The effect of *CAPN1* and *CAST* gene variations on meat quality traits in Finnish Aberdeen Angus and Nordic Red Cattle populations

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High meat quality and specifically meat tenderness are desired traits by the consumers, however the environmental impact of meat production is becoming a relevant factor in the industry. Therefore, breeding of dual purpose cattle breeds may answer the high demand of meat production in the future. In this study we identified statistical differences between genotypes of *CAST* and *CAPN1* gene variants with meat quality traits in a dairy breed (Nordic Red Cattle) and compared the results with beef breed (Aberdeen Angus). Our results show that the favorable alleles have not been selected in the studied dairy breed and thus could be used as a tool for improvement of meat quality. The genes were associated with specific meat quality traits (i.e. sensory juiciness, marbling score and meat color) also in the dairy breed. This supports the utility of known meat quality associated genetic variants to improve meat quality in dairy breeds.

Key words: meat production, dairy cattle, beef cattle, μ-calpain, calpastatin

Introduction

The consumption of animal-based food proteins is predicted to increase (FAO 2011), which emphasizes the importance to improve the environmental performance of livestock system (Herrero et al. 2013). Vellinga and de Vries (2018) evaluated four commonly used mitigation strategies for dairy systems and concluded that dual purpose systems can be advantageous over specialized dairy systems. Therefore, breeding of high quality dual purpose cattle breeds could answer the demand of meat production in the future.

Meat tenderness is considered as the most important meat quality trait (Lian et al. 2012). Meat tenderization is a complex process affected by several elements including pre-and post-slaughter factors and genetic background (Lian et al. 2012). *Post-mortem* tenderization of the meat is due to enzymatic degradation of myofibrillar proteins. The CAPN system plays a major role in this degradation since μ-calpain (*CAPN1*) gene encodes μ-calpain protease that is responsible for degradation of myofibrillar proteins (Koohmaraie 1996, Lian et al. 2012) whereas calpastatin protein encoded by calpastatin (*CAST*) gene binds directly to μ-calpain inhibiting its activity (Barendse et al. 2007).

In this study, we aimed to analyze the allelic and genotypic frequencies of *CAPN1* and *CAST* genes in two different cattle breeds, a dairy breed (Nordic Red Cattle, NRC) and a beef breed (Aberdeen Angus, FAA). We genotyped altogether five variations: rs1109555059, rs41255587, rs17871051, rs109221039, and rs17872050 located in genes *CAPN1* and *CAST*, which have been previously associated with tenderness of meat (Page et al. 2002, White et al. 2005, Schenkel et al. 2006) in several cattle breeds (e.g. Casas et al. 2006, Morris et al. 2006, Johnston and Graser 2010, McClure et al. 2012). To our knowledge, the influence of the *CAPN1* and *CAST* gene variations have not been studied in NRC that is mainly bred for milk production, but can be considered as a dual purpose breed. Therefore, the objective of this study was to evaluate the influence of genetic variation within these genes on meat quality traits and their frequencies in NRC and FAA.

Materials and methods

Animal material and analyzed meat quality traits

The study samples consisted of 40 pure-bred NRC bulls and 40 pure-bred FAA bulls from Finland. The FAA bulls were from 14 and the NRC bulls from 21 different sires. Bulls were fed according to EU organic farming regulation (CEC 1999) and kept in the experimental barn at the Natural Resources Institute Finland in Ruukki, Finland

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and managed according to the Finnish legislation regarding the use of animals in scientific experimentation. The average slaughter age was 495 and 514 d for FAA and NRC, respectively, and slaughtering was done at the Atria Ltd commercial slaughterhouse in Kauhajoki, Finland. The bulls had approximately 18 h fasting and lairage time before slaughtering. Carcasses were analyzed for hot carcass weight, EUROP conformation and fat score, marbling score (IMF), pH value and meat color (L, a, b) of the loin (*longissimus lumborum*). The carcasses were classified for conformation and fatness using the EUROP quality classification (EC 2006). After classification carcasses were chilled overnight below 7 °C. On the day after slaughter the carcasses were commercially cut as described by Pesonen et al. (2013). Loin was cut at the level of the first lumbar vertebra, and the 3 kg loin sample between the first and fifth lumbar vertebra was used for further analysis. Meat quality measurements after 12 d ageing at 4 °C were drip loss, Warner-Bratzler shear force, sensory tenderness, sensory juiciness and beef flavor. Carcass and meat quality measurements have been reported fully by Huuskonen et al. (2017).

DNA extraction and sequencing

DNA was extracted from meat samples using Qiagen blood and tissue kit following manufacturer's instructions. PCR conditions and primer sequences are given in Supplementary file 1. Five genetic single nucleotide polymorphism (SNP) variations were amplified; rs17872050 and rs17871051for *CAPN1* and rs41255587, rs109221039 and rs110955059 for *CAST*. PCR products were separated on a 1% agarose gel, purified and directly sequenced using the Big Dye terminator cycle sequencing kit (Applied Biosystems). Electrophoresis of sequencing reactions was performed on a 3500xL Genetic Analyzers (Applied Biosystems). Sequences were analyzed with the Sequencher 5.4.6 software (Gene Codes Corporation, USA).

Statistical analysis

Associations between meat quality phenotypes and genotypes at selected loci were tested using a non-parametrical test tailored for directional alternatives as they are present in association studies (Fischer et al. 2014). The advantages above classical associations tests based on linear models are that the impact of different group sizes is reduced as well as the impact of outliers in the phenotype data. There is a user-friendly version of the test, implemented in the R-package GenomicTools (Fischer 2017).

Results and discussion

As expected the meat quality based on all analyzed traits was lower in the NRC breed compared to FAA (Suppl. file 2). Our results show that alleles associated with tender meat (Page et al. 2002, White et al. 2005, Schenkel et al. 2006) were more frequent in FAA samples than in NRC samples seen from the low minor allele frequency (MAF) values for non-favorable alleles (Table 1). This indicates that variations investigated in this study are not intensively selected in NRC breed. According to previous studies, SNPs in CAST or CAPN1 genes do not show association for milk yield, protein yield or fat yield (Iso-Touru et al. 2016) or female fertility (Höglund et al. 2015) within NRC breed that might partly explains the lack of selection. For two variations (rs110955059 and rs41255587) alleles in *CAST* gene predicted to be beneficial were minor alleles in NRC breed. This further demonstrates the lack of selection pressure.

Table 1. Allele frequencies of CAPN1 and CAST variations in studied Finnish Aberdeen Angus (FAA) and Nordic Red Cattle (NRC) populations

SNP	gene	SNP consequence	alleles	minor allele FAA	minor allele NRC	MAF FAA	MAF NRC
rs17871051	CAPN1	missense	**G**/A	A	A	0.025	0.15
rs17872050	CAPN1	intron variant	**C**/T	T	T	0.138	0.35
rs110955059	CAST	intron variant	**C**/G	G	**C**	0.175	0.325
rs41255587	CAST	3' UTR variant	**G**/A	A	**G**	0.175	0.35
rs109221039	CAST	3' UTR variant	**A**/G	G	G	0.113	0.363

Identified differences (*p*-value <0.1) in associated genotypes with certain meat traits are presented in Table 2, Table 3 and in Supplementary files 3 and 4. In FAA, differences (*p*-value <0.1) were identified between bulls having

beneficial alleles as homozygous (for variations rs109221039 [*CAST*], rs1109555059 [*CAST*], rs41255587 [*CAST*], and rs17871051 [*CAPN1*]) compared to those having a single copy of the beneficial allele. Homozygous FAA bulls had higher sensory tenderness and juiciness and lower shear force (Table 2, Suppl. file 2). However, since beneficial alleles seem to be almost fixed in FAA population (Table 1), *CAST* and *CAPN1* gene variations are not a powerful tool for selection.

Table 2. Meat quality traits for FAA showing differences (*p*-value <0.1) with *CAPN1* and *CAST* gene variations

Trait	SNP	SNP consequence	*p*-value	AA mean	n (AA)	AB mean	n(AB)	BB mean	n(BB)
shear force	rs109221039	3' UTR variant	0.043	48.35 (sd 8.71)	31	54.68 (sd 8.26)	9	-	-
shear force	rs1109555059	intron variant	0.057	47.68 (sd 7.55)	26	53.66 (sd 10.19)	14	-	-
shear force	rs41255587	3' UTR variant	0.057	47.68 (sd 7.55)	26	53.66 (sd 10.19)	14	-	-
sensory juiciness	rs109221039	3' UTR variant	0.063	5.79 (sd 0.32)	31	5.57 (sd 0.28)	9	-	-
sensory tenderness	rs17871051	missense	0.034	5.81 (sd 0.4)	38	5.1 (sd 0.28)	2	-	-
sensory juiciness	rs17871051	missense	0.092	5.35 (sd 0.07)	38	5.76 (sd 0.31)	2	-	-

sd = standard deviation

In NRC breed, bulls that were homozygous for beneficial alleles (rs1109555059 [*CAST*] rs109221039 [*CAST*], rs41255587 [*CAST*), and rs17872050 [*CAPN1*]) had trend for more yellow tones in meat color, higher sensory juiciness and higher marbling score (*p*-value <0.1, Table 3, Suppl. file 4) but no difference was found for tenderness and shear force. The results obtained for FAA are consistent with the earlier studies, but results from NRC are more discordant.

Table 3. Meat quality traits for NRC showing differences (*p*-value <0.1) with *CAPN1* and *CAST* gene variations

Trait	SNP	SNP consequence	*p*-value	AA mean	n (AA)	AB mean	n (AB)	BB mean	n (BB)
color b	rs1109555059	intron variant	0.013	5.36 (sd 0.73)	5	6.15 (sd 0.97)	16	6.49 (sd 1.4)	19
sensory juiciness	rs1109555059	intron variant	0.025	5.68 (sd 0.34)	5	5.43 (sd 0.43)	16	5.38 (sd 0.32)	19
color a	rs1109555059	intron variant	0.04	19.82 (sd 2.1)	5	21.34 (sd 2.13)	16	21.91 (sd 2.44)	19
IMF	rs109221039	3' UTR variant	0.052	1.33 (sd 0.7)	18	1.3 (sd 0.55)	15	1.11 (sd 1.03)	7
color b	rs41255587	3' UTR variant	0.093	5.36 (sd 0.73)	5	6.39 (sd 1.16)	18	6.27 (sd 1.31)	17
sensory juiciness	rs41255587	3' UTR variant	0.065	5.68 (sd 0.34)	17	5.41 (sd 0.42)	18	5.4 (sd 0.33)	5
color b	rs17872050	intron variant	0.05	5.96 (sd 0.98)	13	6.26 (sd 1.27)	26	8.3 (sd NA)	1

MAF = minor allele frequency. Allele marked in A is the beneficial allele for meat tenderness (based on the literature). AA= homozygote for allele A; AB=heterozygote for allele A; BB=homozygote for allele B; sd =standard error; IMF = marbling score; a = redness; b = yellowness

The large variation of meat quality traits in NRC could be a result of minor selection pressure on these traits. The income from beef has been generally low for dairy farms in Finland and this has reduced motivation to improve beef quality traits of dairy herds (Hietala and Juga 2017). Therefore beef traits are not used for dairy cattle breeding schemes (Hietala and Juga 2017) and they are not included in the current Nordic Total Merit (NTM) index for NRC (Kargo et al. 2014). However, Hietala and Juga (2017) concluded that in production systems similar to Finland, where the majority of produced beef originates from dairy herds and beef production fall below consumption, adding beef traits in the breeding program for dairy breeds could enable more sustainable and profitable milk and beef production.

Conclusions

In conclusion, there were differences in NRC in various meat quality traits depending on the genotype individual had for *CAST* or *CAPN1* gene variations, which indicates a possible selection tool for meat quality in this population. However, since the causality of these variations has not been demonstrated, the effects should be studied in larger population and associations confirmed.

Our study indicates that in Finnish Aberdeen Angus population the known variations have low frequencies for non-favorable alleles and thus selection for the favorable allele does not benefit the population. However, in dairy breed (NRC) the MAFs are higher suggesting that known meat quality associated variations could be used for selection to produce dual purpose cattle breed. Furthermore, at least the analyzed gene variations have not been shown to associate with milk production traits or with female fertility, which introduce the possibility to improve meat quality in NRC without compromising milk production.

Supplementary material

Supplementary file 1. Primer names, sequences, SNPs within the PCR amplicon, PCR product length, used PCR enzyme and PCR conditions
Supplementary file 2. Boxplot figures from all measured traits shown per breed
Supplementary file 3. Boxplot figures from variations having *p*-value <0.10 for Finnish Aberdeen Angus
Supplementary file 4. Boxplot figures from variations having *p*-value <0.10 for Nordic Red Cattle

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