-3 (1st group), lanes 7-9 (2nd group), and lanes 4-6 (3rd group).

3.3. Analysis of PCR-RFLP

PCR-RFLP is a rapid, easy, and efficient method for genotyping single nucleotide polymorphisms (SNPs). It has previously been used to determine the genotype of production traits in sheep (**Wilson *et al.*, 2001**). Msp1 restriction enzymes were used to digest the PCR products of the CLPG and CAST genes in order to detect any mutations. Digested products were electrophoresed on 2.5% agarose gel, and approximately 450 bp in length were obtained for the CLPG gene in all ossimi lamb samples (Figure 3). The results showed that the Ossimi lambs had a single band at ~450 bp, and revealed no mutation in the CLPG gene (++), Figure (3). On the other hand, PCR products of the CAST gene digested by Msp1 enzymes (Figure 4) were used to determine molecular markers of body weight (meat quality). The results showed that the 2nd group, which had the highest lamb body weight, had a polymorphic restriction pattern of 3 distinct bands with ~620, ~330, and ~280 bp. In the 1st group (average of the flock) and 3rd group (the lowest body weight), the restriction pattern consisted of 2 bands with ~330 and ~280 bp. Table 3 shows the genotype of the CLPG gene and allele frequencies based on Msp1 enzyme digestion. According to the results of Msp1 enzyme, the allele frequencies of CLPG gene were 1.0 and 0.0 in Ossimi lambs for + and L, and the frequencies of genotype ++, +L, and LL were 1.0, 0.0, and 0.0, respectively. The genotype and allele frequencies of the CAST gene, based on Msp1 digestion, are shown in Table 3. The allele's frequencies of the CAST gene were 0.83 and 0.17 in the M and N genotypes of Ossimi lambs, and the frequencies of genotype MM, MN, and NN were 0.689, 0.282, and 0.029, respectively. Previous studies and our study on the polymorphism of CAST-MspI in different sheep breeds suggest the M allele is most likely the wild-type allele, while the N allele appears the mutant form **(Daldaban *et al.*, 2025)**.

Table 3. Expected genotype and allele frequencies of the CLPG and CAST genes in Ossimi breed

Restriction enzyme	Sheep breed	No of Lambs	Gene	Expected genotype frequencies			Allele frequencies	
Msp1	Ossimi	27	*CLPG*•	++	+L	LL	+	L
				1.0	0.0	0.0	1.0	0.0
			CAST••	MM	MN	NN	M	N
				0.689	0.282	0.029	0.83	0.17

• Observed genotype: ++= 27 Lambs, +L= Zero lambs, and LL= Zero lambs (CLPG gene).
•• Observed genotype: MM= 18 Lambs, MN= 9 Lambs, and NN= Zero lambs (CAST gene).

The polymorphism of the CLPG gene (++), Figures (3), indicates the absence of mutation in this gene. It can be assumed that the cause of an increase in body weight in Ossimi lambs at the weaning age (90 days) in the 2nd group might be due to the effect of CAST gene mutations. The results obtained are consistent with those reported by **Pomitun *et al.*** (**2019**) in Prydniprovska meat sheep and in Egyptian sheep breeds (**Ibrahim *et al.*, 2015; Mahrous *et al.*, 2016**).

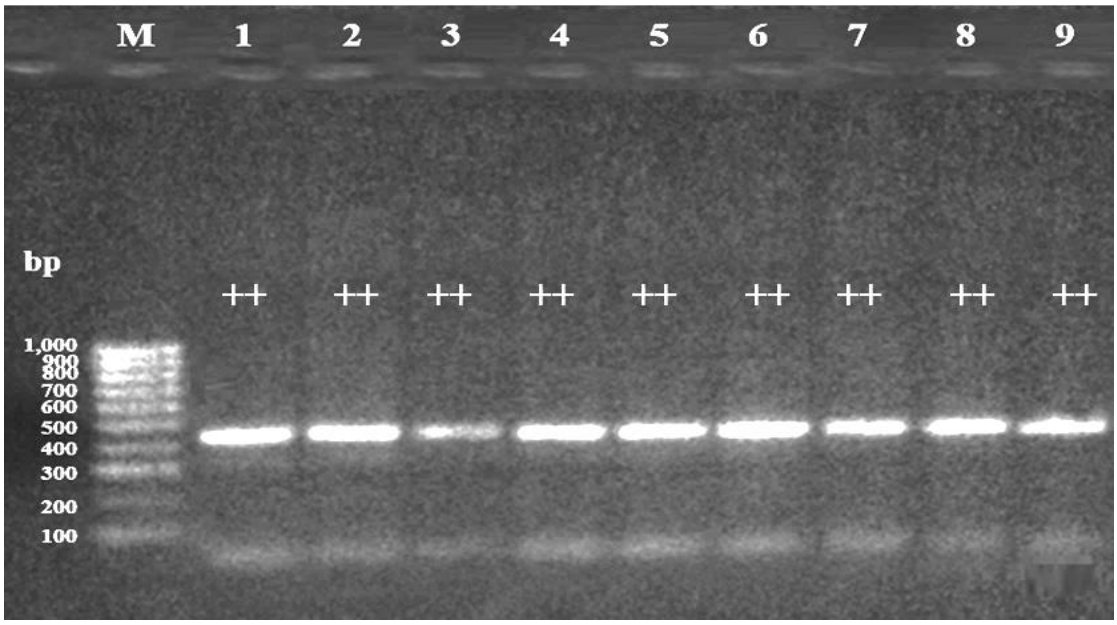

Fig. 3: Restriction fragments of the CLPG gene (~450 bp) were partially digested with Msp1 enzyme from Ossimi breed.

M: 100 bp DNA ladder.

Lanes 1- 9: Ossimi lambs of the ++ genotype (homozygous genotypes).

lanes1-3 (1st group), lanes 7-9 (2nd group), and lanes 4-6 (3rd group).

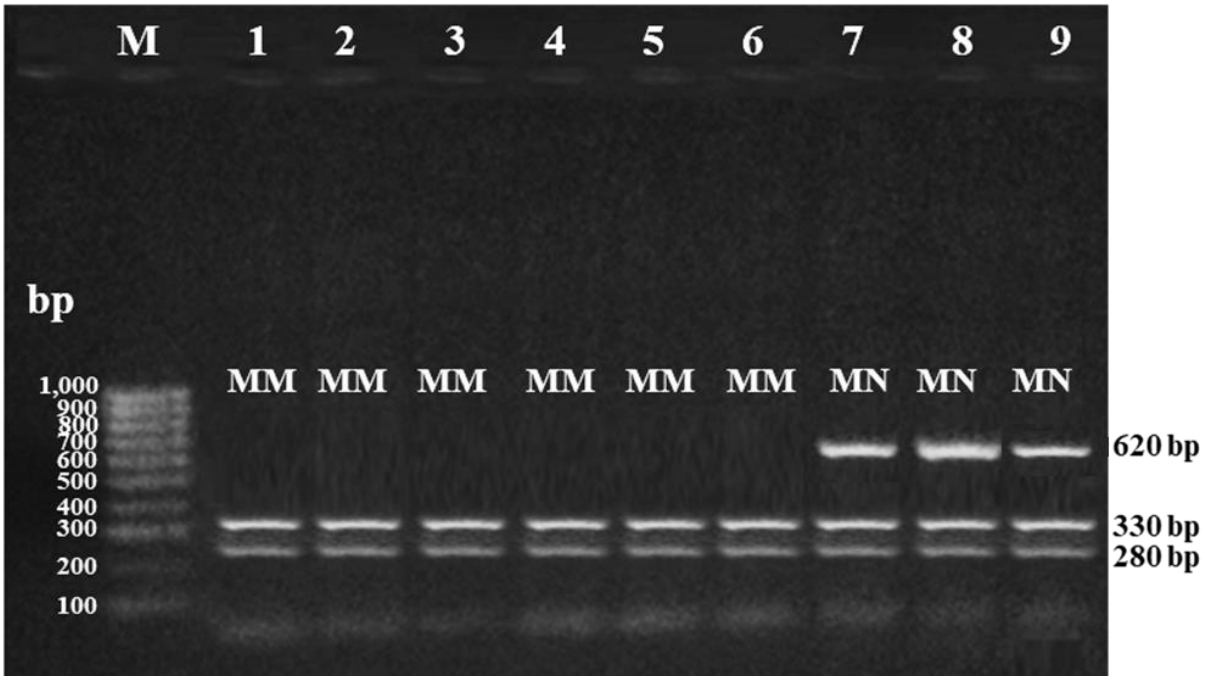

Fig. 4: Restriction fragments of the CAST gene (~620 bp) were partially digested with Msp1 enzyme from Ossimi breed.

M: 100 bp DNA ladder.

Lanes 1-3 (1st group), lanes 7-9 (2nd group), and lanes 4-6 (3rd group)

Lanes 1-6: Ossimi of the MM genotype, fragments (330 and 280 bp).

Lanes 7-9: Ossimi lambs of the MN genotype, fragments (~620, ~330 and 280 bp).

The genotypic distribution of the main genes in livestock must be evaluated to provide a basic genetic data set through several sheep breeds raised in Egypt. In this regard, one of the most well-known and widely used major genes in animal breeding is CAST. The polymorphic calpastatin gene affected longissimus muscle width and final body weight in Awassi sheep (**Jawasreh *et al.*, 2019**). Sheep's body weight and growth were influenced by the genes for Thyrotropin Releasing Hormone Degrading Enzyme (TRHDE) and Myocyte Enhancer Binding Factor 2B (MEF2B) (**Zhang *et al.*, 2016; Gebreselassie *et al.*, 2020; Pelmus *et al.*, 2020; Hoda *et al.*, 2022**). Calpastatin gene mutations can cause the inhibition of its activity; this may lead to an increase in calpain activity, which in turn may increase myoblast proliferation in living cells and protein proliferation, both of which may improve meat productivity's quantitative and qualitative indices (also known as "meat tenderness") (**Pomitun *et al.*, 2019**). Several studies have demonstrated that CAST-MspI and CLPG-MspI polymorphisms are crucial for achieving an individual's genetic potential with regard to early live weight gain and enhancing sheep production efficiency **(Daldaban *et al.*, 2025)**.

4. Conclusions

This research emphasizes the importance of molecular markers in Egyptian Ossimi sheep breeding programs. No mutations or polymorphisms were recorded in the CLPG gene, indicating that it cannot be used as a molecular marker for production traits in this breed. On the other hand, polymorphisms and mutations were identified in the CAST gene, indicating that it represents a reliable molecular marker for predicting meat production in the Ossimi breed. We also recommend conducting future experiments to explore new mutations and their potential impact on productive traits in Egyptian sheep.

References

Alnajm, H. R., Imari, Z.K., Alrubaye. T.A. (2024). Study of Genetic Variation of Myostatin (MSTN) and Calpastatin (CAST) Genes in Two Native Iraqi Sheep by PCR-RFLP Technique." Iraq. J. Vet. Sci., 38 (1): 97–103.

Andersson, L. (2001). Genetic dissection of phenotypic diversity in farm animals. Nat Rev Genet. 2:130-138.

Ardicli, S., Dincel, D., Samli, H., Balci, F. (2017). Effects of polymorphisms at LEP, CAST, CAPN1, GHR, FABP4 and DGAT1 genes on fattening performance and carcass traits in Simmental bulls. Arch. Ani. Breed., 60(2): 61-70.

Bozhilova S.M., Dimitrova, I., Tzonev, T.I. Petrov, N. (2020). Genotype frequencies in calpastatin (CAST) and callipyge (CLPG) genes in Northeast Bulgarian Merino sheep breed using PCR-RFLP method. Bulg. J. Agri. Sci., 26(2): 475-479.

Casas, E., White, S., Wheeler, T., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, C.C., Johnson, D.D., Smith, T.P. L. (2006). Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. J. Ani. Sci., 84(3): 520-525.

Cockett, N.E., Smit, M.A., Bidwell, C.A., Segers, K., Hadfield, T.L., Snowder, G.D., et al. (2005). The callipyge mutation and other genes that affect muscle hypertrophy in sheep. Genet Selec Evol. 37: 65‑82.

Daldaban, F., Karakus, F., Arslan, K., Cinar, M. U., Akyuz, B. (2025). The Role of DGAT1-AluI andCAST-MspI Polymorphisms in Early Growth Traits of Morkaraman Lambs. Vet. Med. Sci, 11:e70395.

Duncan, D.B. (1955).The multiple range and multiple F test. Biom.; 11:1‑42.

Falconer, D.S., and Mackay T.F.C. (1996). Introduction to quantitative genetics, Pearson Education Ltd., Harlow, England.

Gebreselassie, G., Berihulay, H., Jiang, L., Yuehui, M. (2020). Review on Genomic Regions and Candidate Genes Associated with Economically Important Production and Reproduction Traits in Sheep (*Ovies aries*). J. Ani., 10-33.

Goll D.E., Thompson, V.F., Taylor, R.G., Ouali, A. (1998). The calpain system and skeletal muscle growth. Cana. J. Ani. Sci., 78: 503-512.

Hoda, A., Mecaj, R., Hykaj, G. (2022). Morphometric characterization of four sheep breeds reared in South East of Albania. Agr. Est, 68:241–253. https://doi.org/10.17707/AgricultForest.68.3.18

Ibrahim, H.M., Ismail, I.M., Shehata, M.F., El-Beltagy, A.R. (2015). Calpastatin Polymorphism in Barki Lambs and their Effects on Growth and Carcass Traits. J. Amer. Sci., 11 (3).

Jackson, S.P., Miller, M.F., Green, R.D. (1997). Phenotypic characterization of rambouillet sheep expressing the callipyge gene: II. Carcass characteristics and retail yield. J. Ani. Sci., 75:125‑132.

Jawasreh, K.I., Al-Amareen, A.H., Aad, P.Y. (2019). Relationships between Hha1 calpastatin gene polymorphism, growth performance and meat characteristics of Awassi sheep. Ani. 9: 667.

Khederzadeh, S. (2011). Polymorphism of calpastatin gene in crossbreed Dalagh sheep using PCR-RFLP. Afri. J. Biot., 10:10839-10841.

Kolosov, Y.A., Gorlov, I. F., Kolosov, A.Y., Shirokova, N.V., Kulikova, A.Y., Kolosova, M.A., Slozhenkina, M.I., Vorontsova, E.S., Kolosova, N.N. (2021). Determination of CAST gene polymorphism in sheep of the Volgograd breed. IOP Conf. Series: Ear. Envi. Sci., (677) 052112.

Koohmaraie, M. (1992). The role of CA2+-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. Biochimie, 74 (3): 239-245.

Lee R.C., Wang, Z.M., Heymsfield, S.B. (2001). Skeletal muscle mass and aging: regional and whole-body measurement methods. Cana. J. Appl. Phys., 26:102-22.

Mahrous, K.F., Hassanane, M.S., Mordy, M. A., Shafey, H.I. (2015). Polymorphism of Some Genes Associated With Meat-Related Traits in Egyptian Sheep Breeds. Iran. J. App. Ani. Sci., 5 (3): 655–663.

Mahrous, K.F., Hassanane, M.S., Shafey, H.I., Abdel Mordy, M. Rushdi, H.E. (2016). Association between single nucleotide polymorphism in ovine Calpain gene and growth performance in three Egyptian sheep breeds. J. Gene. Eng. Biot., 14: 233‑240.

Mishra, C. (2014). Genetic basis of prolificacy in sheep. Int. J. Liv. Res., 4:46‑57.

Pelmus, R.S., Horia, G., Mircea, C.R., Mihail, A.G., Cristina, L., Florin, P. (2020). The Genetic Influence on Sheep Meat Quality, Growth and Body Weight. A rev., Asi. J. Dair. Food Res., 39(3): 225-231.

Pomitun, A.I., Boyko, Y.A., Rossokha, V. I., Guzevatyi, O.E. Shpilka, M.V., Kulibaba, R.O. (2019). Analysis of Calpastatin and Callipyge Genes Polymorphism in Prydniprovska Meat Sheep. Agri. Sci. Prac., 6: 2312-3370.

Ramadevi, B., Kumari, B.P., Sudhakar, K., Gangaraju, G., Vinod, U. (2020). Polymorphism of the Ovine Calpastatin (CAST) Gene and Its Association with Productive Traits in Nellore Sheep. J. Ani. Res. 10(6):30954.

Safdar, L.B., Foulkes, M. J., Friedrich, H.K., Searle, I.R., Bhosale, R.A., Fisk, I.D., Boden, S.A. (2023). Challenges facing sustainable protein production: Opportunities for cereals. Plant Comm., 4 (6): 100716.

Sahu, A. R., Jeichitra, V., Rajendran, R., Raja, A. (2017). Polymorphism in exon 3 of myostatin (MSTN) gene and its associations with growth traits in Indian sheep breeds. Small Rumin. Res. 149: 81-84.

Sansyzbayeva, B., Adylkanova, S., Sadykulov, T., Kırıkçı, K. (2024). Determination of CAST/MspI Gene Polymorphism and Its Effects on 180-Day Weight in Meat Production Line of Kazakh Saryarka Lambs. Pak. J. Zoo., 56 (6):2977–2980.

SPSS. (2007). Statistical software Package for the Social Sciences, SPSS Inc., version 16, Chicago;

Surov, A.I., Skorykh, L.N., Skokova, A.V., Omarov, A.A., Fominova, I.O. (2023). Polymorphisms of Growth Hormone and Calpastatin Genes and their Association with Sheep Meat Quality. Rus. Agri. Sci. 49(2):333–338.

Talebi, R., Ghaffari, M.R., Zeinalabedini, M., Abdoli, R., Mardi, M. (2022). Genetic Basis of Muscle-Related Traits in Sheep. *Ani. Gene.*, 53(6):723–739.

Tillett, D., Neilan, B.A. (2000). Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol., 36:251‑8.

Wang, H., Zhang, L., Cao, J., Wu, M., Ma, X., Liu, Z., Liu, R., Zhao, F., Wei, C., Du, L. (2015). Genome-wide specific selection in three domestic sheep breed. PLOS ONE. 10: e0128688.

Wilson T., Xi-Yang, W.U., Juengel, J.L., Ross, I.K., Lumsden, J. M., Lord, E. A., et al. (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol Reprod. 64:1225‑35.

Zhang, L., Ma, X., Xuan, J., Wang, H., Yuan, Z., Wu, M., Liu, R., Zhu, C., Wei, C., Zhao, F. (2016). Identification of MEF2B and TRHDE gene polymorphisms related to growth traits in anew Ujumqin sheep population. PLOS ONE. 11:e0159504.

Zhou, H., Hickford, J., Gong, H. (2007). Polymorphism of the ovine calpastatin gene. Mole. Cellu. Prob., 21(3): 242-244.