GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Identification and association of polymorphisms in *CAPN1* and *CAPN3* candidate genes related to performance and meat quality traits in chickens

A.M. Felício[1], C. Boschiero[2], J.C.C. Balieiro[3], M.C. Ledur[4], J.B.S. Ferraz[3], T. Michelan Filho[5], A.S.A.M.T. Moura[6] and L.L. Coutinho[1]

[1]Departamento de Zootecnia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil
[2]Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, Edinburgh, Scotland
[3]Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil
[4]Embrapa Suínos e Aves, Concórdia, SC, Brasil
[5]Aviagen do Brasil Ltda., Rio Claro, SP, Brasil
[6]Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brasil

Corresponding author: L.L. Coutinho
E-mail: llcoutinho@usp.br

Genet. Mol. Res. 12 (1): 472-482 (2013)
Received May 3, 2012
Accepted November 26, 2012
Published February 8, 2013
DOI http://dx.doi.org/10.4238/2013.February.8.12

ABSTRACT. Meat quality is an important feature for the poultry industry and is associated with consumer satisfaction. The calpain 1 (*CAPN1*) gene is related to the tenderness process of meat post-mortem, and the calpain 3 (*CAPN3*) gene plays an important role in

myofibrillar organization and growth. The objective of the present study was to identify polymorphisms in these genes and to determine the association between these polymorphisms and traits of economic interest in poultry. Eleven animals (F_1) from an experimental poultry population at Embrapa Swine and Poultry were used to identify the polymorphisms. Four single nucleotide polymorphisms (SNPs) were found in the *CAPN1* gene, and one SNP was found in the *CAPN3* gene. A polymorphism from each gene was selected for genotyping in 152 chickens from the Embrapa F_2 experimental population and 311 chickens from a commercial population. Polymorphism g.2554T>C (*CAPN1*) was associated with body weight at 35 to 42 days, thigh weight, breast weight, carcass weight, and meat lightness content. SNP g.15486C>T (*CAPN3*) was associated with thigh yield, thawing-cooking loss, and shear force. Results suggest the possibility of using molecular markers in *CAPN1* and *CAPN3* genes as a tool for performance and meat quality traits in poultry breeding programs.

Key words: Calpain; Genetic marker; Poultry; Shear force

INTRODUCTION

Genetic improvement has been crucial for production efficiency and economic growth in agriculture. This is no less true for the poultry industry. Over the last few decades, the poultry industry has shown a high rate of genetic progress.

Success of chicken meat production is strongly related to improvements in growth and carcass yield. The poultry market has excelled in the production of noble carcass parts and processed products (Le Bihan-Duval et al., 2003). Therefore, intense selection for these traits may have also led to changes in meat quality attributes, such as tenderness in particular (Dransfield and Sosnicki, 1999).

According to Park et al. (2002), meat quality traits are essential for the processing industry and end consumers. As a result, these qualitative traits have been widely studied in breeding programs.

The use of genomics in animal production has allowed important advances in the quality of animal products by increasing the efficiency in crosses and in recognition of animal physiology (Hocquette et al., 2007).

The advancement of biotechnology has brought new methods to poultry research. Today, single nucleotide polymorphisms (SNPs) are extensively used in poultry breeding research programs. SNPs can be used as genetic markers to track inheritance patterns of chromosomal regions across generations. They are also considered effective tools in the study of genetic factors associated with traits of economic interest (Brookes, 1999). SNP markers are crucial in the discovery of new strategies for quantitative trait locus mapping and candidate gene studies.

In the present study, the calpain 1 (*CAPN1*) and calpain 3 (*CAPN3*) genes were selected based on previous findings demonstrating that these genes are related to performance,

carcass, muscle development, and meat quality traits.

The *CAPN1* gene encodes a cysteine protease protein that degrades myofibrillar proteins post-mortem; it is considered to be the primary enzyme involved in the post-mortem meat tenderness process (Koohmaraie, 1996). According to the National Center for Biotechnology Information database (NCBI, 2012), there are 118 SNPs in the *CAPN1* gene in chickens.

The *CAPN3* gene is primarily related to muscular dystrophy and growth and plays an important role in myofibrillar integrity (Poussard et al., 1996). This gene is specifically linked to connective muscle in regions where proteolysis has been associated with post-mortem meat tenderness (Taylor et al., 1995). One hundred and fifty-two SNPs have already been described in chickens (NCBI public database, 2012).

The purposes of the present study were 1) to identify polymorphisms in the *CAPN1* and *CAPN3* candidate genes, and 2) to study the association of these SNPs with traits of performance, carcass, muscle development, and meat quality in an experimental chicken population and in a commercial broiler population.

MATERIAL AND METHODS

Populations

In this study, we used 2 populations of chickens, an experimental F_2 population (Embrapa Swine and Poultry) and a commercial broiler population. The experimental population was an F_2 line (TCTC) developed by Embrapa Swine and Poultry through crosses between males from the broiler line (TT) and females of the layer line (CC). CC is a layer line breed of White Leghorn and the TT is a broiler line originated from the breeds White Plymouth Rock, New Hampshire, and White Cornish. Rosário et al. (2009) previously described these 2 lines (TT and CC) in detail. To study the commercial population, a database of an elite commercial flock (also called pedigree flock) of broilers from a breeding company was used. This commercial population was described in detail by Gaya et al. (2006, 2011).

Phenotypes and DNA extraction

Details of the F_2 Embrapa population and phenotypic traits evaluated were described by Nones et al. (2006).

Genomic DNA from birds of the F_1 and F_2 experimental populations was extracted from blood samples using the DNAzol reagent (Life Technologies, Carlsbad, CA, USA).

In the commercial population, we evaluated the following traits: selection body weight at 38 days, slaughter body weight, eviscerated carcass weight (EBW), breast weight without bones and skin (BRT), leg weight (LW), pH 24 h after slaughter, colorimetric values measured 24 h after slaughter [L* (lightness), a* (redness content), b* (yellowness content)], drip loss (DL), thawing loss (TL), thawing-cooking loss (TCL), total water loss (TWL), muscle fiber diameter, muscle fiber area, muscle fiber number per histological field, and shear force (SF). These characteristics were evaluated in the pectoralis major muscle in the skull-ventral portion. From the fragments of muscular tissue embedded in paraffin, genomic DNA was extracted by histological sectioning, and then a lysis solution (200 mM

NaOH 200) and a neutralizing solution (200 mM HCl 200, 100 mM Tris-HCl, pH 8.5) were added. The samples were stored at -18°C.

Selection of candidate genes

The *CAPN1* and *CAPN3* genes were selected based on previous functional evidence indicating that these genes are associated with performance, carcass, muscle development, and meat quality traits.

PCR conditions and sequencing

A pair of primers were designed for each gene to amplify exon and intron regions of chicken sequences of the *CAPN1* and *CAPN3* genes deposited in GenBank (accession Nos. NC_006090.3 and NC_006092.3; NCBI, 2012) using the Primer3 program (http://frodo.wi.mit.edu/). The quality of these primers was analyzed using the NetPrimer program (http://www.premierbiosoft.com/netprimer).

Gene fragments were amplified by PCR using 20 ng genomic DNA, 2.5 µL 10X buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.5), 0.3 mM $MgCl_2$, 0.4 mM dNTP, 2 pmol of each primer, and 1 U Platinum *Taq* DNA polymerase (Life Technologies) in a final volume of 25 µL. Fragment amplification was performed as follows: initial denaturation at 95°C for 1 min, 30 cycles of 95°C for 1 min, the specific annealing temperature for each primer pair for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The amplified fragments were evaluated on an agarose gel (1%).

PCR products were purified and sequenced according to the protocol of the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Life Technologies). Sequencing reactions were purified and applied in the automated ABI PRISM 3100 Genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were edited, assembled, and analyzed using the Phred, Phrap, and Consed computer programs, respectively (Ewing et al., 1998; Gordon et al., 1998).

Genotyping of polymorphisms

After detecting the polymorphisms in the Embrapa F_1 animal population, 2 SNPs, 1 of each gene (*CAPN1* and *CAPN3*), were selected based on 2 more informative full-sib families (heterozygous genotypes) to genotype 152 F_2 animals from the Embrapa population and 311 broilers from the commercial population using allelic discrimination via the TaqMan detection system (Applied Biosystems).

Reactions were performed in a LightCycler 480 System II (Roche, Mannheim, Germany), using the endpoint genotyping method. Results were analyzed using the LightCycler 480 SW 1.5 software (Roche).

Statistical analysis

Allelic and genotypic frequencies were estimated for each locus by simple counting of alleles and different genotypes, respectively, as described by Falconer and Mackay (2001).

To assess the implications of molecular results on all of the relevant traits in chickens, individual analyses were used, and each trait was considered to be a dependent vari-

able. Genotypic effects found for both markers (*CAPN1* and *CAPN3*) were evaluated from the general model presented below:

$$Y_{ijkl} = \mu + C_i + S_j + M_k + e_{ijkl}$$

where Y_{ijkl} is the phenotypic value observed for each trait; μ is a constant inherent to all observations; C_i is the fixed effect of contemporaneous groups; S_j is the random effect of male j with average 0 and variance σ^2_s; M_k is the fixed effect of the genotype for each marker of the SNPs that were evaluated (*CAPN1* and *CAPN3*); e_{ijkl} is the residual random effect associated with trait Y_{ijkl}, with average 0 and σ^2_e.

According to Falconer and Mackay (2001), estimates of additive effects (α) for each polymorphism can be obtained from the difference between the averages of homozygous genotypes, as the deviations attributable to dominance (δ) are estimated by the difference between the mean of the heterozygous genotype in relation to the average of the 2 homozygous genotypes. Estimates of additive effects and deviations attributed to dominance were assessed using a Student *t*-test ($P < 0.05$ was considered to be significant).

Estimates of medium effects of allele substitution for the polymorphisms evaluated in terms of genotypes were calculated using linear regression analyses, considering the number of favorable alleles (Falconer and Mackay, 2001).

All analyses were performed using PROC FREQ and PROC MIXED functions of Statistical Analysis System version 9.2 (SAS Institute, Cary, NC, USA, 2004).

RESULTS AND DISCUSSION

Embrapa experimental population

Identification of polymorphisms in the CAPN1 and CAPN3 genes

Sequencing of target regions identified 5 polymorphic sites in the 2 populations that were studied. Four polymorphisms were identified in intron 5 of the *CAPN1* gene (GGA3): g.2456T>A (ss494474888), g.2460C>T (ss494474889), g.2554T>C (ss494474890), and g.2629C>T (ss494474891). SNP g.2554T>C was selected for association studies. Only 1 polymorphism, g.15486C>T (ss494474891) located in exon 3, was identified in the *CAPN3* gene (GGA5). This polymorphism is a synonymous mutation, and no amino acid change was detected.

In chickens, 118 and 152 SNPs have been described in *CAPN1* and *CAPN3* genes, respectively (NCBI, 2012). The new SNPs identified in this study were deposited in the NCBI SNP database (dbSNP -http://www.ncbi.nlm.nih.gov/snp/).

Descriptive phenotype, genotype and allele frequencies

The descriptive statistics (number of observations, mean estimates, standard deviations, coefficients of variation, and the minimum and maximum values) of the phenotypic traits are presented in Table 1. Results from the F_2 population were highly variable for the traits under study, which was expected given that this population originated from a cross between a broiler line and a layer line.

Table 1. Descriptive information of phenotypic traits evaluated in the Embrapa F_2 population.

Trait	N	Mean	SD	CV	Minimum	Maximum
BW35 (g)	152	788.20	123.52	15.67	554.00	1085.00
BW41 (g)	152	1001.92	170.80	17.05	578.00	1398.00
BW42 (g)	152	963.82	169.91	17.63	549.00	1391.00
Drums and thighs (g)	152	206.15	41.89	20.32	109.00	329.00
Breast (g)	152	157.66	30.96	19.64	77.00	237.00
Carcass (g)	152	625.61	116.32	18.59	341.00	944.00
Yield of drums and thighs (%)	152	21.32	1.35	6.34	18.31	31.40

BW35 = body weight at 35 days; BW41 = body weight at 41 days; BW42 = body weight at 42 days; Drums and thighs = weight of drums and thighs; Breast = breast weight with skin and bones; Carcass = carcass weight obtained from the sum of carcass parts; N = number of observations; SD = standard deviations; CV = coefficients of variation.

Genotypic and allelic frequencies associated with SNPs g.2554T>C (*CAPN1*) and g.15486C>T (*CAPN3*) are presented in Table 2.

Table 2. Genotypic and allelic frequencies of polymorphisms g.2554T>C (*CAPN1*) and g.15486C>T (*CAPN3*) in chicken.

Polymorphism	Genotypic frequencies			Allelic frequencies	
g.2554T>C	f(TT) = 30.20	f(TC) = 46.31	f(CC) = 23.49	f(T) = 0.53	f(C) = 0.47
g.15486C>T	f(CC) = 11.54	f(CT) = 53.85	f(TT) = 34.62	f(C) = 0.38	f(T) = 0.62

SNP association

Association results of SNPs in the *CAPN1* and *CAPN3* genes with traits evaluated in the experimental population are shown in Tables 3 and 4, respectively. For SNP g.2554T>C, additive, dominance, and allelic substitution effects ($P < 0.05$) were observed for all traits described in Table 3, with exception of the yield of drums and thighs, which exhibited a significant effect only for dominance ($P < 0.05$). SNP g.15486C>T showed significant additive and allelic substitution effects on yield of drums and thighs. Animals with the TT genotype had 0.90% higher yield of drums and thighs compared to those with the CC genotype (Table 4).

Table 3. Estimates of additive effects (α), deviation of additivity (δ) and average effects of allele substitution (β_{li}) of phenotypic traits of SNP g.2554T>C (*CAPN1*) in the Embrapa F_2 population.

Trait	Additive effect			Deviation of additivity			Allele substitution effect		
	a	SE	P > |t|	δ	SE	P > |t|	β_{1i}	SE	P > |t|
BW35 (g)	72.48	22.99	0.002**	-53.53	18.19	0.004**	30.46	11.68	0.01*
BW41 (g)	91.55	27.98	0.001**	-65.51	22.20	0.004**	39.11	14.22	0.01*
BW42 (g)	89.66	27.80	0.002**	-64.93	22.05	0.004**	38.24	14.12	0.01*
Drums and thighs (g)	19.07	7.11	0.01*	-18.76	5.64	0.001**	7.63	3.64	0.04*
Breast (g)	18.26	5.77	0.002**	-11.01	4.58	0.02*	8.02	2.90	0.01*
Carcass (g)	60.27	19.88	0.003**	-46.85	15.77	0.004**	25.39	10.11	0.01*
Yield of drums and thighs (%)	-0.07	0.27	0.81	-0.45	0.22	0.04*	-0.08	0.14	0.57

*P < 0.05; **P < 0.001. BW35 = body weight at 35 days; BW41 = body weight at 41 days; BW42 = body weight at 42 days; Drums and thighs = weight of drums and thighs; Breast = breast weight with skin and bones; Carcass = carcass weight obtained from the sum of carcass parts.

Table 4. Estimates of additive effects (α), deviation of additivity (δ) and average effects of allele substitution (β_{li}) of phenotypic traits of SNP g.15486C>T (*CAPN3*) in the Embrapa F_2 population.

Trait	Additive effect			Deviation of additivity			Allele substitution effect		
	a	SE	P > |t|	δ	SE	P > |t|	β_{li}	SE	P > |t|
BW35 (g)	42.17	31.28	0.18	26.46	18.04	0.14	10.90	14.24	0.45
BW41 (g)	46.33	41.63	0.27	20.39	23.63	0.39	15.42	18.80	0.41
BW42 (g)	46.46	40.90	0.26	20.78	23.25	0.37	15.33	18.48	0.41
Drums and thighs (g)	21.09	10.42	0.05	6.18	5.94	0.30	8.21	4.71	0.08
Breast (g)	6.29	8.27	0.45	2.24	4.72	0.64	2.29	3.73	0.54
Carcass (g)	36.98	28.84	0.20	14.13	16.41	0.39	13.12	13.03	0.32
Yield of drums and thighs (%)	1.02	0.36	0.01*	0.17	0.22	0.44	0.45	0.16	0.01*

*P < 0.05. BW35 = body weight at 35 days; BW41 = body weight at 41 days; BW42 = body weight at 42 days; Drums and thighs = weight of drums and thighs; Breast = breast weight with skin and bones; Carcass = carcass weight obtained from the sum of carcass parts.

Commercial population

A commercial population was used to investigate the SNPs identified in the Embrapa F_2 population. Genotypes of the commercial animals were based on 2 polymorphisms selected in the *CAPN1* and *CAPN3* genes from the experimental population.

Descriptive phenotype, genotype and allele frequencies

The descriptive statistics (number of observations, mean estimates, standard deviations, coefficients of variation, and the minimum and maximum values) of the phenotypic traits are presented in Table 5. Genotypic and allelic frequencies of the 2 polymorphisms g.2554T>C (*CAPN1*) and g.15486C>T (*CAPN3*) evaluated in the commercial population are presented in Table 6.

Table 5. Descriptive information of the traits evaluated in the commercial population.

Trait	N	Mean	SD	CV	Minimum	Maximum
SW (g)	310	1528.90	160.00	10.46	970.00	1980.00
SBW (g)	310	3101.37	278.09	8.97	1231.00	3753.00
EBW (g)	310	2214.41	196.59	8.88	1318.00	2762.00
BRT (g)	310	641.76	81.71	12.73	356.00	892.00
LW (g)	310	757.13	75.81	10.01	358.00	918.00
pHf	311	6.18	0.20	3.31	5.44	6.83
DL (%)	310	2.20	0.75	34.35	0.96	6.27
TL (%)	310	3.92	2.75	70.16	0.28	28.36
TCL (%)	311	14.13	4.01	28.41	4.63	25.16
TWL (%)	311	20.19	5.68	28.10	8.63	56.18
L*	308	54.98	3.29	5.98	44.61	63.90
a*	308	5.94	1.24	20.92	3.42	10.42
b*	308	14.41	1.57	10.92	10.35	20.16
MFA (μm^2)	311	1791.91	546.33	30.49	776.35	3714.60
MFD (μm)	311	46.50	6.95	14.95	31.11	67.92
MFN	311	9.60	3.40	35.46	2.00	19.50
SF (kgf)	311	1.59	0.51	31.98	0.50	4.00

SW = selection body weight at 38 days; SBW = slaughter body weight; EBW = eviscerated carcass weight; BRT = breast weight; LW = leg weight; pHf = pH 24 h after slaughter; DL = drip loss; TL = thawing loss; TCL = thawing-cooking loss; TWL = total water loss; L* = lightness content; a* = redness content; b*= yellowness content; MFA = muscle fiber area; MFD = muscle fiber diameter; MFN = muscle fiber number; SF = shear force; N = number of observations; SD = standard deviation; CV = coefficients of variation.

Table 6. Genotypic and allelic frequencies of polymorphisms g.2554T>C (*CAPN1*) and g.15486C>T (*CAPN3*) in the commercial population.

Polymorphism	Genotypic frequencies			Allelic frequencies	
g.2554T>C	f(TT) = 24.44	f(TC) = 49.84	f(CC) = 25.72	f(T) = 0.49	f(C) = 0.51
g.15486C>T	f(CC) = 75.00	f(CT) = 21.10	f(TT) = 3.90	f(C) = 0.86	f(T) = 0.14

SNP association

Estimates of additive, deviation of additivity and allelic substitution effects for polymorphism g.2554T>C in the *CAPN1* gene are presented in Table 7. Allelic substitution of SNP g.2554T>C (*CAPN1*) had a significant effect on lightness (L*). Presence of the C allele, on average, increased meat lightness by 0.39 units in heterozygous animals. There was no significant effect (P > 0.05) of the other phenotypic traits that were evaluated. The same polymorphism showed additive and dominance effects (P < 0.05) on redness of meat (a*) and LW.

Table 7. Estimates of additive effect (α), deviation of additivity (δ) and average effects of allele substitution (β_{li}) of phenotypic traits of SNP g.2554T>C (*CAPN1*) in the commercial population.

Trait	Additive effect			Deviation of additivity			Allele substitution effect		
	a	SE	P > |t|	δ	SE	P > |t|	β_{1i}	SE	P > |t|
SW (g)	10.99	26.22	0.68	13.16	17.65	0.46	5.65	13.10	0.65
SBW (g)	-19.22	43.24	0.66	23.25	30.52	0.45	-9.76	21.61	0.06
EBW (g)	-59.53	31.56	0.06	37.26	21.34	0.08	-29.52	15.81	0.36
BRT (g)	-11.20	12.19	0.36	2.65	8.05	0.74	-5.55	6.08	0.20
LW (g)	-16.59	12.63	0.19	17.99	8.64	0.04*	-8.19	6.35	0.57
pHf	-0.02	0.03	0.56	-0.03	0.02	0.23	-0.01	0.02	0.94
DL (%)	0.01	0.13	0.95	0.17	0.09	0.05	0.00	0.06	0.47
TL (%)	0.31	0.44	0.47	-0.01	0.30	0.98	0.16	0.22	0.21
TCL (%)	0.65	0.52	0.22	0.55	0.36	0.13	0.33	0.26	0.14
TWL (%)	1.13	0.79	0.15	0.53	0.53	0.32	0.58	0.40	0.09
L*	-0.79	0.45	0.08	0.59	0.31	0.06	-0.39	0.23	0.01*
a*	0.41	0.17	0.01*	-0.11	0.12	0.35	0.21	0.08	0.81
b*	0.06	0.22	0.80	0.23	0.15	0.14	0.03	0.11	0.33
MFA (μm^2)	79.78	69.04	0.25	-53.52	46.64	0.25	39.47	34.57	0.25
MFD (μm)	0.88	0.89	0.32	-0.64	0.60	0.28	0.43	0.44	0.51
MFN	0.06	0.40	0.89	0.28	0.27	0.29	0.03	0.20	0.87
SF (kgf)	0.05	0.07	0.51	0.002	0.05	0.97	0.02	0.03	0.51

P < 0.05. SW = selection body weight at 38 days; SBW = slaughter body weight; EBW = eviscerated carcass weight; BRT = breast weight; LW = leg weight; pHf = pH 24 h after slaughter; DL = drip loss; TL = thawing loss; TCL = thawing-cooking loss; TWL = total water loss; L = lightness content; a* = redness content; b* = yellowness content; MFA = muscle fiber area; MFD = muscle fiber diameter; MFN = muscle fiber number; SF = shear force.

Association results of SNP g.15486C>T in the *CAPN3* gene are shown in Table 8. A dominance effect (P < 0.05) was observed on TL% and TWL%. The allelic substitution effect (P < 0.05) was detected on TCL% and SF. Animals with the TT genotype had meat tenderness improved by 0.22 kg, and TCL decreased by 1.46% compared to the other genotypes.

In the present study, four SNPs were identified in the *CAPN1* gene and one SNP in the *CAPN3* gene, and the associations between these polymorphisms and performance, carcass and meat quality traits indicated the viability of using these markers as molecular tools to increase body weight, commercial cut weights, and meat tenderness.

Table 8. Estimates of additive effect (α), deviation of additivity (δ) and average effects of allele substitution (β_{li}) of phenotypic traits of SNP g.15486C>T (*CAPN3*) in the commercial population.

Trait	Additive effect			Deviation of additivity			Allele substitution effect		
	a	SE	P > |t|	δ	SE	P > |t|	β_{1i}	SE	P > |t|
SW (g)	51.65	48.57	0.29	-15.90	30.32	0.60	17.14	17.78	0.34
SBW (g)	72.14	79.69	0.37	-25.93	51.64	0.62	22.17	28.79	0.44
EBW (g)	8.33	59.61	0.89	-30.45	37.45	0.42	-12.33	21.84	0.57
BRT (g)	19.98	22.93	0.38	-12.69	14.17	0.37	3.06	8.45	0.72
LW (g)	7.41	23.73	0.76	-16.00	15.05	0.29	-4.92	8.69	0.57
pHf	0.01	0.06	0.88	0.04	0.04	0.25	0.03	0.02	0.20
DL (%)	0.15	0.23	0.53	-0.13	0.14	0.38	0.00	0.08	0.96
TL (%)	0.90	0.81	0.27	-1.18	0.51	0.02*	-0.19	0.30	0.53
TCL (%)	-0.64	0.96	0.51	-0.77	0.62	0.22	-0.73	0.35	0.04*
TWL (%)	0.28	1.47	0.85	-1.94	0.92	0.04*	-0.92	0.54	0.09
L*	1.01	0.86	0.24	-1.00	0.54	0.06	-0.04	0.32	0.91
a*	-0.32	0.32	0.31	0.13	0.20	0.51	-0.09	0.12	0.45
b*	0.15	0.41	0.73	0.05	0.27	0.86	0.10	0.15	0.52
MFA (μm^2)	32.52	129.74	0.80	11.58	81.32	0.89	22.56	47.45	0.63
MFD (μm)	0.39	1.67	0.81	0.34	1.04	0.74	0.38	0.61	0.53
MFN	-0.10	0.76	0.90	-0.75	0.47	0.11	-0.46	0.28	0.10
SF (kgf)	-0.04	0.13	0.74	-0.16	0.08	0.05	-0.11	0.05	0.02*

P < 0.05. SW = selection body weight at 38 days; SBW = slaughter body weight; EBW = eviscerated carcass weight; BRT = breast weight; LW = leg weight; pHf = pH 24 h after slaughter; DL = drip loss; TL = thawing loss; TCL = thawing-cooking loss; TWL = total water loss; L = lightness content; a* = redness content; b* = yellowness content; MFA = muscle fiber area; MFD = muscle fiber diameter; MFN = muscle fiber number; SF = shear force.

The selection for performance traits may induce several changes in broiler meat quality (Dransfield; Sosnicki, 1999), which has been one of the main factors considered by the poultry industry and consumers.

In this study, polymorphism g.2554T>C identified in the *CAPN1* gene was associated with body weight at 35, 41 and 42 days, weight of drums and thighs, and carcass weight in the experimental population. This SNP, in the commercial population, was associated with body weight at 42 days, eviscerated weight, leg weight, water loss of meat by drip, and lightness and redness content of meat. Our results corroborate previous report by Zhang et al. (2008), that described association of different SNPs in the *CAPN1* gene with body weight, leg weight, carcass weight, and breast weight.

Le Bihan-Duval et al. (2008) found high, negative genetic correlations between breast yield and L*, DL, TCL, and SF (-0.55 ± 10, -0.65 ± 0.10, -0.80 ± 0.06, and -0.60 ± 10, respectively) indicating that selection for increased breast weight could improve water retention capacity and meat tenderness in chickens. According to Gaya et al. (2006, 2011), the estimate of genetic correlation found between DL and a* (0.50) was indicative of a genetic association between these traits. DL and L* (0.19) did not show a significant genetic association. The traits EBW, LW and BRT showed high genetic correlations with BW42. According to these correlation estimates, the traits seem to be associated and able to respond to genetic selection, and SNP g.2554T>C may be used in broiler breeding programs.

The *CAPN3* gene is expressed in skeletal muscle (Sorimachi et al., 1989) and is related to meat tenderness (Kemp et al., 2010), muscular dystrophy and growth, and plays an important role in myofibrillar integrity (Poussard et al., 1996).

The results of this study showed that the SNP g.15486C>T (*CAPN3*) located on chromosome 5 in chickens was associated with yield of drums and thighs in the experimental

population. The same SNP was associated with meat water loss by drip and thawing, lightness content, and shear force in the commercial population.

According to Gaya et al. (2011), genetic correlation estimates found between TL and TCL (0.44) and between SF and L* (0.58) were indicative of significant genetic associations. TL and TCL showed high association with SF (0.77 and 0.80, respectively). However, these traits are able to respond to genetic selection, since the meat water losses can reduce shear force (Anadón, 2002; Gaya et al., 2011). The SNP identified in the *CAPN3* gene had significant effects on the growth and meat quality traits of broilers, and this mutation could be used as a candidate molecular genetic marker for genotypic selection.

Zhang et al. (2009) identified 2 SNPs (11818T>A and 12814T>G) in the *CAPN3* gene in outbred strains of chicken and found associations with body weight, carcass weight, breast muscle weight, and leg muscle weight. In chickens, the *CAPN3* gene was highly expressed in muscle tissues of breast and legs at ages 0, 2, 4, 6, 8, 10, and 12 months (Zhang et al., 2012).

In conclusion, the polymorphisms identified in the *CAPN1* and *CAPN3* genes appear to be in linkage disequilibrium with important economic traits in the populations studied and could be used in poultry genetic selection programs.

ACKNOWLEDGMENTS

A.M. Felício and C. Boschiero received scholarships from the National Council for Scientific and Technological Development, Brazil (CNPq). J.C.C. Balieiro, J.B.S. Ferraz, A.S.A.M.T. Moura, and L.L. Coutinho are recipients of productivity scholarships from CNPq. Research supported by Embrapa/Agricultural Technology Development Project for Brazil (PRODETAB).

REFERENCES

Anadón HLS (2002). Biological, Nutritional, and Processing Factors Affecting Breast Meat Quality of Broilers. Doctoral thesis, Virginia Polytechnic Institute and State University, Virginia.

Brookes AJ (1999). The essence of SNPs. *Gene* 234: 177-186.

Dransfield E and Sosnicki AA (1999). Relationship between muscle growth and poultry meat quality. *Poult. Sci.* 78: 743-746.

Ewing B and Green P (1998). Base-calling of automated sequencer traces using *Phred* II. Error probabilities. *Genome Res.* 8: 186-194.

Falconer DS and Mackay TFC (2001). Introducción a la Genética Cuantitativa. 4ª ed. Acribia, Zaragoza.

Gaya LG, Ferraz JB, Rezende FM, Mourao GB, et al. (2006). Heritability and genetic correlation estimates for performance and carcass and body composition traits in a male broiler line. *Poult. Sci.* 85: 837-843.

Gaya LG, Mourão GB, Ferraz JBS, Mattos EC, et al. (2011). Estimates of heritability and genetic correlations for meat quality traits in broilers. *Sci. Agric.* 68: 620-625.

Gordon D, Abajian C and Green P (1998). Consed: a graphical tool for sequence finishing. *Genome Res.* 8: 195-202.

Hocquette JF, Lehnert S, Barendse W, Cassar-Malek I, et al. (2007). Recent advances in cattle functional genomics and their application to beef quality. *Animal* 1: 159-173.

Kemp CM, Sensky PL, Bardsley RG, Buttery PJ, et al. (2010). Tenderness - an enzymatic view. *Meat Sci.* 84: 248-256.

Koohmaraie M (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci.* 43S1: 193-201.

Le Bihan-Duval E, Berri C, Baeza E, Sante V, et al. (2003). Genetic parameters of meat technological quality traits in a grand-parental commercial line of turkey. *Genet. Sel. Evol.* 35: 623-635.

Le Bihan-Duval E, Debut M, Berri CM, Sellier N, et al. (2008). Chicken meat quality: genetic variability and relationship with growth and muscle characteristics. *BMC Genet.* 9: 53.

NCBI, National Center for Biotechnology Information (2012). Available at [http://www.ncbi.nlm.nih.gov/]. Accessed March 30, 2012.

Nones K, Ledur MC, Ruy DC, Baron EE, et al. (2006). Mapping QTLs on chicken chromosome 1 for performance and carcass traits in a broiler x layer cross. *Anim. Genet.* 37: 95-100.

Park GB, Moon SS, Ko YD, Ha JK, et al. (2002). Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. *J. Anim. Sci.* 80: 129-136.

Poussard S, Duvert M, Balcerzak D, Ramassamy S, et al. (1996). Evidence for implication of muscle-specific calpain (p94) in myofibrillar integrity. *Cell Growth Differ.* 7: 1461-1469.

Rosário MF, Ledur MC, Moura ASMT, Coutinho LL, et al. (2009). Genotypic characterization of microsatellite markers in broiler and layer selected chicken lines and their reciprocal F1s. *Sci. Agric.* 66: 150-158.

SAS (2004). SAS/STATUser's Guide. Version 9.1. SAS Institute, North Caroline.

Sorimachi H, Imajoh-Ohmi S, Emori Y, Kawasaki H, et al. (1989). Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and mu-types. Specific expression of the mRNA in skeletal muscle. *J. Biol. Chem.* 264: 20106-20111.

Taylor RG, Geesink GH, Thompson VF, Koohmaraie M, et al. (1995). Is Z-disk degradation responsible for postmortem tenderization? *J. Anim. Sci.* 73: 1351-1367.

Zhang ZR, Liu YP, Jiang X, Du HR, et al. (2008). Study on association of single nucleotide polymorphism of *CAPN1* gene with muscle fibre and carcass traits in quality chicken populations. *J. Anim. Breed. Genet.* 125: 258-264.

Zhang ZR, Liu YP, Yao YG, Jiang XS, et al. (2009). Identification and association of the single nucleotide polymorphisms in calpain3 (*CAPN3*) gene with carcass traits in chickens. *BMC Genet.* 10: 10.

Zhang ZR, Zhu Q, Yao YG, Jiang XS, et al. (2012). Characterization of the expression profile of calpain-3 (*CAPN3*) gene in chicken. *Mol. Biol. Rep.* 39: 3517-3521.