Association of CAST gene polymorphisms with meat texture in Egyptian buffalo

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Aim: the aim of our study was to estimate the effects of a SNP in the *calpastatin* gene on meat quality parameters in Egyptian buffalo. *Subjects and methods:* our study estimated the effect of single nucleotide polymorphisms (SNP) in *calpastatin* gene on meat texture in 60 samples of Egyptian buffalo males by texture profile analysis and Taqman SNP genotyping assay. All data were collected then tabulated and underwent for statistical analysis. *Results:* our study showed that *CAST-2 SNP* had significant effects on the meat texture parameters ($P < 0.0001$). Animals inheriting the homozygous genotype GG had meat that was more tender,lower in firmness, cohesiveness, gumminess, springiness and chewiness than those inheriting the GA genotype. *Conclusion:* from the results, it can be concluded that this SNP in the *calpastatin* gene is associated with meat tenderness, firmness, cohesiveness, gumminess, springiness and chewiness in Egyptian buffalo.

Keywords: *calpastatin,texture profile analysis, Egyptian buffalo*
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Introduction

A number of sensory perception-related characteristics, including appearance, colour, flavour, texture (particularly tenderness), juiciness/water-holding capacity, and odour, are used to determine the quality of meat. The absence of germs, parasites, infectious agents, or poisons makes meat freshness another classic quality criteria. Even though the lack of infectious organisms in meat is an invisible attribute, we identify it with "freshness," and we are hesitant to eat meat that appears to be old. **(Purslow, 2017).**

In Egypt, cattle and buffalo are the most significant animals. Red meat and milk are primarily sourced from them. Due of their higher milk fat content and longer lifespan than cattle, buffaloes play a significant role in Egyptian farmers' lives **(El-Nahrawy, 2011).**

There are 3.9 million heads of buffalo in Egypt, which supply the market with milk and red meat at a rate of 44% and 39%, respectively. In Egypt, the rate of growth in the human population is 2.2–2.5% higher than the rate of growth in the number of buffalo, which is 1.7 percent annually **(Abou El-Amaiem, 2014).**

Buffalo are a kind of bovid, their meat differs significantly from beef in many ways. Compared to the yellow-white fat in beef, the fat in buffalo meat is milky white and has a lower fat percentage. Buffaloes have firmer bones than cows due to their bigger size, and their flesh is deeper in colour. While cow muscle has a pH of 6.40.7, buffalo meat has a lower pH of 5.60.4. Its muscles contain substantially less collagen as well; however the degree of intramuscular collagen cross-linking is not significantly different between the species. **(Valin et al., 1984)**

The *calpain/calpastatin* system is an endogenous, calcium-dependent proteinase system that mediates the proteolysis of key myofibrillar proteins during the postmortem storage of carcasses and cuts of meat at refrigerated temperatures **(Koohmaraie et al., 1995)**.The *calpain* gene is responsible for the breakdown of myofibrillar proteins, which is closely related to meat tenderness**(Wheeler & Koohmaraie, 1994).** The *calpastatin* gene *(CAST)* has five SNPs (*CAST1, CAST2, CAST3, CAST4, CAST5*) which act as endogenous inhibitors of the proteolytic activity of *calpains***(Calvo et al., 2014)**

2. Subjects and methods

2.1. Animals

The present study was carried out on meat and blood samples from sacrificed 60 males (fourteen months old). All animals previously raised under Egyptian condition at El-saadany private farm at Giza governorate. The animals were apparently healthy and free from any clinical disorders or diseases.

2.2 Texture profile analysis

The approach outlined by **Bourne (2003)** was used to conduct a texture profile analysis to evaluate the tenderness of the meat. 48 hours after death, meat samples (longissimus lumborum muscle) were put in polyethylene bags. The bags were heated until the internal temperature of the beef samples reached 70°C in a water bath set at 80°C. The samples were cooked and then allowed to cool for 30 minutes at room temperature. After the crust was peeled off, (40* 40* 30) mm-sized samples were used to measure the texture. TPA was computed using software and a universal testing device (Cometech, B type, Taiwan). An aluminium cylindrical probe with a 40 mm diameter was used in the TPA double compression test to reach a depth of 50% at a speed of 1mm/s.Firmness (N), gumminess (N), chewiness (N), cohesiveness (Ratio) and springiness (mm) were calculated from TPA graphic.

2.3. SNP genotyping

One SNP in CAST gene was genotyped in this study. Detailed information on the locations and positions of this marker is provided in **Table 1**. All of the animals' blood samples (500 µl) were used to extract genomic DNA. Rotor-Gene Q2 Plex Real-time PCR was used to genotype samples using TaqMan SNP genotyping assays created by Metabion.. The primers were used are shown in**Table 2**.

Table (1): Genetic descriptions of the SNPs genotyped

Gene	Chromosome no.	SNP name	Position on GCF_019923925.1 (NDDB_DH_1)	SNP
CAST	9	CAST 2	14461198	G/A

Table (2): The used primers and probes

Primer forward	Primer reverse	Probe 1 (VIC)	Probe 2 (FAM)
CTCACGTGTTCTTCAGTGTTCTG	CAACCCAAAGAAACATCAAACACAGT	CCTTTCCTCTTAGACTTG	CTTTCCTCTTGGACTTG

2.3. Statistical analysis

ANOVA using a single SNP was applied to test the null hypothesis, that there is no difference between the trait means of any genotype group. All analysis was conducted by R **(R Core Team, 2020)**. P-value < 0.05 was considered statistically significant.

3. Results

The genotypic and allelic frequencies of the *CAST-2* were calculated for 60 males of Egyptian buffalo and are presented in **Table 3**. In the *CAST-2* gene, the genotypic frequencies for GG and GA genotypes were 0.4 and 0.6, respectively, while the allelic frequencies for the G and A alleles were 0.7 and 0.3 respectively.

Table (3): Genotypes and allelic frequencies of *CAST-2* gene in Egyptian buffalo

Marker	Genotype	NO.	Frequency	Allele	NO.	Frequency
CAST-2	GG	24	0.4	G	42	0.7
	AG	36	0.6	A	18	0.3

One-way ANOVA test revealed that the genotype GG of *the CAST-2* gene is associated with meat that is more tender, lower in firmness, cohesiveness, gumminess, springiness and chewiness than GA genotype (P < 0.0001) (**Table 4)**.

Table (4): Effect of the *CAST-2* on meat texture parameters

Genotypes of CAST-2	Meat texture parameters					
	Tenderness	Firmness	Cohesiveness	Gumminess	Springiness	chewiness
	LSM ±SE					
GG	76.8 ± 2.58	26.1 ± 3.4	0.3± 0.02	8.25±4.48	0.38 ± 0.09	3.01 ±0.8
GA	29.8 ± 2.1	51.5± 2.78	0.7 ± 0.02	39.1±3.66	0.72 ± 0.08	19.29±0.7
[1]P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.009	< 0.0001

[1]Significant effect ($P < 0.05$) after modified Bonferroni correction for trait-wise multiple tests.
LSM = Least Square Mean

4. Discussion

This study's main goal was to assess the allelic frequencies of the genotyped marker *CAST-2,* as well as the relationship between meat texture parameters and the polymorphism of this marker in Egyptian buffalo which has not previously been investigated in buffalo. We closely examined the informational content of the marker used in commercial trials.

The frequency of the G allele in this study was about 70%, which was greater than the estimates for the populations of Bos taurus crossbred GPE Cycle VII and B. taurus B. indicus crossbred Cycle VIII as well as the pure French Charolais, Limousin, and Blonde d'Aquitaine breeds. **(Casas, White et al. 2006, Allais, Journaux et al. 2011)**.

The frequency of the G allele of the *CAST-2* marker was estimated to be 29% in the CRC1 population of Brahman, Santa Gertrudis, and Belmont Red breeds, compared to an extremely low frequency of 6% in the crossbred Charolais Angus population **(Van Eenennaam, Li et al. 2007)**, 12% in the pure Angus breed, and 13% in the CRC1 population of temperate breeds **(Johnston and Graser 2010)**.

In this study, it was found that the G allele is associated with meat that is more tender, less in firmness, cohesiveness, gumminess, springiness and chewiness than animals with A allele and this result is the opposite of what has been found in cattle by**Barendse 2002; Casas, White et al. 2006; Allais, Journaux et al. 2011**.

In conclusion, we centred on a published gene marker in the CAST gene to determine if this marker was associated with the meat texture parameters in Egyptian buffalo. The CAST-2 genetic marker was found associated with the meat texture parameters.

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