Animal Science Papers and Reports vol. 38 (2020) no. 4, 369-380
Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

Post-mortem carcass traits are associated with μ-calpain and calpastatin variants in Santa Inês sheep*

Ariana Nascimento Meira[1], Adriana de Farias Jucá[1], Taiana Cortez de Souza[1], Tatiana Cortez de Souza[1], Luiz Lehmann Coutinho[2], Gerson Barreto Mourão[2], Hymerson Costa Azevedo[3], Evandro Neves Muniz[3], Victor Breno Pedrosa[4], Luís Fernando Batista Pinto[1]**

[1] Federal University of Bahia – 500, Av. Adhemar de Barros, Salvador, BA, 40170110, Brazil

[2] Brazilian Agricultural Research Corporation – Embrapa Coastal Tablelands – 3250, Av. Beira Mar, Aracaju – SE, 49025040, Brazil

[3] University of São Paulo – 11, Av. Pádua Dias, Piracicaba – SP, 13635900, Brazil

[4] State University of Ponta Grossa – 4748, Av. General Carlos Cavalcanti, Ponta Grossa – PR, 84030900, Brazil

(Accepted August 7, 2020)

This study aimed to test the association between variants in the *CAPN1* and *CAST* genes with meat and carcass traits in Santa Inês sheep. Hot and cold carcass yields, biometric measures of the carcass, finishing and conformation carcass scores, rib eye area, primal cut yields and longissimus lumborum traits (pH, a*, b*, L*, tenderness and water-holding capacity) were measured in 192 lambs. Single-locus and haplotype association analyses were performed, using 64 variants in these genes. Additive effects of variants in the *CAPN1* gene on rib eye area (-0.5317±0.2316), pH24 (5.3558±1.5422), water-holding capacity (0.2926±0.1282), internal carcass length (0.6507±0.3080), conformation carcass score (0.1053±0.0469), leg length (1.0045±0.4605), leg yield (-2.4015±0.8787), L* (-1.4828±0.6202) and b* (-0.5907±0.2650) were found, while variants in the *CAST* gene had additive effects on pH0 (-0.0700±0.0239), rump (1.5625±0.5245) and thoracic widths (2.9299±1.1052), L* (-0.9409±0.4757), a* (-0.8445±0.2554), b* (-0.4414±0.2074), water-holding capacity (0.0203±0.0051), neck yield (-0.3021

*This study has been financed with support of FAPESB (project APP0116/2009); CNPq (projects 562551/2010-7 and 474494/2010-1). Study was also financed in part by the CAPES – Finance Code 001 and Embrapa Tabuleiros Costeiros (infrastructure of the experimental farm). LL Coutinho, LFB Pinto, GB Mourão, and VB Pedrosa are recipients of a CNPq Productivity Scholarship.
**Corresponding authors: luisfbp@ufba.br

±0.1318) and finishing carcass score (0.1738±0.0752). Haplotypes in *CAPN1* were not associated with any trait; however, some linkage disequilibrium blocks in the *CAST* gene were associated with external and internal carcass lengths, rump girth, rib yield, pH0, pH24, a*, L* and water-holding capacity. Therefore, variants in the *CAPN1* and *CAST* genes are associated with carcass traits in Santa Inês sheep and are new sources of information to improve these traits via selection schemes.

KEYWORDS: lamb / primal cuts / selection / meat quality traits

The Santa Inês is a meat sheep breed from Northeast of Brazil, which has superior resistance to both heat [McManus *et al*. 2009] and parasitosis [Amarante *et al*. 2004], as well as a low degree of reproductive seasonality [Balaro *et al*. 2014]. However, attributes such as hot and cold carcass yields and primal cut yields need to be improved in this breed [Jucá *et al*. 2016]. While carcass and meat quality traits are difficult to record on a large scale, variants in candidate genes can improve the selection schemes.

The *CAPN1* gene encodes the μ-calpain, a proteolytic enzyme with activity on myofibrillar proteins [Geesink *et al*. 2006]. An increase in the size and number of fast-twitch glycolytic muscle fibers was observed in *CAPN1*-knockout mice [Kemp *et al*. 2013], which suggests that knockout mice exhibit an increased capacity to accumulate and maintain protein in the skeletal muscle. An association study with *CAPN1* variants and hot carcass weight, carcass eye muscle depth, fat thickness and intramuscular fat percentage was reported in Australian crossbreed sheep, but no significant effect was found [Knight *et al*. 2014]. However, a previous study with the *CAPN1* gene in Santa Inês sheep revealed additive effects of some variants on weaning weight, croup and withers heights, body depth as well as ultrasound images of rib eye area and fat thickness [Machado *et al*. 2020].

The *CAST* gene encodes calpastatin, an inhibitor of calpains, with activity closely linked to β-agonist-induced muscle hypertrophy [Smith *et al*. 2000]. Therefore, the *CAST* gene also plays a vital role in muscle growth. Previous studies found *CAST* variants to be associated with average daily gain and fat thickness in Kıvırcık sheep [Yilmaz *et al*. 2014], while association between *CAST* variants and carcass traits in Australian lambs were non-significant [Knight *et al*. 2014]. However, the *CAST* variant *rs418818682* was found to be in association with carcass finishing score measured *in vivo* in Santa Inês sheep [Machado *et al*. 2020].

Although previous association studies have analyzed *CAPN1* and *CAST* genes in sheep [Knight *et al*. 2014, Yilmaz *et al*. 2014, Machado *et al*. 2020], their effects on primal cut yields and several meat quality traits remain unknown in sheep. Thus, the present study aimed to estimate the association between variants in the *CAPN1* and *CAST* genes with carcass and meat quality traits in Santa Inês lambs.

Material and methods

Population and phenotypes

The current study was performed with the approval of the Ethical Committee for Animal Use from the Veterinary Medicine and Animal Science School of Federal

University of Bahia (protocol number 02/2010). The sample population used in the study comprised 192 no full sibling lambs, all males approximately 240 days of age. Of these, 106 lambs born in Embrapa Tabuleiros Costeiros, in Frei Paulo – SE, Brazil, were progenies of seven unrelated sires, with 9 and 17 progenies in the smallest and biggest half-sib (sire) family, respectively. The other 86 lambs were raised in the experimental farm of Federal University of Bahia. For this group no pedigree control was performed, because mating occurred at pastures; however, only unrelated sires were used on that farm. In addition, a principal component analysis using the *CAPN1* and *CAST* variants found no structuration problem in the sample used in the present study [Machado *et al*., 2020]. The animals of both farms were raised on pasture with access to areas containing *Panicum maximum* and received diets formulated to meet the nutritional requirements for lambs with estimated weight gains of 170 g/day, according to the National Research Council [NRC 2007]. Water and mineral salt were available ad libitum on both farms.

Lambs were slaughtered at approximately eight months of age in two abattoirs after a 16-h fasting period, with an average live weight of 36.12 kg and a standard deviation of 4.4 kg. Lambs were slaughtered in four groups. The first three groups (68 lambs in 2010, 15 in 2011, and 17 in 2012) were slaughtered in an abattoir located in the municipality of Propriá, Sergipe State, while the remaining group (86 lambs in 2014) was slaughtered in an abattoir located in the municipality of Feira de Santana, Bahia State. Both abattoirs are under the control of the Federal Sanitary Inspection Service. The animals were slaughtered through cerebral concussion using a non-penetrative method, according to procedures followed by the Sanitary and Industrial Inspection Regulation for Animal Origin Products. Totally, 24 traits were registered (Tab. 1).

Approximately 45 minutes after slaughter the carcasses were weighed to record the hot carcass weight and had the initial pH (pH0) measured. Thereafter, the carcasses were chilled to a final temperature of 3-4°C for 24 h. Approximately 24 h after slaughter the carcasses were weighed again to record the cold carcass weight, while final pH (ph24) was also determined. The pH was measured on the left side of each carcass in the region of the longissimus lumborum muscle (LL) between the 12th and 13th ribs using a Testo 205 pH meter (Testo Instrument Co. LTD., Germany). The pH meter was calibrated before use to pH 7.0 and 4.0 using buffer solutions. Three sequential pH records were obtained at three different points in the LL of each carcass, with the average of this triplicate used as a reference value. The hot and cold carcass yields were calculated as a function of hot and cold carcass weights on body weight at slaughter.

At 24 hours after slaughter, morphometric carcass traits were recorded using a measure tape as follows: internal and external carcass lengths, leg length, carcass width in both the thoracic and rump region, thoracic depth and rump girth. Visual scores from 1 to 5 were used to classify the carcasses for conformation and finishing. Afterwards the carcasses were sectioned in the middle; in the left half portion a transversal cut was made between the 12th and 13th rib for the measurement of the rib eye area. The left half portion of the carcass was subdivided into five sections: palette,

Table 1. Number of recorded individuals (N), mean and standard deviation (SD) of the traits in Santa Inês sheep

Trait	N	Mean	SD
Hot carcass yield (%)	185	44.70	7.25
Cold carcass yield (%)	185	45.07	7.19
Carcass conformation score (cm)	185	2.22	0.35
Carcass finishing score (cm)	185	2.06	0.56
Carcass internal length (cm)	185	60.47	5.78
Carcass external length (cm)	185	64.20	7.34
Leg length (cm)	185	40.21	6.66
Rump girth (cm)	185	61.93	7.49
Carcass width at rump region (cm)	185	22.68	3.75
Carcass width at thoracic region (cm)	178	20.62	2.15
Thoracic depth (cm)	185	25.31	3.57
Palette yield (%)	185	18.80	2.96
Neck yield (%)	185	9.50	1.49
Rib yield (%)	184	28.44	8.33
Loin yield (%)	185	9.73	2.37
Leg yield (%)	176	31.99	6.34
Rib eye area (cm^2)	184	11.33	3.53
pH0	99	6.63	0.18
pH24	99	5.46	0.27
Lightness (L*)	185	44.59	5.51
Redness (a*)	185	20.9	5.69
Yellowness (b*)	185	8.38	2.42
Water-Holding Capacity	99	0.24	0.02
Tenderness measured by shear-force (N)	185	17.55	9.03

neck, rib, leg and loin, which were used to calculate the yields of commercial cuts. Details concerning carcass morphometric traits and primal cuts yields were previously reported [Meira *et al*. 2019].

The LL of each animal was removed from both sides of carcasses, packed and frozen (stored for up to 1 week at -20 °C) until further analyses of color traits, tenderness and water-holding capacity. Three days after slaughter the LL was defrosted at 4°C for 12 h. Then, LL samples were allowed to bloom for 30 min at 4°C, for chromatic characterization [Hunt *et al*., 1991]. A Minolta chromameter (CR400, Minolta Inc., Osaka, Japan) was used to measure the color of each LL sample, which was expressed as CIE/Lab lightness (L*), redness (a*) and yellowness (b*). Black and white reference standards provided by the manufacturer were used to calibrate the chromameter. The light source was set at the D65 standard illuminant with the observer set to 10°. A measuring aperture area of 8 mm was used. Three readings were performed on the cranial end of each LL, the mean value of which was used in the analyses.

After chromameter evaluation, the lumbar section of LL was sliced into steaks of 2.5 cm. A thermocouple probe was then inserted into the geometric center of each steak to monitor the cooking temperature. The steaks were then placed on an electric

grill. When steak temperature reached 40°C, they were turned over and the other side was grilled until it reached 71°C, according to the methodology reported by [Ramos and Gomide 2007]. After steak samples were cooled to room temperature, tenderness evaluations were performed using a shear force test with a Warner Bratzler Shear Force device. Using the cylindrical punch of the device, five cuboidal strips with a diameter of 1.27 cm were removed from the center of the steak samples, perpendicular to the meat fibers (with no fat or nerves). Peak shear force (N) was calculated as the mean of five measurements. Meat samples were also placed on a filter paper to determine their water-holding capacity using the press method [Trout, 1988]. In this method the samples were placed between two acrylic plates with a 10 kg weight placed on this structure for 5 minutes. Subsequently, the difference in weight was used to calculate water loss.

Genotyping

Blood samples (5 ml) were collected from all animals and placed in vacutainer tubes containing EDTA and refrigerated at 4°C. DNA extraction was performed using the salt precipitation method and proteinase K solutions [Oliveira *et al*. 2007]. The primer design for amplification of the *CAPN1* and *CAST* genes fragments was performed based on the gene sequences (ID: 443130) and (ID: 443364), respectively, available in the NCBI (National Center for Biotechnology Information). For the *CAPN1* gene the primers 5′TGTGCTGCGTTTCTTCTCAG3′(forward)and3′AAGGTCACCACTCCATCCAG5′ (reverse) were used to amplify 3,927 bp, located between the positions 42625107 and 42629033. The primers 5′AAAAGCCAAAGAAGAGGATCG3′ (forward) and 3′GGGAAACCACTTCAGAGACG5′ (reverse) were used to amplify a fragment of 4,107 bp of the *CAST* gene, located between the positions 93395596 and 93399703. After PCR the amplified products were sequenced on the MiSeq platform (Illumina, San Diego, CA, USA). In this Santa Inês population, 45 variants in *CAPN1* and 49 in *CAST* were detected. More details concerning PCR conditions, method of sequencing and bioinformatic approach to detect these variants can be found in Machado *et al*. [2020].

Association analysis

The single-locus association analysis was performed with the Qxpak-5 software [Pérez-Enciso and Misztal 2011], which performs a likelihood ratio test. A likelihood ratio test (LRT) was used to compare reduced versus full hierarchical models, where the difference between them was the inclusion of genetic effects (additive and dominance). A higher value of LRT indicates a significant difference between the reduced and full models, where the full model is more suitable to explain the variance of the trait. The univariate general model used can be expressed as:

$$\mathbf{y} = \boldsymbol{\beta}\mathbf{X} + \sum_{k=1}^{n} \mathbf{Z}\boldsymbol{\delta}_k + \boldsymbol{\varepsilon}$$

where:

y – vector containing the records of the trait;

β – vector of the fixed effects;

δ_k – vector of the genetic effects for any of the *n* quantitative trait loci (QTLs) that affect the trait, and is the vector of the errors. **X** and **Z** are the incidence matrices that associate observations in **y** to the vectors in β and δ_k, respectively.

A contemporary group effect was constructed using farm (two levels), year (four levels) and month of birth (12 levels). Moreover, the covariate animal's age was included in the model. The same statistical model was used in haplotype regression analysis, which was performed with the Haplo.stat package version 1.7.7 [Lake *et al*. 2003]. Only haplotype copies with a frequency ≥4% were used. The linkage disequilibrium blocks and the frequencies of haplotype copies were previously reported by Machado *et al*. [2020].

Single-locus analyses were performed with 64 variants, which showed both Hardy-Weinberg equilibrium ($p>0.05$) and minor allele frequency (MAF) >1%. These variants were used to found five linkage disequilibrium blocks in *CAST* and three in *CAPN1*. Thus, nominal p-values of 0.0008 and 0.0063 were calculated using the Bonferroni correction to adjust an overall significance level of 0.05 in single locus analysis and haplotype regression analysis, respectively.

Results and discussion

CAPN1 gene

The estimates of additive and dominance genetic effects of gene variants are listed in Table 2. At the Bonferroni level, no additive effect was found with variants in the *CAPN1* gene ($P>0.05$). However, four variants in this gene (*rs401662939*, *rs407017992*, *rs418468486*, and *rs420860201*) had suggestive additive effects ($P<0.05$) on eight carcass traits (Tab. 2). The genotype frequencies of these variants were as follows: GG (49.2%), GA (39.9%), and AA (10.9%) for the *rs401662939*, TT (65.0%), TC (32.8%), and CC (2.2%) for *rs420860201*, TT (36.6%), TC (41.5 %), CC (21.9%) for *rs418468486*, and CC (61.2%), CT (33.3%), and TT (5.5%) for *rs407017992*. Haplotype regression analysis showed no association ($P>0.05$).

In a previous study concerning Santa Inês sheep, variants in the *CAPN1* gene were associated with weaning weight (*rs417258958*), rib eye area (*rs403953588* and *rs430307080*), fat thickness (*rs408790217*), body depth (*rs420860201*), and heights at croup and withers (*rs408790217*) [Machado *et al*. 2020], but all of these traits were recorded *in vivo*. The present study revealed that variants in these genes are also associated with meat physicochemical traits such as pH, color, tenderness and water-holding capacity. Moreover, our results revealed that important primal cuts, such as rib and leg yields, may also have part of their variation controlled by the calpain/calpastatin system. These results are supported by the key role of μ-calpain and calpastatin on the protein turnover. A lower proteolysis of skeletal muscle proteins

Table 2. Statistically significant estimates of additive (a) and dominance (d) effects of *CAPN1* and *CAST* variants on carcass traits in Santa Inês sheep

Trait	Variant	a (SE)	d (SE)	*p*-value
		CAPN1		
Rib eye area	*rs401662939*	-0.5317 (0.2316)	-	0.023
Water Holding Capacity	*rs407017992*	0.2926 (0.1282)	0.3050 (0.1283)	0.042
Internal carcass length	*rs418468486*	0.6507 (0.3080)	-	0.036
Conformation carcass score	*rs420860201*	0.1053 (0.0469)	-	0.026
Leg length	*rs420860201*	1.0045 (0.4605)	-	0.031
Leg yield	*rs420860201*	-2.4015 (0.8787)	-	0.007
L*	*rs420860201*	-1.4828 (0.6202)	-	0.018
b*	*rs420860201*	-0.5907 (0.2650)	-	0.027
		CAST		
pH0	*rs414639908*	-0.0700 (0.0239)	-	0.001
Rump width	*rs415186098*	1.5625 (0.5245)	-	0.003
Thoracic width	*rs415186098*	2.9299 (1.1052)	-	0.009
L*	*rs415186098*	-0.9409 (0.4757)	-	0.049
a*	*rs415186098*	-0.8445 (0.2554)	-	0.001
b*	*rs415186098*	-0.4414 (0.2074)	-	0.034
Water Holding Capacity	*rs418161864*	0.0203 (0.0051)	-	0.0001#
Neck yield	*rs428230968*	-0.3021 (0.1318)	-	0.023
Finishing carcass score	*rs430517308*	0.1738 (0.0752)	-	0.022

a* – redness; #Significant effect at Bonferroni correction threshold; β – estimate of regression coefficient; SE – standard error of β.

in μ-calpain knockout mice when compared to a no-knockout group was reported [Geesink *et al*., 2006]. Additionally, an increase in the size and number of fast-twitch glycolytic muscle fibers, as well as lower expression of proteins associated with muscle regeneration in knockout mice was observed [Kemp *et al*. 2013].

Only two previous studies tested the association between *CAPN1* variants and phenotypic traits in sheep [Knight *et al*. 2012, Knight *et al*. 2014]. Using targeted next generation sequencing, 1252 animals from an Australian Sheep Nucleus Flock were genotyped for 13 variants in the *CAPN1* gene, but the effects on omega-3 fatty acid content and meat tenderness were non-significant [Knight *et al*. 2012]. Other meat quality traits were not evaluated in this study, but in another study [Knight *et al*., 2014] new traits such as hot carcass weight, carcass eye muscle depth, carcass C-site fat depth and intramuscular fat percentage were evaluated, with no significant effect found.

lthough few association studies with *CAPN1* variants in sheep have been reported, results found in other livestock species revealed effects of variants in this gene on both carcass and meat quality traits. In beef cattle, variants in the *CAPN1* gene were reported in association with some carcass traits [Juszczuk-Kubiak *et al*. 2004, Cheong *et al*. 2008, Hou *et al*. 2011, Barendse 2011]. A variant within intron 14 of the bovine *CAPN1* gene was studied in 141 bulls of seven breeds, with the *TT* genotype showing the lean share in valuable cuts to be more favorable than in the *CC* animals [Juszczuk-Kubiak *et al*. 2004]. In Korean beef cattle, 421 animals were genotyped with 39 variants to test

the association with carcass trait and a variant in the 3'UTR region (*c.2151*479C>T*) showed significant association with marbling score (P=0.0007), but no effect was found on cold carcass weight [Cheong *et al*. 2008]. The variant *3553A>G* in the *CAPN1* gene was associated with marbling score and tenderness in a beef cattle population compound of nine breeds [Hou *et al*. 2011], where *AA* had a higher marbling score than *AG* and *GG* (P<0.05) and higher tenderness value than *GG* (P<0.01). Moreover, a SNP in the *CAPN1* gene was found to be in association with intramuscular fat percentage in a cattle population comprising seven breeds [Barendse 2011].

The effects of *CAPN1* variants are also known in non-ruminant species. In pigs, the variant *rs196951250* in intron 3 was associated with REA in the Polish Large White and FT in Duroc [Ropka-Molik *et al*. 2016]. In chickens haplotype and single-locus association analyses performed with variants in exons 5, 6, and 16 of the *CAPN1* gene found associations with live weight, carcass weight, breast muscle weight, leg muscle weight, eviscerated percentage and breast muscle fiber density [Zhang *et al*. 2008]. Moreover, the variant *ss494474890*, located in intron 5 of the *CAPN1* gene, was associated with BW at 35 and 42 days of age, thigh, breast and carcass weights in a chicken F2 population [Felício *et al*. 2013].

CAST gene

Regarding the *CAST* gene, only the variant *rs418161864* showed association at the Bonferroni level (P<0.0063). This variant is in intron 20 and had an additive effect on water-holding capacity (Tab. 2). Moreover, four other variants (*rs414639908*, *rs415186098*, *rs428230968*, and *rs430517308*) in intron 20 showed suggestive additive effects (P < 0.05) on some carcass trait. Genotype frequencies of these variants were as follows: CC (79.0%), CG (19.4%), and GG (1.6%) for rs418161864, TT (18.9%), TC (49.7%), and CC (31.4%) for *rs428230968*, CC (61.1%), CT (30.9%), and TT (7.9%) for *rs415186098*, TT (81.1%), TC (16.2%), and CC (2.6%) for *rs430517308*, and GG (35.1 %), GA (47.6%), and AA (17.3%) for rs414639908. Additionally, three linkage disequilibrium blocks in *CAST* were found to be in association (P<0.05) with some traits (Tab. 3), being three regression coefficients significant.

The block-1 is 200 bp long and includes the variants (*rs413442067*, *rs424912630*, *rs403339381*, *rs414639908*, and *rs425997700*), which formed three haplotype copies with frequencies higher than 5% as follows: *GGGGA* (45.3%), *GGGAA* (40.3%), *AAAGG* (5.8%). The replacement *GGGGA>AAAGG* was associated with a lower water-holding capacity. The block-2 is 556 bp long and includes the variants (*rs406915912*, *rs422402447*, *rs419473804*, *rs399204438*, and *rs425885251*), which formed four haplotype copies with frequencies higher than 5% as follows: *CTAAT* (38.2%), *TCGGC* (28.1%), *TCAGC* (20.0%), and *TCAAT* (5.4%). The replacement *CTAAT>TCGGC* was associated with a higher value of meat redness (a*). The block-3 is 290 bp and includes the variants *rs411571641*, *rs422744326*, *rs418161864*, *rs403866848*, and *rs415186098*, which formed the haplotype copies as follows: *CGCCC* (49.7%), *TGCCT* (22.7%), *TGCCC* (12.9%), and *TAGTC* (10.2%). The replacement *CGCCC>TAGTC*

Table 3. The effect of haplotype substitution in the *CAST* gene on carcass traits in Santa Inês sheep

Trait	Haplotype substitution	Linkage block	β	SE	*p*-value
Water holding capacity	GGGGA>AAAGG	1	-0.0094	0.0032	0.003#
a*	CTAAT>TCGGC	2	0.6422	0.3219	0.048
External carcass length	CGCCC>TAGTC	3	-1.491	0.733	0.043
Rump girth	CGCCC>TAGTC	3	-1.781	0.866	0.041
Rib yield	CGCCC>TAGTC	3	-2.332	0.801	0.004#
Water holding capacity	CGCCC>TAGTC	3	-0.0062	0.0026	0.019
External carcass length	CGCCC>TGCCC	3	-1.977	0.770	0.011
Internal carcass length	CGCCC>TGCCC	3	-1.223	0.597	0.042
Rib yield	CGCCC>TGCCC	3	-2.029	0.841	0.017
pH0	CGCCC>TGCCC	3	0.4670	0.2345	0.048
pH24	CGCCC>TGCCC	3	0.4327	0.2034	0.035
a*	CGCCC>TGCCT	3	0.8973	0.3093	0.004#

a* – redness; #Significant effect at Bonferroni correction threshold; β – estimate of regression coefficient; SE – standard error of β.

was associated with lower values of external carcass length, rump girth, rib yield and water-holding capacity; the replacement *CGCCC>TGCCC* was associated with lower values of external carcass length, internal carcass length and rib yield, as well as higher values of both pH0 and pH24; while the replacement *CGCCC>TGCCT* was associated with higher values of meat redness (a*).

Calpastatin is a specific inhibitor of calpain and some variants in the *CAST* gene have been essentially associated with meat tenderness in some domestic species, including sheep [Knight *et al*. 2014]. There are also several reports of *CAST* variants associated with growth traits in sheep [Dehnavi *et al*. 2012, Yilmaz *et al*. 2014, Gorlov *et al*. 2016, Jawasreh *et al*. 2017]. In Santa Inês sheep, Machado *et al*. [2020] reported the effect of *CAST* variants on growth traits such as weaning weight and average daily gain, which generated the hypothesis that carcass attributes were also associated with *CAST* variants. The results of the current study confirmed this hypothesis.

In addition, our results support previous findings concerning other sheep breeds. For instance, the variant *rs430517308* showed an additive effect on carcass finishing score in Santa Inês sheep, while a previous study on Kivircik lambs reported a PCR-RFLP in the *CAST* gene to be associated with fat thickness, where the genotypes *MM* and *MN* were characterised by greater fat thickness than the *NN* genotype [Yilmaz *et al*. 2014]. Moreover, an intronic variant *rs404358363* in the *CAST* gene was also associated with fat thickness in Texel sheep [Armstrong *et al*. 2018]. Moreover, the variant *rs415186098* was associated with meat lightness (L*) in Santa Inês sheep, while a PCR-RFLP in the *CAST* gene were found to be in association with this trait in Awassi sheep [Jawasreh *et al*. 2017].

Variants in the *CAST* gene were also found to be in association with meat and carcass quality traits in other livestock species. In beef cattle, *CAST* variants were

associated with meat tenderness [Li *et al*. 2010, Enriquez-Valencia *et al*. 2017]. In pigs, a PCR-RFLP polymorphism in the intron-6 of the *CAST* gene was associated with fat thickness, shoulder weight, carcass length and loin weight [Krzecio *et al*. 2008]. The *CAST/RsaI* polymorphism was found to be in association with meat content in carcass, ultimate pH and cooking yield in pigs [Boruszewska *et al*. 2016]. The single nucleotide polymorphism *g.16443397T>G* located in intron 8 in the Hyla, Champagne and Tianfu Black rabbit breeds was found to be in association with pH and intramuscular fat in the longissimus dorsi and biceps femoris muscles [Wang *et al*. 2016]. Rabbits with the *GG* and *TT* genotypes had lower pH and intramuscular fat content than *GT* rabbits. Therefore, there is evidence of *CAST* variants associated with carcass and meat quality traits in multiple livestock species.

The current study revealed that variants in the *CAPN1* and *CAST* genes are associated with meat and carcass traits in Santa Inês sheep and that these variants may be used to improve these traits. However, it is recommended that more studies be conducted to validate the effects found here. In addition, while the current study sequenced long fragments of both *CAPN1* and *CAST* genes, many regions of these genes have not been explored here. Therefore, other regions of these genes need to be investigated to identify the causal variant.

Acknowledgments. *To Dr. Luiz Lehmann Coutinho for providing access to the infrastructure of Functional Genomics Core facility of ESALQ/USP.*

REFERENCES

1. AMARANTE A.F.T., BRICARELLO P.A., ROCHA R.A., GENNARI R.M., 2004 – Resistance of Santa Inês, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. ***Veterinary Parasitology*** 120, 91-106.
2. ARMSTRONG E., CIAPPESONI G., IRIARTE W., DA SILVA C., MACEDO F., NAVAJAS E.A., BRITO G., SAN-JULIÁN R., GIMENO D., POSTIGLIONI A., 2018 – Novel genetic polymorphisms associated with carcass traits in grazing Texel sheep. ***Meat Science*** 145, 202-208.
3. BALARO M.F.A., FONSECA J.F., OBA E., CARDOSO E.C., BRANDÃO, F.Z., 2014 – Is the Santa Inês sheep a typical non-seasonal breeder in the Brazilian Southeast? ***Tropical Animal Health and Production*** 46, 1533-1537.
4. BARENDSE W., 2011 – Haplotype analysis improved evidence for candidate genes for intramuscular fat percentage from a genome wide association study of cattle. ***Plos One*** 6, e29601.
5. BORUSZEWSKA K., PRZYBYLSKI W., GRZYWACZ L., JAWORSKA D., ŻELECHOWSKA E., TERMAN A., 2016 – Effect of calpastatin gene (CAST) polymorphism on pork quality. ***Animal Science Papers and Reports*** 34, 61-72.
6. CHEONG H.S., YOON D.H., PARK B.L., KIM L.H., BAE J.S., NAMGOONG S., LEE H.W., HAN C.S., KIM J.O., CHEONG I.C., SHIN H.D., 2008 – A single nucleotide polymorphism in CAPN1 associated with marbling score in Korean cattle. ***BMC Genetics*** 9, 33.
7. DEHNAVI E., AZARI M.A., HASANI S., NASSIRY M.R., MOHAJER M., AHMADI A.R.K., SHAHMOHAMADI L., YOUSEFI S., 2012 – Association between yearling weight and calpastatin and calpain loci polymorphism in Iranian Zel sheep. ***Iranian Journal of Applied Animal Science*** 2, 131-135.

8. ENRIQUEZ-VALENCIA C.E., PEREIRA G.L., MALHEIROS J.M., SILVA J.A.I.I.V., ALBUQUERQUE L.G., OLIVEIRA H.N., CHARDULO L.A.L., CURI R.A., 2017 – Effect of the g.98535683A>G SNP in the CAST gene on meat traits of Nellore beef cattle (Bos indicus) and their crosses with Bos taurus. ***Meat Science*** 123, 64-66.
9. FELÍCIO A.M., BOSCHIERO C., BALIEIRO J.C.C., LEDUR M.C., FERRAZ J.B.S., MICHELAN-FILHO T., MOURA A.S.A.M.T., COUTINHO L.L., 2013 – Identification and association of polymorphisms in CAPN1 and CAPN3 candidate genes related to performance and meat quality traits in chickens. ***Genetics and Molecular Research*** 12, 472-482.
10. GEESINK G.H., KUCHAY S., CHISHTI A.H., KOOHMARAIE M., 2006 – Micro-calpain is essential for postmortem proteolysis of muscle proteins. ***Journal of Animal Science*** 84, 2834-2840.
11. GORLOV I.F., SHIROKOVA N.V., RANDELIN A.V., VORONKOVA V.N., MOSOLOVA N.I., ZLOBINA E.Y., KOLOSOV Y.A., BAKOEV N.F., LEONOVA M.A., BAKOEV S.Y., KOLOSOV A.Y., GETMANTSEVA L.V., 2016 – CAST/MspI gene polymorphism and its impact on growth traits of Soviet Merino and Salsk sheep breeds in the South European part of Russia. **T*urkish Journal of Veterinary and Animal Sciences*** 40, 399-405.
12. HOU G., HUANG M., GAO X., LI J., GAO H., REN H., XU S., 2011 – Association of Calpain 1 (CAPN1) and HRSP12 allelic variants in beef cattle with carcass traits. ***African Journal of Biotechnology*** 10, 13714-13718.
13. HUNT M.C., ACTON J.C., CALKINS C.R., JEREMIAH L.E., SALM C.P., SAVELL J.W., SHIVAS S.D., 1991 – Guidelines for meat color evaluation. Chicago, USA: National livestock and meat board.
14. JAWASREH K.I., JADALLAH R., AL-AMAREEN A.H., ABDULLAH A.Y., AL-QAISI A., ALRAWASHDEH I.M., AL-ZGHOUL M.B.F., AHAMED M.K.A., OBEIDAT B., 2017 – Association between MspI calpastatin gene polymorphisms, growth performance, and meat characteristics of Awassi sheep. ***Indian Journal of Animal Sciences*** 87, 635–639.
15. JUCÁ A.F., FAVERI J.C., MELO-FILHO G.M., RIBEIRO-FILHO A.L., AZEVEDO H.C., MUNIZ E.N., PEDROSA V.B., PINTO L.F.B., 2016 – Effects of birth type and family on the variation of carcass and meat traits in Santa Inês sheep. ***Tropical Animal Health and Production*** 48, 435-443.
16. JUSZCZUK-KUBIAK E., SAKOWSKI T., FLISIKOWSKI K., WICIÑSKA K., OPRZADEK J., ROSOCHACKI S.J., 2004 – Bovine u-calpain (CAPN1) gene: new SNP within intron 14. ***Journal of Applied Genetics*** 45, 457-460.
17. KEMP C.M., OLIVER W.T., WHEELER T.L., CHISHTI A.H., KOOHMARAIE M., 2013 – The effects of CAPN1 gene inactivation on skeletal muscle growth, development, and atrophy, and the compensatory role of other proteolytic systems. ***Journal of Animal Science*** 91, 3155-3167.
18. KNIGHT M.I., DAETWYLER H.D., HAYES B.J., HAYDEN M.J., BALL A.J., PETHICK D., MCDONAGH M.B., 2014 – An independent validation association study of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers in Australian lamb. ***Meat Science*** 96, 1025-1033.
19. KNIGHT M.I., DAETWYLER H.D., HAYES B.J., HAYDEN M.J., BALL A.J., PETHICK D.W., MCDONAGH M.B., 2012 – Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. ***Animal Production Science*** 52, 591-600.
20. KRZECIO E., KÓCWIN-PODSIADŁA M., KURYŁ J., ZYBERT A., SIECZKOWSKA H., ANTOSIK K., 2008 – The effect of interaction between genotype CAST/RsaI (calpastatin) and MYOG/MspI (myogenin) on carcass and meat quality in pigs free of RYR1T allele. ***Meat Science*** 80, 1106-1115.
21. LAKE S.L., LYON H., TANTISIRA K., SILVERMAN E.K., WEISS S.T., LAIRD N.M., SCHAID D.J., 2003 – Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. ***Human Heredity*** 55, 56-65.

22. LI J., ZHANG L.P., GAN Q.F., LI J.Y., GAO H.J., YUAN Z.R., GAO X., CHEN J.B., XU S.Z., 2010 – Association of CAST Gene Polymorphisms with Carcass and Meat Quality Traits in Chinese Commercial Cattle Herds. Asian-**Australian Journal of Animal Science** 23, 1405-1411.
23. MACHADO A.L., MEIRA A.N., MUNIZ E.N., AZEVEDO H.C., COUTINHO L.L., MOURÃO G.B., PEDROSA V.B., PINTO L.F.B., 2020 – Single loci and haplotypes in CAPN1 and CAST genes are associated with growth, biometrics, and in vivo carcass traits in Santa Inês sheep. ***Annals of Animal Science*** https://doi.org/10.2478/aoas-2020-0007.
24. MCMANUS C., PALUDO G.R., LOUVANDINI H., GUGEL R., SASAKI L.C.B., PAIVA S.R., 2009 – Heat tolerance in Brazilian sheep: physiological and blood parameters. ***Tropical Animal Health and Production*** 41, 95-101.
25. MEIRA A.N., MONTENEGRO H., COUTINHO L.L., MOURÃO G.B., AZEVEDO H.C., MUNIZ E.N., MACHADO A.L., SOUSA-JR L.P., PEDROSA V.B., PINTO L.F.B., 2019 – Single nucleotide polymorphisms in the GH and IGF1 genes associated with carcass traits in Santa Inês sheep. ***Animal*** 13, 460-468.
26. NRC – National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids (2007) Washington, DC: The National Academy Press.
27. OLIVEIRA M.C.D.S., REGITANO L.C.A., ROESE A.D., ANTHONISEN D.G., PATROCINIO E., PARMA M.M., SCAGLIUSI S.M.M., TIMOTEO W.H.B., BELICUAS S.N.J., 2007 – Fundamentos teórico-práticos e protocolos de extração e de amplificação de DNA por meio da técnica de reação em cadeia da polimerase. São Carlos, SP: EMBRAPA. (in Portuguese).
28. PÉREZ-ENCISO M., MISZTAL I., 2011 – Qxpak5: Old mixed model solutions for new genomics problems. ***BMC Bioinformatics*** 12, 202.
29. RAMOS E.M., GOMIDE L.A.M., 2007 – Avaliação da qualidade de carnes: fundamentos e metodologia. 1. ed. Viçosa, MG: Universidade Federal de Viçosa.(in Portuguese).
30. ROPKA-MOLIK K., ECKERT R., TYRA M., PIÓRKOWSKA K., OCZKOWICZ M., SZYNDLER-NĘDZA M., BERETA A., 2016 – CAPN1 gene as a potential marker for growth performance and carcass characteristics in pigs. ***Animal Production Science*** 57, 1014-1021.
31. SMITH T.P.L., CASAS E., REXROAD C.E. 3RD, KAPPES S.M., KEELE J.W., 2000 – Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. ***Journal of Animal Science*** 78, 2589-2594.
32. TROUT G.R., 1988 – Techniques for measuring water-binding capacity in muscle foods - A review of methodology. ***Meat Science*** 23, 235-252.
33. WANG J., ELZ M.A., YAN L., CHEN S., JIA X., ZHANG M., LAI S., 2016 – A single nucleotide polymorphism in CAST gene is associated with meat quality traits in rabbits. ***Animal Science Papers and Reports*** 34, 269-278.
34. YILMAZ O., CEMAL I., KARACA O., ATA N., 2014 – Association of Calpastatin (CAST) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kıvırcık Lambs. Kafkas Universitesi Veteriner Fakultesi Dergisi 20, 675-680.
35. ZHANG Z.R., LIU Y.P., JIANG X., DU H.R., ZHU Q., 2008 – Study on association of single nucleotide polymorphism of CAPN1 gene with muscle fibre and carcass traits in quality chicken populations. ***Journal of Animal Breeding and Genetics*** 125, 258-64.