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The Open Agriculture Journal, 2017, *11*, 1-10

The Open Agriculture Journal

Content list available at: www.benthamopen.com/TOASJ/

DOI: 10.2174/1874331501711010001

RESEARCH ARTICLE

Detection of Allelic and Genotypic Frequencies of Polymorphisms Associated with Meat Quality in the Mediterranean Baladi Cattle

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Received: August 24, 2016 Revised: November 15, 2016 Accepted: December 02, 2016

Abstract: Baladi, (*B taurus;* DAGRIS) a native cattle breed found throughout the entire Southern Mediterranean basin, is known for its high disease resistance and hardiness. Baladi cows in Israel and Southern Mediterranean basin are endangered due to the introduction of larger and more productive European breeds in these regions. In order to promote conservation initiatives of Baladi by stakeholders, the yet unexplored production traits, over their well accepted adaptation to the harsh Mediterranean conditions, were sought in the current study. Aiming at locating the genetic potential of Baladi for meat quality, the allelic and genotypic frequencies of four polymorphisms in *CAST, CAPN1, DGAT1,* and *FASN* genes, previously reported to be associated with meat quality traits, were compared to four cattle breeds. The other four breeds included Limousine, Holstein, Simmental and Brahman cattle, which represent beef, dairy, dual-purpose and indicine bovine members, respectively. Relative to the four bovine members, Baladi cattle exhibited high frequencies of the increasing alleles and genotypes in all four SNPs associated with meat tenderness or fat deposition. These findings, along with future phenotyping and genomic profiling of meat quality related markers, and the well-established adaptability to the challenging Mediterranean pasture conditions, may promote conservation initiatives of Baladi cattle by stakeholders.

Keywords: Baladi Cattle, SNPs, Meat Quality, Endangered breed, Tenderness.

INTRODUCTION

The search for new food sources has promoted, along the years, a global spread of well-marketed breeds [1]. As a result, the current domesticated animal dispersion is essentially restricted to a few breeds, and almost exclusively involves transfers from developed to developing countries, thereby imposing a major threat to the conservation and utilization of indigenous animal genetic resource [2].

The need to conserve livestock genetic diversity within breeds has been recognized already in the early 1990s [3], and is widely reflected by FAO initiatives [2]. However, in order to encourage breeders and policy makers to keep and preserve indigenous breeds, sound arguments related to their superior performance over the exotic breeds, in terms of economic and biological efficiencies, should be provided. While basic conservation programs focus on managing production performance through the control of population size and genetic variability, to ensure maintenance of adaptability to a given environment in favor of persistent production under non-favorable conditions, less attention has been directed to product quality [4].

Baladi (*B taurus;* DAGRIS), a native cattle found throughout the entire Southern Mediterranean basin [5], is facing the danger of extinction, due to the introduction of larger and more productive European breeds in these regions. Baladi (BAL) cows are commonly reputed to be tolerant of poor care, meager diet, adverse climate conditions [6], and high disease resistance [7]. Recently, its unique foraging behavior was explored by Dolev *et al.* [8]. Comparing to a larger frame breeds, the small-framed BAL cows were found to travel longer distances between foraging habitats and

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extended foraging periods, reflecting their physiological plasticity to cope with conditions of changing herbage quality [9]. Moreover, BAL cows were more efficient in conditions of low herbage quality [10]. Although the above highlights the improved adaptability of BAL cattle to the harsh Mediterranean ecosystems, near future scenario predicts potential dilution of its genetic diversity and hence, the need for its conservation becomes critical.

Due to the small population size of the BAL, an ill-conceived conservation strategy could be detrimental to its survival [6]. In order to promote practical conservation decisions for the BAL in addition to its integration into the local herd, it is crucial to study yet unexplored production quality traits of the breed, and estimate their interaction with adaptability traits.

While European beef cattle breeds introduced to the Mediterranean ecosystems are superior to BAL in their productive performance by means of growth rate and carcass weight, a comparative analysis of their meat quality characteristics haven't been carried out yet. Since population size of BAL cattle in the Southern Mediterranean basin has been largely reduced during the last decades [6], it limits the use of phenotypic characterization of meat quality, and turns the genomic approach, at this stage, prerequisite.

From a genetic perspective, determination of allelic and genotypic distribution of markers linked with economically important traits may be a powerful tool to gain an immediate knowledge as for the productive potential of livestock breeds and populations [4]. Indeed, several genes have been successfully identified as markers for meat quality characteristics. Polymorphisms in the calpastatin (*CAST*) and μ-calpain (*CAPN1*) genes were significantly associated with meat tenderness in several beef cattle breeds [11 - 15]. Tenderness is classified as one of the most important organoleptic quality traits affecting customer's choice [16]. Both calpastatin and μ-calpain are proteolytic enzymes involved in the process of meat tenderization following rigor mortis [17]. A lysine to alanine polymorphism encoding diacylglycerol O-acyltransferase 1 (*DGAT1*), a microsomal enzyme that catalyzes the final step of triglyceride synthesis, has been shown to be associated both with milk fat (percentage of lipid content of milk) [18, 19] and intramuscular fat content (IMF; percentage of lipid content within muscle) [20]. The last is an important characteristic of cooked meat, affecting both juiciness and flavor [21]. Fatty acid synthase (*FASN*) is a multifunctional enzyme complex that regulates *de-novo* biosynthesis of long chain saturated fatty acids, and the thioesterase (TE) domain within this complex is responsible for termination of fatty acid synthesis [22]. Polymorphisms in the TE domain have been shown to affect fatty acid composition [23].

In the current study, we estimated the allelic and genotypic frequencies of single nucleotide polymorphisms (SNPs) from *CAST, CAPN1, DGAT1* and *FASN* genes in five cattle breeds: BAL, Limousine (LIM; beef breed; *Bos taurus*), Holstein (HOL; dairy breed; *Bos taurus*), Simmental (SIM; dual-purpose breed; *Bos taurus*), and Brahman (BRH; beef breed; *Bos indicus*). During the course of evolution and domestication, the classification of BAL cattle as either dairy or beef breed has still been obscured. Hence, the inter-breed comparison carried out in the current study was also meant to locate the genetic potential of BAL for meat quality within dairy, beef, dual-purpose and indicine bovine members.

MATERIALS AND METHODS

DNA samples of 5 cattle breeds were analyzed in the current study: BAL, (n=92; females and males); LIM, (n=33; males); HOL, (n=216; males); SIM, (n=135; females and males) and BRH, (n=24; females).

Genomic DNA was extracted from blood samples of each animal using a commercial kit (Sigma, NA2020-1KT). Four single nucleotide polymorphisms (SNPs) were genotyped, one in each of the following genes: *CAST*, *CAPN1*, *DGAT1* and *FASN* (Table **1**). The *CAST* SNP is an A→G variation, present in exon 7 of the gene, causing an amino acid substitution of threonine to alanine at position 182 (*T182A*). This polymorphism was previously associated with meat tenderness (Table **1**) [24]. The *CAPN1* SNP is a C→G polymorphism, causing an amino substitution of glycine to alanine at position 316 (*G316A*) of the protein (Table **1**). This polymorphism was previously reported to be associated with meat tenderness in cattle [12 - 14]. The *DGAT1* polymorphism is a lysine to alanine amino acid substitution at position 232 (*K232A*), presents in exon 8 of the gene (Table **1**). This polymorphism was first linked to a quantitative trait locus (QTL) in *BTA14*, associated with milk fat content and other milk characteristics [18, 19]. Later, the *K232A* polymorphism was associated with intramuscular fat content in cattle [20]. The fourth tested variant is an A→G polymorphism present in the *FASN* gene, causing an amino acid substitution of alanine to threonine at position 2266 of the protein (*A2266T*) (Table **1**). This polymorphism was previously shown to be associated with fatty acid composition in cattle, as animals carrying the GG genotype had significantly higher oleic acid content and total mono unsaturated fatty acid concentration comparing to these carrying the AA genotype of the SNP [23].

Table 1. SNPs from four genes tested on five cattle breeds.

SNP / Gene	BTA	SNP Position *UMD 3.1*	Allele	*AA Substitution*	SNP rs#	Associated trait	Reference
CAST	7	98,535,683	A/G	*T182A*	rs210072660	Tenderness	Calvo *et al.* 2014;
CAPN1	29	44,069,063	C/G	*G316A*	rs17872000	Tenderness	Page *et al.* 2002, 2004; Lee *et al.* 2014
DGAT1	14	1,802,265 - 1,802,266	A/K*	*K232A*	rs109234250; rs109326954	Intramuscular Fat deposition	Winter *et al.* 2002
FASN	19	51,402,032	A/G	*A2266T*	rs41919985	Fatty acid composition	Zhang *et al.* 2008

DGAT1* A allele (CG);** K (AA). BTA – *Bos taurus* chromosome.

SNP genotyping was carried out using custom TaqMan allelic discrimination assay, designed by Applied Biosystems according to manufacturer's protocol. Primers and TaqMan® MGB probes were designed to amplify and target the two alleles of each SNP, using Primer Express® software (Applied Biosystems, Foster City, CA). The list of oligos and probes used for genetic analysis is presented in Table **2**.

Table 2. Primers and MGB probes used for PCR and allelic discrimination.

Gene	Product	Sequence	Primer / Probe
CAST	Calpastatin	TGTCGATCTTTTAGACCAAGTCACA	Forward
		AGCTGGTTCGGCAGATGCT	Reverse
		AAAGAGC**A**CTGTTCC	VIC
		AAGAGC**G**CTGTTCC	FAM
CAPN1	μ-Calpain	AGCTGCTCCCGCATGTAAG	Forward
		GGCTGGGCAGGTCAGT	Reverse
		TCCACG**C**CGTTCCA	VIC
		CCACG**G**CGTTCCA	FAM
DGAT1	Diacylglycerol-O-acyltransferase1	CCGCTTGCTCGTAGCTTTG	Forward
		CCGCGGTAGGTCAGGTTG	Reverse
		AGGTAAG**GC**GGCCAA	VIC
		CAGGTAAG**AA**GGCCAAC	FAM
FASN	Fatty acid synthase	GGCTCCACCACCGTGTTC	Forward
		ACCTCCTGTACACTGTAGGCCATAG	Reverse
		TGGCC**A**CCAAGCT	VIC
		TGGCC**G**CCAAGCT	FAM

All statistical analyzes were performed using JMP statistical software (JMP10 software, SAS institute). Gender-based allelic and genotypic frequencies for each polymorphism were studied in the different breeds. Pair-wise tests were performed to calculate for genotypic frequencies. For females the test included a comparison between BAL, SIM and BRH breeds. For males BAL, LIM, HOL and SIM were compared. Deviations from Hardy-Weinberg (HW) equilibrium were calculated using a contingency analysis - Chi Square likelihood test with a threshold of $P \leq 0.05$.

RESULTS

The populations examined in the current study were composed of females and males. While in BAL and SIM there was a mix of both genders, BRH was exclusively represented by females, and LIM and HOL by males. Inter-gender comparison was performed for the BAL and SIM breeds. Genotypic and allelic frequencies identified in females (n=74) versus males (n=18) of the BAL breed are presented in Table **3**. As seen, significant differences were found between genotypic frequencies in the *CAST* SNP ($P \leq 0.01$), showing predominance of the heterozygous genotype AG in females (Table **3**).

In the *DGAT1* SNP, significant differences were identified between males and females for both, allelic and genotypic frequencies ($P \leq 0.05$; Table **3**). The AK genotype was the highest for both genders. As for the homozygous genotypes, while the AA was higher in females, the KK was higher in males ($P \leq 0.05$). Within the allelic frequencies the A allele was higher in females and the K allele was higher in males ($P \leq 0.05$).

Unlike for *CAST* and *DGAT1*, no differences were detected between BAL females and males in the *CAPN1* and

FASN SNPs (Table **3**).

In the SIM breed, no significant differences were detected between females (n=30) and males (n=105); gender-based analysis revealed the following P values for the following polymorphisms: *CAST* (P=0.17), *CAPN1* (P=0.71), *DGAT1* (P=0.39) and *FASN* (P=0.58). In the *DGAT1* polymorphism, none of the SIM females and only 3 SIM males were homozygous for the KK genotype. Similarly, in the *FASN* SNP, none of the females and only 2 males were homozygous for the AA genotype (Table **4A**, **4B**).

Table 3. Comparison test of genomic and allelic frequencies of four polymorphisms in males *vs.* females of the BAL breed. SNPs present in: (a) *CAST* and *CAPN1* genes; (b) *DGAT1* and *FASN* genes.

(a).

			CAST					***CAPN1***				
Breed	Sex	N	GG	AG	AA	G	A	CC	CG	GG	C	G
BAL	Female	74	0.49	0.41	0.10	0.70	0.30	0.16	0.84	0.06	0.20	0.74
	Male	18	0.50	0.13	0.37	0.56	0.44	0.88	0.12	0.00	0.94	0.06
	X^2		n.s.	8.9**	n.s.			n.s.			n.s.	

(b).

			DGAT1					***FASN***				
Breed	Sex	N	AA	AK	KK	A	K	GG	AG	AA	G	A
BAL	Female	74	0.33	0.54	0.13	0.60	0.40	0.55	0.35	0.10	0.73	0.27
	Male	18	0.6	0.63	0.31	0.38	0.62	0.63	0.31	0.06	0.78	0.22
	X^2		6.17*			5.35 *		n.s.			n.s.	

Abbreviations: BAL, Baladi; n.s. – not significant; *P ≤0.05; **P ≤0.01.

Due to potential confounding effect of gender, highlighted by the differences found in BAL (Table **3**), separate estimations of genotypic and allelic frequencies were carried out for females (BAL, SIM and BRH; Table **4A** and males (BAL, LIM, HOL and SIM; Table **4B**).

For females, no differences were revealed in genotypic and allelic frequencies of the *CAST* SNP, between BAL, SIM and BRH (Table **4A**).

In the case of the *CAPN1* marker, allelic (P≤0.05) and genotypic (P≤0.01) frequencies of BAL females were significantly lower than those of SIM and BRH (Table **4A**). It is noteworthy, that all three breeds favored the CC genotype and C allele.

Interestingly, both, the allelic and genotypic frequencies of the *DGAT1* marker differed among females of BAL, SIM and BRH (P≤0.0001; Table **4A**). Estimated genotypic and allelic frequencies in the SIM showed the highest values of the AA genotype and A allele, followed by those of BAL. As expected, females of the BRH breed presented the highest frequency of the KK genotype and K allele (Table **4A**).

Table 4A. Genotypic and allelic frequencies of four polymorphisms estimated in females (BAL, LIM, HOL and SIM), present in: (a) *CAST* and *CAPN1* genes; (b) *DGAT1* and *FASN* genes.

(a).

		CAST					***CAPN1***				
Breed	N	AA	AG	GG	A	G	CC	GC	GG	C	G
BAL	74	0.49	0.41	0.10^a	0.69	0.31^a	0.74	0.20	0.06^a	0.84	0.16^a
SIM	30	0.39	0.35	0.26^a	0.56	0.44^a	0.90	0.10	0.00^b	0.95	0.05^b
BRH	24	0.54	0.32	0.14^a	0.70	0.30^a	1.00	0.00	0.00^b	1.00	0.00^b
	X^2	n.s			n.s.		15.2**			8.5*	

(b).

		DGAT1					***FASN***				
Breed	N	AA	AK	KK	A	K	GG	AG	AA	G	A
BAL	74	0.33	0.54	0.13^a	0.60	0.40^a	0.56	0.34	0.10^a	0.73	0.27^a

(Table 4A) contd.....

		DGAT1					*FASN*				
SIM	30	0.86	0.14	0.00^{b}	0.93	0.07^{b}	0.65	0.35	0.00^{a}	0.83	0.17^{a}
BRH	24	0.04	0.42	0.54^{c}	0.25	0.75^{c}	0.68	0.27	0.05^{a}	0.81	0.19^{a}
	X^2	63.7***			33.1***		n.s			n.s	

Abbreviations: BAL, Baladi; SIM, Simmental; BRH, Brahman. Genotypic frequencies within columns with different superscript letters ($^{a-c}$) are significantly different; n.s. – not significant; *P ≤0.05; **P ≤0.01; ***P≤0.0001

For the *FASN* marker, females of BAL, SIM and BRH presented higher GG genotype and G allele frequencies compared to the AA genotype and A allele, but without significance among breeds (Table **4A**).

For males, no differences were revealed in genotypic and allelic frequencies of the *CAST* SNP, between BAL and SIM (Table **4A**). Of all tested males, LIM presented the highest AA genotype and A allele, followed by BAL, SIM and HOL (P≤0.0001; Table **4B**).

In the case of the *CAPN1* marker, genotypic frequencies of BAL males were significantly higher than those of LIM and HOL, but similar to those of SIM (P≤0.0001; Table **4B**). With the exception of HOL the other three breeds favored the CC genotype.

The allelic and genotypic frequencies of the *DGAT1* marker was similar in BAL and LIM, but differed from HOL and SIM males (P≤0.0001; Table **4B**). Interestingly, unlike in females, BAL males exhibited higher frequencies of the KK genotype and K allele, not as in HOL and SIM, which favored higher values of the AA genotype and A allele Table **4B**).

For the *FASN* marker, BAL and SIM males presented higher GG genotype and G allele frequencies compared to the LIM and HOL males (P≤0.0001; Table **4A**).

Table 4B. Genotypic and allelic frequencies of four polymorphisms estimated in males (BAL, LIM, HOL and SIM), present in: (a) *CAST* and *CAPN1* genes (b) *DGAT1* and *FASN* genes.

(a).

		CAST					*CAPN1*				
Breed	N	AA	AG	GG	A	G	CC	GC	GG	C	G
BAL	18	0.50	0.12	0.38^{a}	0.56	0.44^{a}	0.88	0.12	0.00^{a}	0.94	0.06^{a}
LIM	33	0.88	0.09	0.03^{b}	0.93	0.07^{b}	0.52	0.45	0.03^{b}	0.74	$0.26^{a,b}$
HOL	216	0.26	0.44	0.30^{c}	0.48	0.52^{a}	0.34	0.46	0.20^{c}	0.57	0.43^{b}
SIM	105	0.46	0.19	0.35^{a}	0.55	0.45^{a}	0.92	0.08	0.00^{a}	0.96	0.04^{a}
	X^2	66.9*			26.5*		128.2*			68.3*	

(b).

DGAT1						*FASN*					
Breed	N	AA	AK	KK	A	K	GG	AG	AA	G	A
BAL	18	0.06	0.63	0.31^{a}	0.38	0.62^{a}	0.63	0.31	0.06^{a}	0.78	0.22^{a}
LIM	33	0.27	0.58	0.15^{a}	0.56	0.44^{a}	0.18	0.40	0.42^{b}	0.38	0.62^{b}
HOL	216	0.72	0.23	0.05^{b}	0.84	0.16^{b}	0.28	0.48	0.24^{b}	0.52	0.48^{b}
SIM	105	0.77	0.20	0.03^{b}	0.87	0.13^{b}	0.65	0.33	0.02^{a}	0.81	0.19^{a}
	X^2	58.5*			28.8*		74.4*			18.2*	

Abbreviations: BAL, Baladi; LIM, Limousine; HOL, Holstein; SIM, Simmental. Genotypic frequencies within columns with different superscript letters (a-d) are significantly different; *P≤0.0001

In order to evaluate the genetic dynamics of meat quality in the above populations, with respect to the four polymorphisms, departures from HW equilibrium were estimated. Deviations from HW were identified for the *CAST* polymorphism in the SIM breed (P≤0.0001; Table **5**). For the *CAPN1* polymorphism, departures were observed in the BAL (P≤0.05), and HOL (P≤0.001) populations, as a lower than expected number of heterozygous was identified. Such departure was identified also in the BRH population (P≤0.0001), but in the opposite direction (Table **5**). Both *DGAT1* and *FASN* polymorphisms were in HW equilibrium in all breeds. In LIM, none of the four tested markers were in HW disequilibrium (Table **5**).

DISCUSSION AND CONCLUSION

The indigenous breed of Israel and other Southern Mediterranean countries like Egypt is the BAL. The BAL populations in these regions are facing the danger of extinction. In the current study we aimed at checking whether in addition to its superb adaptability to the harsh Mediterranean conditions, BAL conservation may be justified also on basis of the yet unexplored meat quality traits. Lacking a sufficient sample size that would enable phenotypic characterization of meat quality parameters, we concentrated herein on a genetic approach. It is well established that meat quality is determined by a large number of genetic and environmental factors [25, 26]. Therefore, in the current study, we focused on four SNPs in genes involved in meat tenderness (*CAST* and *CAPN1* genes), intra-muscular fat content (*DGAT1*) and fatty acid composition (*FASN*).

As previously reported, the *CAST* genotypes (AA, AG) and A allele have been associated with improved meat tenderness [24]. In the current study, frequencies of the AA genotype and A allele were highest in LIM males, a breed selected for meat tenderness [27, 28]. In HOL males, a breed selected for milk production, frequencies of the AA genotype were the lowest (Table **4B**). Interestingly, in females and males of the BAL breed, frequencies of the AA genotype and A allele were higher, comparing to the GG genotype and G allele, respectively. In addition, the AG genotype was found at high frequency in BAL females. A gender-based comparison between BAL and SIM (the predominant beef breed in Israeli herds), revealed similar frequencies of the AA genotype and A allele in females and males. Altogether, these findings imply a possible genetic potential for meat tenderness in the BAL breed. According to Rodero *et al.* [4], similarities only in *CAST* allele frequencies, between continental and indigenous cattle, might not fulfill the requirements to improve meat tenderness of local breeds.

Table 5. Departures from Hardy-Weinberg (HW) equilibrium: Observed and expected heterozygosities in five cattle breeds and four polymorphisms present in: (a) *CAST* and *CAPN1* genes (b) *DGAT1* and *FASN* genes.

(a).

	CAST			***CAPNI***		
Breed	Ho	He	HW[a]	Ho	He	HW[a]
BAL	0.367	0.449	n.s.	0.178	0.269	*
LIM	0.091	0.140	n.s.	0.455	0.382	n.s.
HOL	0.457	0.499	n.s.	0.457	0.585	**
SIM	0.228	0.491	***	0.081	0.076	n.s.
BRH	0.318	0.405	n.s.	0.290	0.000	***

(b).

	DGAT1			***FASN***		
Breed	Ho	He	HW[a]	Ho	He	HW[a]
BAL	0.556	0.492	n.s.	0.322	0.385	n.s.
LIM	0.576	0.496	n.s.	0.394	0.471	n.s.
HOL	0.249	0.274	n.s.	0.477	0.499	n.s.
SIM	0.191	0.200	***	0.331	0.299	n.s.
BRH	0.409	0.438	n.s.	0.273	0.305	n.s.

Abbreviations: Ho, Observed heterozygosity; He, Expected heterozygosity, BAL, Baladi; LIM, Limousine; HOL, Holstein; SIM, Simmental; BRH, Brahman. [a] Estimated P-values associated with the null hypothesis of Hardy-Weinberg equilibrium; *P≤0.05; **P≤0.001; ***P≤0.0001; n.s: not significant.

The *CAPN1* polymorphism was significantly associated with increased meat tenderness in several cattle breeds and populations [12, 29, 30]. In the study of Van Eenennaam *et al.* [30] this marker *(G316A*) was evaluated for meat tenderness within a two marker haplotype together with the marker *CAPN1* 4751-T3. The *CAPN1* 316/4751 C-C haplotype was significantly associated with increased meat tenderness in a mixed cattle population [31]. In the current study, a high frequency of the *CAPN1* C allele was detected in both genders of the BAL and SIM breeds. For SIM, this is not surprising, as in addition to dairy this breed has also been selected for meat production. In BRH females, a fixation towards the CC genotype and C allele was detected. Assuming C is the favorable allele [29, 31 - 35], this finding is intriguing since BRH breed is apparently known for its low meat tenderness, emphasizing the need to test additional markers associated with the trait.

In males, *CAPN1* CC genotype and C allele frequencies were significantly lower in LIM when compared to BAL

and SIM, but higher as related to HOL, which demonstrated the lowest frequency. The HOL breed was characterized by a close to normal genotypic distribution in the *CAPN1* marker, supporting the common notion that HOL was not selected for meat quality. An interaction between the *CAST* and *CAPN* genes was brought by De Tullio *et al.* [36]. Calvo *et al.* [24] suggested that a specific substitution in the *CAST* gene (*T182A*, located in the well conserved protein in its inactive calcium free form) might be responsible for the variation in meat tenderness, found seven days post-mortem in animals from the Parda de Montaña Spanish cattle breed. Although both, *CAPN1* and *CAST* are proteolytic enzymes involved in the process of meat tenderization, calpastatin serves as μ-calpain inhibitor, moderating its activity post-mortem [17, 37]. The substitution of threonine by alanine at position 182 is generating a more stable union between μ-calpain and calpastatin, affecting meat tenderization [38]. Calvo *et al.* [24] has shown strong linkage disequilibrium (LD) between the decreasing G allele in *CAPN1* and the increasing A allele in *CAST* in the Parda de Montaña and Pirenaica breeds. It is possible, that a similar situation exists in the LIM population tested herein, and this might explain the relatively low frequency of the *CAPN1* G allele in this breed, known for its meat quality. Future studies should be conducted in the Israeli herd and BAL population to verify whether this LD exists in one or both populations, and if it does, which is the proper strategy to break it.

In females, the estimated frequency of *DGAT1* AA genotype was significantly lower in the BAL than in SIM. BRH females showed the lowest frequencies of the alanine variant (A allele) and highest frequencies of the lysine variant (K allele). There is a lack of consensus regarding the association of the *DGAT1* polymorphism with IMF [39]. In the study of Thaller *et al.* [20], the K allele was found to be significantly associated with higher IMF content in German Holstein animals. In a different study, Pannier *et al.* [39] found no association of the K allele with IMF content. Allelic frequencies estimated in the study of Pannier *et al.* [39] for several purebred cattle breeds were similar to these identified in our study for the SIM (both genders) and HOL (males) breeds, showing higher A allele frequency. The findings of Pannier *et al.* [39] were in agreement with these reported by Casas *et al.* [15], who found no association of the K allele with IMF content. Winter *et al.* [19]. suggested that the K allele is likely the ancestral state of *DGAT1*, as the lysine variant was identified in yak (*Bos grunniens*) and water buffalo (*Bubalus bubalus*), in addition to several *Bos taurus* and *Bos indicus* breeds. The presence of the alanine variant in the Anatolian Black local breed might point out that the *K232A* substitution probably raised early in the history of cattle domestication or even previous to that, especially since this breed is indigenous in a region known as a site of domestication of the European *Bos taurus* [40]. As for the alanine variant, it was not detected in subspecies of *Bos indicus* which domestication occurred independently [41]. Based on the above and as BAL does not present a fixation for either genotypes, it can be postulated that selection towards each allele might be equally achievable.

The GG genotype of the *FASN* marker is associated with health index and higher proportions of monounsaturated fatty acids [23]. While GG genotype and G allele frequencies were similar in BAL and SIM breeds, their distribution in LIM and HOL males, was significantly lower. Theoretically, the high frequency of GG genotype observed in BAL and Israeli SIM may partially be related to their adaptation to poor nutritional conditions, likely to occur in the Southern Mediterranean basin. Indeed, when lactating SIM cows were exposed to low energy and protein diet, they switched their milk fatty acids in favor of mono- and polyunsaturated ones [42].

A similar finding was detected in the *CAST* polymorphism for which the SIM breed showed significantly lower observed frequencies, presumably as a result of its selection as a dual breed.

Deviation from HW equilibrium may serve to estimate the genetic dynamics of population at given loci. In the current study, it was implemented, in order to evaluate the rate of divergence in allelic frequencies of meat quality related polymorphisms, with respect to *CAST*, *CAPN1*, *DGAT1* and *FASN* markers. Departures from HW equilibrium were revealed only in the case of *CAST* and *CAPN1*. The lower observed frequencies in the *CAST* polymorphism in the SIM breed may presumably stem from its selection for dual purpose. In the case of *CAPN1*, deviations from HW equilibrium were detected in three breeds. While for BAL and HOL populations lower than expected number of heterozygous was identified, in BRH this departure was the other way around. In the BAL population, the favorable allele C of the *CAPN1* marker was significantly higher, emphasizing the plausible potential of the breed with respect to meat tenderness. However, as already indicated, such findings are only speculative until verified by proper meat phenotyping. Similar findings were shown in the study of Rodero *et al.* [4] where two endangered Spanish cattle breeds presented lower observed than expected heterozygosity. As for the HOL breed, the deviation from HW could be explained by the fact that this breed is selected for dairy, with less emphasize on meat related traits.

In summary, for the first time, the current study examines the potential of BAL cattle with respect to meat quality traits, by means of genotypic / allelic frequencies of four genetic polymorphisms. Relative to cattle lineages that

represent various classifications and agricultural specializations [*Bos taurus* (HOL, LIM, SIM) and *Bos indicus* (BRH), [dual purpose (SIM), dairy (HOL) and meat (LIM) breeds], the BAL population exhibited high frequencies of increasing alleles and genotypes in all four SNPs. Although from a conservation point of view this information sounds very encouraging, the upcoming research should focus on additional polymorphisms associated with these traits. In the future, adding these meat quality related markers to the already established repertoire of adaptive traits may add a great value to the "conservation initiative index" and promote conservation policy of BAL by stakeholders. However, this could be attained only when population size exceeds the critical number that defines a breed as endangered.

LIST OF ABBREVIATIONS

BAL	=	Baladi
BRH	=	Brahman
CAST	=	Calpastatin
CAPN1	=	μ-calpain
DGAT1	=	Diacylglycerol O-acyltransferase 1
FASN	=	Fatty acid synthase
HOL	=	Holstein
LIM	=	Limousine
LD	=	Linkage disequilibrium
Simmental	=	SIM
SNPs	=	Single nucleotide polymorphisms
TE	=	Thioesterase

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

We thank Mr. Ali Zoabi and Mr. Rame Kaabia for their assistance with handling the animals of Newe Ya'ar cowshed. We also thank Dr. Erin K. Wagner for her statistical advise.

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