

# ALLELIC FREQUENCIES OF POLYMORPHISMS IN GENES ASSOCIATED WITH MEAT QUALITY IN THE LOCAL MEDITERRANEAN BALADI CATTLE

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**Abstract – Baladi, a native cattle breed found throughout the entire Southern Mediterranean basin, is known for its high disease resistance and hardiness. Baladi cows in Israel are in danger of extinction due to the introduction of larger and more productive European breeds in these regions. In order to promote practical conservation decisions of Baladi, the yet unexplored production traits, over their well accepted adaptation to cope with harsh local conditions, should be revealed. In the current study, the allelic and genotypic frequencies of two SNPs from two genes, *CAST* and *DGAT1*, associated with meat tenderness and intra-muscular fat (IMF) content, respectively, were estimated in four cattle breeds, in order to locate the genetic potential of Baladi for meat quality within dairy, dual-purpose and incidine bovine members. Significant allelic and genotypic results were obtained for both, *CAST* and *DGAT1* SNPs, in the four cattle breeds. The Baladi cattle showed high allelic and genotypic frequencies of the A allele and AA, AG genotypes in the *CAST* SNP, which previously found to be associated with meat tenderness. Based on the estimation of allelic frequencies of the *DGAT1* SNP we are suggesting that no selection for high IMF was performed in the Baladi cattle.**

**Key Words – Baladi cattle, Meat quality, Intra muscular fat, Genetic markers.**

## I. INTRODUCTION

Baladi, a native cattle breed found throughout the entire Southern Mediterranean basin [1], is known for its high disease resistance and hardiness [2]. Baladi cows in Israel, and in other Mediterranean countries, are in danger of extinction due to the introduction of larger and more productive European breeds in these regions. In order to promote practical conservation decisions of Baladi, the yet unexplored production traits, over their well accepted adaptation to cope with harsh local conditions, should be revealed.

Looking at given genetic markers, the genomic approach is a powerful tool through which one can gain an immediate estimation as for the productive potential

of untested breeds, by means of carcass yield and meat quality. This is even of more pronounced significance for endangered breeds, in which the number of individuals is too little to support phenotypic evidence. Indeed, several genes have previously been identified as markers for meat tenderness and intra muscular fat (IMF) content [3, 4].

The aim of the current study was to estimate the allelic and genotypic frequencies of single nucleotide polymorphisms (SNPs) from two genes, Calpastatin (*CAST*) and diacylglycerol O-acyltransferase 1 (*DGAT1*), previously found to be associated with meat tenderness and IMF content, respectively, in Baladi, Holstein (*Bos taurus*), Simmental (dual-purpose; *Bos taurus*) and Brahman (*Bos indicus*) cattle breeds.

During the course of evolution and domestication, the classification of Baladi cattle as either dairy or beef breed has still been obscured. Hence, the inter-breed comparison carried out in the current study was meant to locate the genetic potential of Baladi for meat quality within dairy, dual-purpose and incidine bovine members.

## II. MATERIALS AND METHODS

Genomic DNA was extracted from blood samples of animals from 4 cattle breeds: Baladi (BAL), Holstein (HOL), Brahman (BRH) and Simmental (SIM) using a commercial kit. Two single nucleotide polymorphisms (SNPs) were genotyped, one in each gene. The SNP in *CAST* is an A>G substitution, present in exon 7 of the gene, causing an amino acid change of Threonine to Alanine at position 182 (*T182A*) [3]. The second SNP referred to as *DGAT1*, is a Lysine to Alanine amino acid substitution at position 232 (*K232A*), present in exon 8. This mutation is linked to a quantitative trait locus (QTL) in BTA14, associated with milk fat content and other milk characteristics [5, 6].

SNP genotyping was carried out using custom TaqMan allelic discrimination assay designed by Applied Biosystems according to manufacturer's protocol. Statistical analysis was performed using JMP software.

## III. RESULTS AND DISCUSSION

Genotypic and allelic frequencies were estimated for the *CAST* and *DGAT1* SNPs and presented in **TABLE 1**. Chi-square tests revealed significant differences in genotypic ( $P<0.01$ ) and allelic frequencies ( $P<0.05$ ) between the four breeds in the *CAST* SNP. Pair-wise test results show that the allelic and genotypic distributions in the BAL breed are similar to these identified in the SIM and BRH breeds, with higher frequencies of A allele and AA and AG genotypes in all 3 breeds. These two genotypes (AA, AG) were previously found to be associated with higher meat tenderness, comparing to the GG genotype which was associated with tough meat [3]. Such genotypic and allelic frequencies might give us a hint with respect to the potential of meat tenderness in the BAL breed. To gain a broader understanding with regard to this trait, other SNP markers should be evaluated as well, together with phenotypic characterization of meat samples. However, the phenotypic characterization could be attained only when population size exceeds the critical number that defines a breed as endangered [7], as in BAL. Genotypic and allelic frequencies of *CAST* SNP in the HOL breed were significantly different comparing to the 3 other tested breeds ( $P\leq 0.05$ ), showing lower frequency of the AA genotype and A allele. Being a significant dairy breed, HOL has apparently not been selected for meat tenderness or other meat related traits. Hence, the observation shown herein of normal *CAST* SNP distribution is well expected. Again, additional markers are needed to verify this assumption. For example, the *T182A* mutation, located in the well conserved region (between mammalian species) of the L-domain of the *CAST* protein, which interacts with DII-domain of calpain protein in its inactive calcium free form [8]. Calvo et al. [1] suggested that this specific mutation in the *CAST* gene might be responsible for the variation in meat tenderness, found seven days post-mortem in animals from the Parda de Montana Spanish cattle breed. Both calpain and *CAST* are proteolytic enzymes involved in the process of meat tenderization, while *CAST* serves as calpain's inhibitor, moderating its activity post-mortem [9, 10]. The substitution of Threonine by Alanine, is generating a more stable union between calpain and *CAST*, affecting meat tenderization [11].

Significant genotypic and allelic differences were also detected in the Chi-square analysis of *DGAT1* mutation (*K232A*). Pair-wise tests for genotypic and allelic differentiation indicated that the frequency distributions for the HOL and the SIM breeds were quite homogenous, while the BAL and BRH breeds were

**TABLE 1.** Comparison of genotype and allele frequencies in four cattle breeds in *CAST* and *DGAT1* SNPs.

|       |          | CAST SNP |      |      |       |                   |
|-------|----------|----------|------|------|-------|-------------------|
| Breed | N        | AA       | AG   | GG   | A     | G                 |
| BAL   | 52       | 0.50     | 0.30 | 0.20 | 0.65  | 0.35 <sup>a</sup> |
| SIM   | 16       | 0.57     | 0.18 | 0.25 | 0.66  | 0.34 <sup>a</sup> |
| BRH   | 24       | 0.58     | 0.30 | 0.12 | 0.73  | 0.27 <sup>a</sup> |
| HOL   | 182      | 0.27     | 0.44 | 0.29 | 0.49  | 0.51 <sup>b</sup> |
|       | $\chi^2$ | 19.45**  |      |      | 9.31* |                   |

|       |          | DGAT1 SNP |      |      |           |                   |
|-------|----------|-----------|------|------|-----------|-------------------|
| Breed | N        | AA        | AK   | KK   | A         | K                 |
| BAL   | 52       | 0.36      | 0.34 | 0.29 | 0.53      | 0.47 <sup>a</sup> |
| SIM   | 16       | 0.82      | 0.12 | 0.06 | 0.85      | 0.15 <sup>b</sup> |
| BRH   | 24       | 0.04      | 0.42 | 0.54 | 0.25      | 0.75 <sup>a</sup> |
| HOL   | 182      | 0.72      | 0.26 | 0.02 | 0.82      | 0.18 <sup>b</sup> |
|       | $\chi^2$ | 92.11**** |      |      | 61.12**** |                   |

Abbreviations: BAL, Baladi; SIM, Simmental; BRH, Brahman; HOL, Holstein. <sup>a,b</sup> Genotypic frequencies within columns with different superscript letters are significantly different ( $P<0.05$ ). \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ . \*\*\*\*  $P<0.0001$ .

significantly different ( $P\leq 0.05$ ). In the literature, there is a lack of consensus regarding the association of *DGAT1* mutation with IMF [12]. In the study of Thaller et al. [4], the *K* allele was found to be significantly associated with higher IMF content in German Holstein animals. In a different study, Pannier et al. [12] found no association of the *K* allele with IMF content tested in two muscles in a crossbred cattle population, as only 2 out of 126 animals were homozygous to the *K* allele. Allelic frequencies estimated in that study for 9 purebred cattle breeds were similar to these identified in our study for the SIM and HOL breeds, showing higher A allele frequency (0.72 and 0.82, respectively). The findings of Pannier et al. were also supported by these of Casas et al. [13] which found no association of the *K* allele with IMF content. The *K* allele is likely the ancestral state of *DGAT1* [6]. As suggested by Medjugorac et al. [14], the *K232A* substitution probably raised early in the history of domesticated cattle or even before domestication as indicated by the presence of the A allele in the Anatolian Black indigenous breed. The last is present in a region known as the site of domestication of the European *Bos Taurus* [6]. In our study, a slightly higher allele frequency was detected in the BAL cattle for the A allele (0.53, Table 2), suggesting no selection for high IMF in this breed. However, an additional set of

markers associated with IMF content, as well as phenotypic characterization of meat cuts in larger number of animals are needed, in order to evaluate the potential of BAL as a beef breed.

#### IV. CONCLUSION

The current study is the first to estimate meat quality traits of BAL cattle, by means of genetic markers. The findings shown herein of the allelic and genotypic frequencies of two SNPs in genes associated with meat quality encourage further scientific efforts that together with the proven physiological advantages to cope with the challenging Mediterranean pasture conditions. These, can contribute to a conservation initiative of the BAL breed. Conservation program will enable to carry out phenotypic characterization along with an additional set of markers, on a larger number of individuals.

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