Animal Science Papers and Reports vol. 33 (2015) no. 3, 287-298
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Effect of newly found polymorphisms in the promoter region of the CAPN1 gene on transcript abundance in broiler chicken breast muscle*

Katarzyna Piórkowska[1], Joanna Nowak[2], Katarzyna Połtowicz[2], Katarzyna Ropka-Molik[1], Tomasz Szmatoła[1]

[1] Laboratory of Genomics, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland

[2] Department of Animal Genetics and Breeding, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland

(Accepted June 23, 2015)

The calpain family includes proteolytic enzymes, which have a high capacity to degrade cytoskeletal and muscle fibre proteins. Thus they play an important role in the fusion of myoblasts and in cell proliferation and growth. The *CAPN1* gene has been selected as a 'candidate gene' for meat quality in many domestic animals, including chickens. Consequently, the aim of our study was to identify new polymorphisms in the promoter region of the *CAPN1* gene in broilers and to investigate their impact on *CAPN1* transcript abundance in breast muscles. The experiment used broilers of two genetic lines, fast- and slow-growing. Five new polymorphisms in the promoter region of the *CAPN1* gene were identified, all of them in linkage disequilibrium (P<0.05). However, the results obtained for their association with expression level were doubtful. Therefore, we surmise that the newly discovered polymorphisms, although they alter the potential sequence binding of transcription factors, probably have just a weak effect on the level of *CAPN1* expression in broiler chickens at the investigated stage of ontogenesis.

KEY WORDS: broilers / *CAPN1* / polymorphism / promoter region

*This work was supported from the National Research Institute of Animal Production statutory activity, research project No. 01-4.02.1.
**Corresponding author: katarzyna.piorkowska@izoo.krakow.pl

Calpains are enzymes requiring suitable concentrations of Ca^{2+} for their activation. Found in cytoplasm, they are involved in a variety of calcium-regulated cellular processes such as signaling, cell proliferation, differentiation, transduction, apoptosis, membrane fusion and platelet activation [Sorimachi and Suzuki 2001, Huang and Wang 2001]. Therefore, deregulation of their function leads to various pathological conditions such as neuronal degeneration, muscle dystrophy, Alzheimer's disease, metastasis and cataract. Biological and molecular studies have shown that calpains constitute a superfamily, which is ubiquitous in organisms ranging from humans to microorganisms [Suzuki *et al*. 2004]. Calpains exist in the cytosol of cells as inactive enzymes and are translocated to membranes in response to increased cellular Ca^{2+} concentrations [Suzuki *et al*. 1995]. A high level of calpain activity increases the rate of protein degradation in muscles [Kawabe *et al*. 1997]. Information on calpain activity in muscles could be useful in selection for improvement of tenderness of the breast muscle and growth rate in broiler chickens.

The literature on the subject contains many reports, in which polymorphisms in calpain genes affect economically important traits in farm animals. In 2007 Yang *et al*. [2007] identified eight missense mutations in five exons of the *CAPN1* gene in pigs and wild boars, recommending them as molecular markers for meat tenderness and pork quality. A year later the same authors discovered polymorphic sites in 3'UTR of the *CAPN1* gene and associated them with lean meat percentage [Yang *et al*. 2008]. In cattle, Page et al. [2002] found that single nucleotide polymorphisms in exons 9 and 14 alter the *CAPN1* protein sequence, which affects meat tenderness. Similarly, Allais *et al*. [2011], testing three French beef breeds, confirmed the influence of *CAPN1* markers (45221250, 45219395 and 45241089 on Btau 4.0) on meat tenderness and hardness.

In broiler chickens, Chinese scientists Zhang *et al*. [2008] and Zhou *et al*. [2011] calculated the effect of *CAPN1* on economically important chicken traits. They found that mutations in the fifth and sixth exons change fibre density, and that the C2546T/G3535A/C7198A haplotype affected live, carcass, breast and leg muscle weights. Moreover, a mutation within exon 4 associated with pH in breast muscle and intramuscular fat content. The latest studies, conducted by Sun *et al.* [2013] and Felício *et al*. [2013], confirmed previous observations on the effects of mutations in the *CAPN1* gene.

Traditional selection of broilers, directed at growth rate, led to a deterioration in meat quality traits. Previous reports demonstrated that the *CAPN1* gene is associated with tenderness and other meat quality traits in chickens [Li *et al.* 2004]. To date, information about *CAPN1* gene expression in broiler chickens is very poor.

Therefore, the aim of our study focused on polymorphic sites in the promoter region of the *CAPN1* gene to examine their effects on gene expression in the pectoralis muscle of two lines of broiler chickens which differ in their growth rate and quality characteristics of meat.

Material and methods

Chicken population

The analyses were conducted on 144 broiler chickens from two lines, the fast-growing (FG) Hubbard Flex (67 chickens) and the slow-growing (SG) Hubbard JA957 (77 chickens). In the study both sexes were included, with 38 and 36 cockerels in the fast- and slow-growing lines, respectively. The chicks were hatched in a commercial poultry hatchery from eggs obtained from selected parent stock farms, delivered to the experimental farm of the National Research Institute of Animal Production, Aleksandrowice (Poland) and maintained in the same feeding and environmental conditions according to on-farm procedures. All broilers were fed ad libitum with complete starter, grower and finisher diets containing 22, 20.5 and 20.5% CP and 2,990, 3,130 and 3,130 kcal/kg ME, respectively. FG chickens were maintained to 42 days of age. During this time male and female chickens reached mean body weights of 2.80 and 2.40 kg, respectively. Broilers characterised by a slower growth rate were slaughtered at 56 days of age, when their body weights approximated the mean weight attained by the FG chickens. The chickens were slaughtered by decapitation. The local Krakow Ethics Committee for Experiments on Animals approved all the experimental procedures relating to the use of live animals (permission no. 878). During dissection, two samples of breast muscle were collected into tubes and stabilised with RNAlater® solution (Ambion). Additionally, two blood samples were drawn into EDTA tubes and frozen at -20°C.

Sequencing and selection of SNPs for genotyping

DNA was extracted from whole blood samples using a Wizard Genomic Purification Kit (Promega, Madison, WI, USA), following instructions provided in the manufacturer`s protocol. To identify new polymorphisms in the promoter region of the *CAPN1* gene, we applied High Resolution Melting (HRM) screening using the Eco Real-Time PCR System (Illumina). Primers were designed based on sequences accessible in the Ensembl database (ENSGALG00000010186) using the Primer3 Input computer program (version 0.4.0) Primer pairs for HRM and sequencing are given in Table 1 and the DNA amplification and sequencing were performed according to Piórkowska *et al*. [2014].

The PCR-RFLP method was used to determine genotype frequencies of five detected polymorphisms (located in the promoter region) for 144 broilers of both lines. The restriction enzymes were selected to detect individual mutations using NEBCutter v 2.0 (New England BioLabs, Frankfurt am Main, Germany) – Table 2.

Gene expression analysis

RNA isolation was performed using the TRI Reagent (Applied Biosystems) in accordance with the method described by Chomczyński [1993]. Samples of breast muscle were homogenised with beads using a Bullet Blender 24 homogeniser (Next

Table 1. Primer sequences used for High Resolution Melting (HRM) analysis and for sequencing PCRs of the promoter region of the *CAPN1* gene in broiler chickens

Item	Sequence of primers used for HRM	PCR product size (bp)	Temperature of annealing (°C)
5' gene flanking region 1 (promoter region1)	F1 ACGCTCATCAGGCAAGTAGG R1 TCACACTCCCAGTGCCTTC	256	55
5' gene flanking region 2 (promoter region2)	F2 CCATGGCCCTCTTCTGAT R2 CTCTCAGGCGGTCCCTCT	254	55
	Sequence of primers used for PCR-sequencing		
Promoter region of *CAPN1* gene	F1 ACGCTCATCAGGCAAGTAGG R2 CTCTCAGGCGGTCCCTCT	525	55

Table 2. Novel polymorphisms detected in the promoter region of the *CAPN1* gene in chickens

Polymorphisms identified	Polymorphism type	Primers (PCR product size, bp)	Restriction enzyme	Length of restriction products (bp)
g.1009 C>T	SNP	256	*Bsm*FI	C: 19/20/83/134 T: 19/83/154
g.1019_1023del GCTTA	INDEL	256	*Dde*I	gctta: 68/69/119 -: 119/137
g.1121G>C	SNP	256	*Mnl*I	G: 9/11/54/82/100 C: 9/11/31/54/80/82
g.1288 T>G	SNP	254	*Hinf*I	T: 62/192 G: 254
g.1362C>T	SNP	254	*Bpm*I	C: 254 T: 120/134

Reference sequence: GenBank AADN03003353.1.
The PCR-RFLP method was adapted for five single nucleotide polymorphisms.

Advance). Quantitative and qualitative evaluation of ribonucleic acid isolated and transcribed into cDNA was performed according to Piórkowska *et al*. [2014].

The expression of *CAPN1* in breast muscles was determined for 144 (67 FG and 77 SG) broiler chickens, including 38 and 36 cockerels in the fast- and slow-growing lines, respectively. Primers and probes for chicken *CAPN1* and endogenous reference genes were designed and synthesised by Applied Biosystems (TaqMan® gene expression assay: *CAPN1*-Gg03346514_m1). As endogenous controls, we used SDHA (TaqMan® gene expression assay: *SDHA Gg03330760_m1*) and RPL4 (TaqMan® gene expression assay: *RPL4-Gg03370187_m1*) genes [Piórkowska *et al.* 2014]. The length of amplicons of *CAPN1*, *SDHA* and *RPL4* genes was in the range of 60-82nt; they extended between two exons: 2-3, 1-2 and 2-3, respectively. One multiplex reaction, consisting of a primer and a probe for the *CAPN1* and RPL4 genes, and one singleplex reaction for the *SDHA* gene were carried out. Reactions were performed in a volume of 20 µl and 3 repetitions with a 7500 Real-Time PCR instrument according to the manual protocol. The relative mRNA abundance was computed according to Piórkowska *et al*. [2013].

Transcription factors and statistical analysis

Statistical analysis was conducted in the Power Marker v 3.25 and SAS Enterprise 9.3 using General Linear Model (GLM) procedure. Grubbs' test was applied to remove outliers. The normality of distribution was verified with the use of the Shapiro-Wilk test (P-value ≤0.05), therefore the test for non-normal distribution was used. Thus, the statistical differences between groups were assessed first by the Kruskal-Wallis test and then by the Wilcoxon test. The unitrait GLM model in the analysis of the effect of genotypes was:

$$yijk = \mu + di + bj + eijk,$$

where:

yijk – observation of gene expression of ijk-individual;

μ – overall mean;

di – fixed effect of kth: g.1009 C>T-*CAPN1* (CC, CT, TT) or g.1019_1023del GCTTA-*CAPN1* (ins, ins/del, del) or g.1121G>C-*CAPN1* (CC, CG, GG) or g.1288 T>G-*CAPN1*(GG, TG, TT) or g.1362C>T-*CAPN1* (CC, CT, TT) genotypes;

bj – fixed effect of j-th line;

eijk – random error connected with ijk-observation.

In turn, in haplotype analysis the GLM model was:

$$yijk = \mu + hi + bj + eijk,$$

where:

hi – fixed effect of kth H2 (0,1,2) or H5 (0,1,2) or H6 (0,1,2) or H13 (0,1,2), 0,1,2 – copies of haplotype, y, μ , bj;

eijk – as above.

Analysis of transcription factors binding site was performed using the MatInspector software (Genomatrix) [Cartharius *et al.* 2005].

Results and discussion

Five polymorphic sites were found in the investigated 525 bp promoter region g.1009 C>T, g.1121G>C, g.1288 T>G, g.1362C>T, and INDEL in position g.1019_1023del GCTTA (*GenBank: AADN03003353.1*). These were all submitted to the Short Genetic Variations database (dbSNP NCBI) and received SNP IDs as follows: ss#647514531 – g.1009 C>T; ss#647514532 – g.1019_1023del GCTTA; ss#647514533 – g.1121G>C; ss#647514534 – g.1288 T>G; and ss#647514535 – g.1362C>T. The highest frequency was shown for allele C in the g.1121G>C *locus*. The FG chickens were characterised by a different frequency of new polymorphisms in the *CAPN1* gene when compared to the SG line. Fast-growing chickens according to polymorphism g.1009C>T have less C allele and G of polymorphism g.1288T>G (Tab. 3). Moreover, the calculation

Table 3. Frequency of genotypes and alleles of newly-discovered polymorphisms in the promoter region of the *CAPN1* gene in broiler chickens

Polymorphisms and genotypes		Genotype and allele frequency				
		fast-growing		slow-growing		total
		male (38)	female (29)	male (38)	female (29)	
g.1009 C>T *Bsm*FI	CC	0.19		0.28		0.23
	CT	0.48		0.36		0.42
	TT	0.33		0.36		0.35
	Allele					
	C	0.43		0.45		0.44
	T	0.57		0.55		0.56
HWE p-value		0.83		**0.005**		
g.1019_1023del GCTTA	Del/Del	0.33		0.36		0.35
*Dde*I	Ins/Del Ins/Ins	0.48		0.36		0.42
	Allel	0.19		0.28		0.23
	Del					
	Ins	0.57		0.55		0.56
		0.43		0.45		0.44
HWE p-value		0.83		**0.005**		
g.1121G>C	CC	0.41		0.74		0.54
*Mnl*I	CG	0.43		0.23		0.37
	GG	0.16		0.03		0.09
	Allel					
	C	0.62		0.86		0.74
	G	0.38		0.14		0.26
HWE p-value		0.44		0.54		
g.1288 T>G *Hinf*I	GG	0.14		0.20		0.17
	GT	0.34		0.42		0.38
	TT	0.52		0.38		0.45
	Allel					
	G	0.30		0.41		0.36
	T	0.70		0.59		0.64
HWE p-value		0.06		0.19		
g.1362C>T	CC	0.36		0.59		0.48
*Bpm*I	CT	0.49		0.32		0.40
	TT	0.15		0.09		0.12
	Allel					
	C	0.61		0.75		0.69
	T	0.39		0.25		0.31
HWE p-value		0.80		0.49		

Bolded are populations in Disequilibrium of Hardy Weinberg (HWE) for particular polymorphisms.

of linkage disequilibrium showed that polymorphisms of *CAPN1* g.1019_1023del GCTTA and g.1009 C>T are strongly linked by D=0.242 (P≤0.05, Tab. 4); thus, the association analysis of g.1009 C>T polymorphism was omitted. The influence of the polymorphisms on *CAPN1* expression was observed only for SNPs detected by the *Mnl*I restriction enzyme (g.1121G>C), where GG homozygotes had a level of *CAPN1* mRNA approximately 30% higher than in chickens with other genotypes (P≤0.01) (Fig. 1). This could have been caused by the fact that homozygotes have no binding site for C2H2 zinc finger transcription factors 2 (ZF02) (Tab. 5), which may suggest

Table 4. Linkage analysis

Polymorphisms1	Polymorphism2	Mutual Information	Multi-Allelic D	p-value
g.1121G>C	g.1362C>T	0.5935	0.1626	≤0.001
g.1362C>T	g.1288 T>G	0.2438	0.1001	≤0.001
g.1288 T>G	g.1019_1023delGCTTA	0.0877	0.0769	≤0.001
g.1019_1023del GCTTA	g.1009 C>T	0.9102	0.2419	≤0.001

In table presented the highest linked polymorphism pairs.

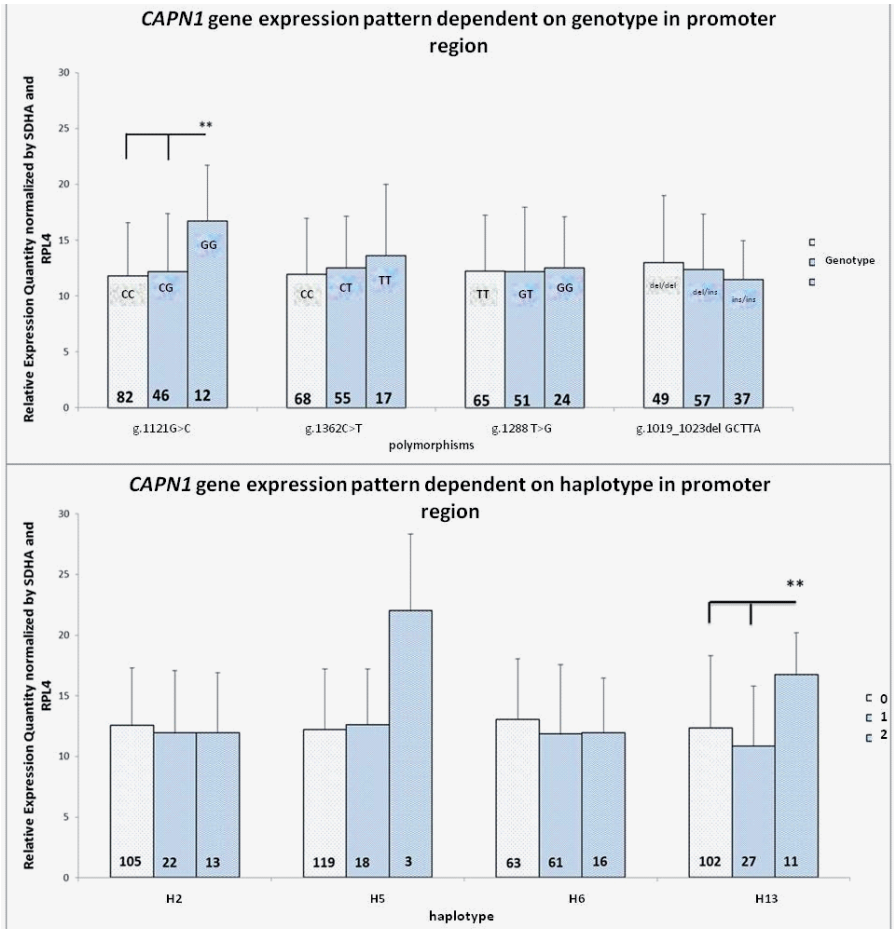

Fig. 1. *CAPN1* gene expression patterns dependent on polymorphic regions detected by specific restriction enzymes and haplotypes in promoters. The number of animals in particular genotypes is shown in the bars. Significant differences between genotype groups are shown as ** $p<0.01$. H2, H5 etc.: particular haplotypes; 0, 1, and 2 in the legend indicate the number of copies of a particular haplotype. The 1 copie of haplotype it means that the pattern of some haplotype occurred in one allele, two copies of some of haplotype it means that in both alleles is polymorphism pattern presented by some haplotype

Table 5. The potential changes in binding transcription factors caused by new polymorphic sites

Polymorphisms identified	Transcription factors			
	additional binding site for	loss of binding site for	family information	specify of transcription factor
g.1009 C>T	1. EREF 2. SORY 3. SF1F		1. Estrogen response elements 2. SOX/SRY-sex/testis determinig and related HMG box factors 3. Vertebrate steroidogenic factor	1. Estrogen related receptor 2. SRY (sex determining region Y)-box 9 homodimer 3. SF1 steroidogenic factor 1
g.1019_1023 del GCTTA	1. TF2D	2. BCDF 3. E2FF 4. PAX5	1. General transcription factor IID, GTF2D 2. Bicoid-like homeodomain transcription factors 3. E2F-myc activator/cell cycle regulator 4. PAX-2/5/8 binding sites	1. Initiator (INR) and downstream promoter element (DPE) with strictly maintained spacing 2. Pituitary Homeobox 1 (Ptx1, Pitx-1) 3. E2F-1/DP-1 heterodimeric complex 4. Paired box protein 2
g.1121G>C		1. ZF02	1. C2H2 zinc finger ranscription factors 2	1. Core promoter-binding protein (CPBP) with 3 Krueppel-type zinc fingers
g.1288 T>G	1. IKRS		1. Ikaros zinc finger family	1. Ikaros 1, potential regulator of lymphocyte differentiation
g.1362C>T	1. AP4R	2. KLFS	1. AP4 and related proteins 2. Krueppel-like transcription factors	1. Activator protein 4 2. Kruppel-like factor 6

Table 6. Frequency of haplotypes (%) of polymorphisms in the promoter region of the *CAPN1* gene, separately for fast – (FG) and slow-growing (SG) lines of broiler chickens

Haplotype		Haplotype frequency broiler line	
		fast-growing	slow-growing
H1	c/c/g/del/c	0	1.27
H2	c/c/g/del/t	5.81	9.56
H3	c/c/g/ins/c	3.23	3.82
H4	c/c/g/ins/t	0	0.64
H5	c/c/t/del/t	12.9	15.29
H6	c/c/t/ins/c	**67.1**	**61.15**
H7	c/c/t/ins/t	0	1.27
H8	c/t/t/del/t	0	3.19
H9	c/t/g/del/t	0	0
H10	c/t/g/ins/c	0	0
H11	c/t/t/ins/c	1.94	1.27
H12	g/c/t/ins/c	1.29	0
H13	g/t/t/del/t	7.74	2.55
H14	g/t/g/del/t	0	0

H1, H2, etc. – following diplotypes; ins – insertion; del – deletion.
Bolded are the highest frequency of haplotype and diplotypes.

that ZF02 modulates *CAPN1* transcription. In other studies it was proven that zinc finger transcription factor increases even 5- to 20-fold the expression level of the *VEGF-A* gene in cultured human skeletal muscle, depending on the type of ZFP and on the location of the binding sequence for this transcription factor [Liu *et al*. 2001]. In other polymorphisms no significant effect on *CAPN1* expression was observed. The analysis of haplotypes showed the presence of 14 variants, with the highest frequency for H6 C/C/T/ins/C (64%; Tab. 6). Our results showed that chickens with haplotype H13 in both alleles had a higher *CAPN1* transcript levels than the others ($P \leq 0.01$, Fig. 1), which could led to a higher proteolytic enzyme level. In that haplotype occurred the G variant of g.1121G>C polymorphism, which also led to higher *CAPN1* expression. Other haplotypes, including the G variant of g.1121G>C polymorphism (H12 and H14), were not statistically analysed due to the low frequencies and difficulty in the organizing a statistical group. Therefore, we do not exactly know if the higher *CAPN1* gene expression is determined only by g.1121G>C polymorphism or by H13 halpotype.

The role of protein, a large subunit of μ-calpain, encoded by the *CAPN1* gene, is not clear as yet, although the activity of m- and μ-calpains is considered essential for maintenance of life functions. In research on human subjects it was observed that calpain 1 is necessary for normal platelet development and function [Azam *et al*. 2001] and that dysfunction of this protein leads to neurodegenerative disease [Branca 2004]. However, a study conducted on the *CAPN1*-/- of knockout mice suggests that a lack of the *CAPN1* gene product could be substituted by a large subunit of m-calpain [Goll *et al*. 2003]. Moreover, it has been shown that *CAPN1* in humans is highly polymorphic: many SNPs were found in coding regions, but the mutations were situated in the region of domains, which do not play a key role in enzymatic activity of calpain 1 or were anonymous (Ensemble browser). In turn, it was observed that human tumor tissue is characterised by many more mutations of *CAPN1* than normal tissue [Imielinski *et al*. 2012, Bell *et al*. 2011, Berger *et al*. 2012].

In farm animals, polymorphisms in the *CAPN1* gene were examined for their impact on meat quality traits. Polymorphic sites were investigated in coding regions to find missense mutations responsible for altering a major part of the amino-acid sequence. In cattle Cheong *et al.* [2008] showed that, out of 39 SNPs identified in the *CAPN1* gene, only one located in 3′UTR affects marbling score. Moreover, in goats Singh *et al*. [2012] discovered a large number of SNPs in exons 3, 4 and 11 and their flanking regions, which will be considered in further association analyses. To date, 118 SNPs were discovered in the *CAPN1* gene in chickens [NCBI 2013], including silent mutations, which were tested for their effect on the weight of meat cuts and breast muscle fibre density [Zhang *et al*. 2008]. Most of the identified polymorphisms were situated in the intronic region; nevertheless, they were also associated with e.g. lightness or the muscles of drums and thighs [Felício *et al.* 2013].

All the polymorphisms identified in our study alter at least one binding site for transcription factors; g.1019_1023del GCTTA changes five sequences of

binding transcription factors (Tab. 6). One of the most important is the INR_DPEl transcription factor included in the general transcription factor IID (TFIID) family, which is responsible for coordination of the components of the transcription initiation complex [Barrett *et al*. 2012] and its binding to the TATA box in the core promoter of the gene prior to the start of transcription [Orphanides et al. 1996]. Nevertheless, in the promoter region of *CAPN1* in chickens, there is another TFIID binding site in position -231 to -193 upstream of the gene (Ensemble browser). Furthermore, the deletion identified in our study also results in the loss of three other binding sites for bicoid-like homeodomain transcription (BCDF), paired box protein 5 (PAX5) and E2F-myc activator/cell cycle regulator (EIIFF) transcription factors (Tab. 6). BCDF is engaged in myogenesis in limbs, as it activates *MyoD* [L'Honoré *et al.* 2010], but its involvement in proteolysis has not been proven.

The not clear influence observed in the present study for new polymorphisms in the promoter region of the *CAPN1* gene on its expression could be due to the age of the broilers, since various transcription factors modulating *CAPN1* gene expression are activated at different stages of ontogenesis. Therefore, additional investigations including younger broilers are necessary. Regardless of this doubts the influence of polymorphisms on gene expression in this investigation, the newly-discovered polymorphisms should receive further consideration in terms of their usefulness as genetic markers, given that the calpain system plays such an important role in *post-mortem* meat maturation [Bilak *et al*. 1998], while also determining water-holding capacity [Melody *et al*. 2004].

Because the important role, which play the calpain system in the modulation of meat quality traits in chickens and other farm animal species, research aimed at investigating the genes encoding proteins belonging to this family should be continued. Our study delivered new information about polymorphic sites in the promoter region of the *CAPN1* gene. Although we were unable to prove their clear impact on gene expression, in further studies they could be examined for their influence on chicken traits.

REFERENCES

1. ALLAIS S., JOURNAUX L., LEVÉZIEL H., PAYET-DUPRAT N., RAYNAUD P., HOCQUETTE J.F., LEPETIT J., ROUSSET S., DENOYELLE C., BERNARD-CAPEL C., RENAND G., 2011 – Effects of polymorphisms in the calpastatin and μ-calpain genes on meat tenderness in 3 French beef breeds. ***Journal of Animal Science*** 89, 1-11.
2. AZAM M., ANDRABI S.S., SAHR K.E., KAMATH L., KULIPOULOS A., CHISTI A.H., 2001 – Disruption of the mouse μ-calpain gene reveals an essential role in platelet function. ***Molecular and Cellular Biology*** 21, 2213-2220.
3. BARRETT L.W., FLETCHER S., WILTON S.D., 2012 – Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. ***Cellular and Molecular Life Sciences*** 69, 3613-3634.
4. BELL D., BERCHUCK A., BIRRER M., CHIEN .J, CRAMER D.W., DAO F., DHIR R., DISAIA P., 2011 – Integrated genomic analyses of ovarian carcinoma. ***Nature*** 474, 609-615.

5. BERGER M.F., HODIS E., HEFFERNAN T.P., DERIBE Y.L., LAWRENCE M.S., PROTOPOPOV A., IVANOVA E., WATSON I.R., et al., 2012 – Melanoma genome sequencing reveals frequent PREX2 mutations. ***Nature*** 485, 502-506.
6. BILAK S.R., SERNETT S.W., BILAK M.M., BELLIN R.M., STROMER M.H., HUIATT T.W., ROBSON R.M., 1998 – Properties of the novel intermediate filament protein synemin and its identification in mammalian muscle. ***Archives of Biochemistry and Biophysics*** 355, 63-76.
7. BRANCA D., 2004 – Calpain-related diseases. ***Biochemical and Biophysical Research Communications*** 322, 1098-1104.
8. CARTHARIUS K., FRECH K., GROTE K., KLOCKE B., HALTMEIER M., KLINGENHOFF A., FRISCH M., BAYERLEIN M., WERNER T. , 2005 – MatInspector and beyond: promoter analysis based on transcription factor binding sites. ***Bioinformatics*** 21, 2933-42.
9. 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.
10. CHOMCZYŃSKI P., 1993 – A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. ***BioTechniques*** 15, 532-537.
11. 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.
12. GOLL D.E., THOMPSON V.F, LI H., WEI W., CONG J., 2003 – The calpain system. ***Physiological Reviews*** 83, 731-801.
13. HUANG Y., WANG K.K.W., 2001 – The calpain family and human disease. ***Trends in Molecular Medicine*** 7, 355-362.
14. IMIELINSKI M., BERGER, A.H., HAMMERMANP.S., HERNANDEZ B., PUGH T.J., HODIS E., CHO J., SUH J., 2012 – Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. ***Cell*** 150, 1107-1120.
15. KAWABE K., MAEDA Y., OKAMOTO S., HASHIGUCHI T., 1997 – Correlation between skeletal muscle calpain activity and fractional rate of muscle degradation of Japanese quail Coturnix coturnix japonica. ***Japanese Poultry Science*** 34, 231-239.
16. L'HONORÉ A., OUIMETTE J.F., LAVERTU-JOLIN M., DROUIN J., 2010 – Pitx2 defines alternate pathways acting through MyoD during limb and somitic myogenesis. ***Development*** 137, 3847-3856.
17. LI T.S., LIU F.M., YIN X.H., LIAN A.L., TANG H., QU J.P., 2004 – The evaluation methods of chicken meat tenderness and their relationship. ***Acta Veterinaria et Zootechnica Sinica*** 35, 171-177.
18. LIU P.Q., REBAR E.J., ZHANG L., LIU Q., JAMIESON A.C., LIANG Y., QI H., LI P.X., CHEN B., MENDEL M.C., ZHONG X., LEE Y.L., EISENBERG S.P., SPRATT S.K., CASE C.C., WOLFFE A.P., 2001 – Regulation of an endogenous locus using a panel of designed zinc finger proteins targeted to accessible chromatin regions: Activation of vascular endothelial growth factor A. ***The Journal of Biological Chemistry*** 276, 11323-11334.
19. MELODY J.L., LONERGAN S.M., ROWE L.J., HUIATT T.W., MAYES M.S., HUFF-LONERGAN E., 2004 – Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. ***Journal of Animal Science*** 82, 1195-1205.
20. NCBI, 2013 – Available at http://www.ncbi.nlm.nih.gov/
21. ORPHANIDES G., LAGRANGE T., REINBERG D., 1996 – The general transcription factors of RNA polymerase II. ***Genes and Development*** 10, 2657-2683.

22. PAGE B.T., CASAS E., HEATON M.P., CULLEN N.G., HYNDMAN D.L., MORRIS C.A., CRAWFORD A.M., WHEELER T.L., KOOHMARAIE M., KEELE J.W., SMITH T.P.L., 2002 – Evaluation of single-nucleotide polymorphisms in *CAPN1* for association with meat tenderness in cattle. ***Journal of Animal Science*** 80, 3077-3085.
23. PIÓRKOWSKA K., NOWAK J., POŁTOWICZ K., ROPKA-MOLIK K., 2014 – New polymorphisms in regulatory region of *CAPN3* gene with no effect on gene expression in breast muscle of broiler chickens. ***Annals of Animal Science*** 14, 511-524.
24. PIÓRKOWSKA K., ROPKA-MOLIK K., ECKERT R., RÓŻYCKI M., 2013 – The expression pattern of proteolytic enzymes of cathepsin family in two important porcine skeletal muscles. ***Livestock Science*** 157, 427-434.
25. SINGH L.V., TRIPATHI V., SHARMA R., PANDEY A.K., MAITRA A., MISHRA B.P., 2012 – Genetic polymorphism of *CAPN1* gene in Sirohi goat. ***International Journal of Meat Science*** 2, 13-19.
26. SORIMACHI H., SUZUKI K., 2001 – The structure of calpain. ***The Journal of Biochemistry*** 129, 653-664.
27. SUN Y., LIU R., LU X., HU Y., ZHAO G., ZHENG M., CHEN J., WANG H., WEN J., 2013 – Associations of polymorphisms in four candidate genes with carcass and/or meat-quality traits in two meat-type chicken lines. ***Animal Biotechnology*** 24, 53-65.
28. SUZUKI K., HATA S., KAWABATA Y., SORIMACHI H., 2004 – Structure, activation, and biology of calpain. ***Diabetes*** 53(Suppl 1), S12-S18.
29. SUZUKI K., SORIMACHI H., YOSHIZAWA T., KINBARA K., ISHIURA S., 1995 – Calpain: novel family members, activation and physiological function. ***Biological Chemistry Hoppe-Seyler*** 376, 523-529.
30. YANG X.Q., LIU H., GUO L.J., XU Y., LIU D., 2007 – The mutation site analysis on *CAPN1* gene of Wild boar, Min pig and Yorkshire. ***Hereditas*** 29, 581-586.
31. YANG X.Q., LIU H., GUO L.J., GUAN Q.Z., XU Y., LIU D., 2008 – Analysis of SNPs in partial exons and 3′UTR of CAPN1 in porcine. ***Hereditas*** 30, 741-746.
32. 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 fiber and carcass traits in quality chicken populations. ***Journal of Animal Breeding and Genetics*** 125, 258-264.
33. ZHOU G.L,GUO Y., FANG X., WU Y.H., 2011 – Polymorphism of *CAPN1* gene and its correlation with meat quality traits in Shouguang chicken. ***Food Science*** 32, 207-210.