Association analysis of c.105G→A SNP in CAPN1 gene with carcass and meat quality traits in goose

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Summary

Meat quality is an important feature for the poultry industry. Domestic geese usually present a though meat and it is necessary to select them to improve meat tenderness. The relation of the *calpain 1* (CAPN1) gene with the tenderness process of meat post-mortem has been demonstrated in several species. Thus, the objective of the present study was to identify polymorphisms in this gene and to determine for first time the possible association between these polymorphisms and related economic traits in goose raised in a *dehesa* ecosystem. For the analysis, forty geese of two different genotypes (20 Embden-Anser anser, EE; 20 Toulouse-Anser anser, TT) were studied. A novel SNP was found in the CAPN1 gene, c.105G→A. This Polymorphism was statistically associated with different carcass and meat quality traits as tight quality (circumference, $p = 0.020$; and length, $p = 0.026$) and b* (meat color, $p = 0.024$) parameters for the global goose population. The association of this gene with meat tenderness (WBSF) was only confirmed in the case of female individuals of the Toulouse breed ($p = 0.043$), where the G allele of *Calpain 1* gene contributes to obtain a tender meat. The results suggest the possibility of using molecular markers in CAPN1 gene as a promising tool for the improvement of carcass and meat quality traits in poultry breeding programs.

Key words: geese; calpain 1; meat production; tenderness; SNP

Association analysis of g.68G>A SNP in CAPN1 gene with carcass and meat quality traits in goose

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INTRODUCTION

Meat quality is an important feature for the poultry industry.

Domestic geese products obtained from free-range systems usually have rather tough meat, and it is necessary to select them to improve meat tenderness. The common domestic breeds used for meat production are the Embden-*Anser anser* and Toulouse-*Anser anser* breeds.

The relation of the *calpain 1* (*CAPN1*) gene with the tenderness (WBSF) process of meat post-mortem has been demonstrated in several species.

MATERIAL Y METHODS

For the analysis of carcass characteristics and meat quality traits (Table 1), forty geese of two different genotypes (20 Embden-Anser anser, EE; 20 Toulouse-Anser anser, TT) were selected. After genotyping, a novel SNP was found in the *CAPN1* gene, **g.68G→A**.

Trait association of allele and genotypic frequencies (Table 2) in each goose subpopulation and in the global population were performed using Maximum-Likelihood Chi-square test and a General Linear Model. Statistical analysis was performed using SAS 9.2 (Statistical Analysis System, Inc. Cary, USA).

The aim of this study was to identify polymorphisms in CAPN1 gene and to determine for first time the association with related economic traits in goose raised in a *dehesa* ecosystem

Table 2 Genotypic and allelic frequencies of polymorphism g.68G→A (*CAPN1* gene) in the two goose subpopulations and in the global population

Breed	Sex	Genotypic frequencies			Allelic frequencies	
		f(GG)	f(AG)	f(AA)	f(G)	f(A)
	Male	1.00	0.00	0.00	1.00	0.00
	Female	0.40	0.60	0.00	0.70	0.30
	Overall	0.70	0.30	0.00	0.85	0.15
Toulouse	*Male*	0.80	0.10	0.10	0.85	0.15
	Female	0.60	0.30	0.10	0.75	0.25
	Overall	0.70	0.20	0.10	0.80	0.20
GLOBAL	*Male*	0.84	0.12	0.04	0.90	0.10
	Female	0.44	0.52	0.04	0.70	0.30
	Overall	0.64	0.32	0.04	0.80	0.20

Table 1 *P-values* corresponding to the association analysis between g.68G→A (*CAPN1* gene) with carcass characteristics and meat quality traits using a General Linear Model in Toulouse and Embden

	Global	Embden		Toulouse		
	Genotype	*Global*	*Female^*	*Global*	*Male*	*Female*
Carcass characteristics						
Carcass weight	0.60	0.19	0.24	0.15	0.10	0.70
Carcass length	0.41	0.82	0.82	0.06	0.07	0.50
Carcass width	0.56	0.40	0.39	0.07	0.13	0.37
Breast muscle weight	0.54	0.33	0.22	0.14	0.19	0.40
Liver weight	0.53	0.045*	0.41	0.38	0.59	0.18
Thigh muscle width	0.020*	0.78	0.81	0.022*	0.05	0.24
Thigh muscle length	0.026*	0.72	0.81	0.015*	0.06	0.16
Meat quality traits						
WBSF (kg/cm2)	0.26	1.00	1.00	0.13	0.94	0.043*
Cooking loss	0.91	0.006**	0.038*	0.19	0.16	0.92
Drip loss	0.31	0.34	0.43	0.12	0.29	0.36
pH 72hours	0.59	0.008**	1.00	0.24	0.63	0.31
L* 72 hours	0.65	0.37	0.37	0.43	0.19	0.83
a* 72 hours	0.47	0.27	0.48	0.98	0.05	0.20
b* 72 hours	0.41	0.61	0.61	0.58	0.84	0.68
pH 5days	0.48	0.011*	0.022*	0.19	0.15	0.74
L* 5days	0.85	0.58	0.32	0.81	0.43	0.94
a* 5days	0.64	0.98	1.00	0.58	0.59	0.93
b* 5days	0.19	0.010**	0.039*	0.82	0.70	0.82
pH 10days	0.41	0.008**	1.00	0.80	0.84	0.67
L* 10days	0.17	0.009**	1.00	0.46	0.94	0.16
a* 10days	0.79	0.32	0.32	0.46	0.94	0.12
b* 10days	0.024*	0.043*	0.038*	0.50	0.93	0.35

^ *Association with male individuals could not be analysed for Embden breed.*

RESULTS AND DISCUSSION

The genotypes at g.68G→A in *CAPN1* gene have a homogeneous distribution among subpopulations Embden and Toulouse, with the GG genotype predominant over others (Table 2). The AA genotype has been identified only in the Toulouse breed (0.10 in both, male and female).

Regardless of the well-documented fact that the *CAPN1* gene is related mainly to the process of meat maturation, the existence of linkage disequilibrium with other carcass and meat characteristics in poultry cannot be excluded. Thus, g.68G→A was statistically associated with different carcass and meat quality traits such as tight muscle parameters (width, $P = 0.020$; length, $P = 0.026$) and the b* 10days (meat color, $P = 0.024$) parameter for the global goose population. The association of this gene with meat tenderness (WBSF) was confirmed in the case of female individuals of the Toulouse breed ($P = 0.043$).

CONCLUSIONS

This research suggests that the novel **g.68G→A** SNP of the *CAPN1* gene is associated with some carcass and meat quality traits in the goose subpopulations raised in *dehesa* ecosystem. This opens the opportunity for selection to improve meat tenderness in goose breeds, but it is important to carry out future analyses in a larger animal sample.

67th EAAP annual meeting Belfast 2016

Short communication: Association analysis of g.68G→A SNP in CAPN1 gene with carcass and meat quality traits in goose raised in organic *dehesa*

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Abstract

Meat quality is an important concern for the poultry industry. Domestic geese products obtained from free-range systems usually have rather tough meat, and it is necessary to select them to improve meat tenderness. The relation of the *calpain 1* (CAPN1) gene with the tenderness process of meat post-mortem has been demonstrated in several species. Thus, the objective of the present study was to identify polymorphisms in this gene and to perform an association analysis between these polymorphisms with related economic traits in goose raised in a *dehesa* ecosystem. For the analysis, fifty geese of three different subpopulations (20 Embden-*Anser anser*; 10 F1 cross; 20 Toulouse-*Anser anser*) were studied. A novel SNP was found in the CAPN1 gene, g.68G→A. This Polymorphism was statistically associated with different carcass and meat quality traits such as tight muscle parameters (width, $p = 0.020$; length, $p = 0.026$) and the b* 10days (meat color, $p = 0.024$) parameter for the global goose population. The association of this gene with meat tenderness (WBSF) was confirmed in the case of female individuals of the Toulouse breed ($p = 0.043$). The results suggest the possibility of using molecular markers in CAPN1 gene as a potential tool for improving carcass and meat quality traits in goose breeding programs.

Key words: *Anser anser*; *calpain 1*; free-range; meat production; tenderness.

In the last few years there has been an increasing demand for the production of high quality poultry meat, with the traditional products such as those obtained from free-range animals the most highly valued (Solé *et al*., 2015). Generally, geese are usually produced on specialized commercial farms, and although the meat from intensively raised stocks has a soft texture, it is considered by many traditional consumers to have less flavour (F.A.O., 2004). Thus, alternative rearing systems such as *dehesa* emerge as ecosystems of high ecological value for the extensive production of high quality meat with outstanding and unique characteristics (Rey *et al*., 2006; Bouderoua *et al*., 2009; Keddam *et al*., 2010). In the case of the geese industry, some commonly domestic breeds used for meat production are the Embden-*Anser anser* and Toulouse-*Anser anser* breeds. Males of the Embden are characterized by their ability to gain weight rapidly, and they are good foragers (Jacob & Pescatore, 2013), while females of the Toulouse breed are mainly used for egg and *foie gras* production (Batty, 1996). Over the last 50 years, there has also been a great advance in the development of hybrid breeds for intensive commercial poultry production (F.A.O., 2004), and the Embden and Toulouse breeds have been popular as stock for generating these hybrid crosses for meat production: for instance, the Embden x Toulouse cross offspring grow rapidly and have good fleshing qualities (Jacob & Pescatore, 2013). The genetic improvement of goose characteristics reared in free-range conditions using classical phenotype recording is extremely complicated. Advances in molecular genetics have led to the identification of genes or genetic markers controlling the variation in reproductive ability or productive

characteristics (Kadarmideen, 2010). Contributions to improving both the efficiency and intensity of selection in many species of special interest for breeders have been provided in cattle (Avilés *et al.*, 2009; Casas *et al.*, 2005) or chickens (Felício *et al.*, 2013, Zhang *et al.*, 2008). In a previous study, in the Embden, the Toulouse and its F1 cross goose breeds, less tenderness was observed in comparison with other specialized poultry meat species (Solé *et a*l., 2015). In this case, a genetic selection of the animals is recommended in order to improve meat tenderness. Here, the CAPN1 gene is related to the biological process responsible for the disappearance of rigor mortis and subsequent meat tenderness (Avilés *et al*., 2009); however, it has not been described previously in *Anser anser*.

Therefore, the main objective of this study was to identify the CAPN1 gene in *Anser anser* and to perform an association analysis of single nucleotide polymorphisms (SNP) with carcass and meat quality traits in different goose breeds raised in organic *dehesa*. For the molecular characterization of the goose population, fifty geese of three different genotypes or subpopulations (half in each sex type: 20 Embden-*Anser anser*; 10 F1 cross; 20 Toulouse-*Anser anser*) were analyzed. All the animals were supplied by the companies Inddeco S.L. and Tierras de Ganso Ibérico S.L.

The following traits were evaluated: carcass characteristics (body weight, carcass weight, carcass length, carcass width, breast muscle weight, liver weight, tight muscle width, tight muscle length) and meat quality traits (Warner–Bratzler shear force; WBSF, kg/cm^2), cooking and drip loss, pH and colorimetric values (L*, lightness; a*, redness content; b*, yellowness content) measured 72hours, 5days and 10days after slaughter. A detailed description of the methodology used can be found in Sole *et al*. (2015).

Genomic DNA was extracted from muscle tissue samples of the breast of the animals using standard laboratory protocols (Roche Diagnostics, GmbH). The sequence of CAPN1 gene has not been previously described in the domestic goose (*Anser anser*). Thus, the primers for this gene fragment amplification (amplicon of 284 pb) were designed through the comparison of conservative regions of CAPN1 gene between *Gallus gallus* (NC_006090.3) and Peking duck *Anas platyrhynchos* (NW_004678518.1) GenBank sequences. The primers (F: 5'-CAGCTGCGGATCTTGTTC-3', R: 5'-GCTGGTTTAAGCATTGAGCT-3') were designed using the software Oligo Primer Analysis System© (Molecular Biology Insights, Inc., USA). The PCR reaction was performed using standard protocols in Eppendorf Thermal Cycler (Eppendorf® AG, Germany). The thermal profiling consisted of a hot starting step at 94º C for 4 min, followed by 35 cycles of 30 s at 95º C, 30 s at the annealing temperature of 58º C, 4 min at 72º C and a final extension step of 10 min at 72º C. The PCR products were analysed with Sequencher^{TM} v.4.6 software.

Allelic and genotypic frequencies and trait association analysis were performed in each goose subpopulation and in the global population using the Maximum-Likelihood Chi-square test in a first approximation. An association analysis of the detected SNP polymorphism was developed to determine marker effect with the different carcass and meat quality traits. For this purpose, the General Linear Model Procedure (proc GLM) was performed (SAS Version 9.2, SAS Institute, Cary, NC, USA). The polymorphism for the global population and for each genetic parental subpopulation was independently evaluated using different fixed effects (breed and sex variables), according to the trait of study.

In the present study, the sequences corresponding to the coding region of CAPN1 gene (GenBank KU363622, KU363624, KU363625) have been identified for the first time in the domestic goose (*Anser anser*). The micromolar calcium-activated neutral protease (CAPN1) gene encodes the μ-calpain protease (Page *et al*., 2002), belonging to the enzymatic complex which degrades myofibrillar proteins post-mortem (Avilés *et al*., 2009). Several SNPs in

CAPN1 gene have been previously associated to tenderness in different livestock species (e.g. Cattle: Avilés *et al*., 2009; Casas *et al*., 2005 or Chickens: Felício *et al*., 2013; Zhang et al., 2008). Moreover, the genotype CC of c.2554T→C polymorphism of this gene has been previously associated with an increase in live body weight in chickens (Felício *et al*., 2013).

Here, a novel SNP polymorphism in CAPN1 gene has been identified, named g.68G→A. The allelic and genotypic frequencies of this SNP in the three goose subpopulations (Embden-*Anser anser*, F1 cross, Toulouse-*Anser anser*) and for the global population were analysed (Table 1). It was shown that the genotypes have a homogeneous distribution among parental subpopulations Embden and Toulouse, with the GG genotype predominant over others. The AA genotype has been identified only in the Toulouse breed (0.10 in both male and female individuals). The allelic frequencies showed that the G allele was the predominant one in all goose subpopulations.

Table 1 Genotypic and allelic frequencies of polymorphism g.68G→A (CAPN1 gene) in the three goose subpopulations and in the global population.

Breed	**Sex**	**Genotypic frequencies**			**Allelic frequencies**	
		f(GG)	**f(AG)**	**f(AA)**	**f(G)**	**f(A)**
Embden	*Male*	1.00	0.00	0.00	1.00	0.00
	Female	0.40	0.60	0.00	0.70	0.30
	Overall	0.70	0.30	0.00	0.85	0.15
F1 cross	*Male*	0.60	0.40	0.00	0.80	0.20
	Female	0.20	0.80	0.00	0.60	0.40
	Overall	0.40	0.60	0.00	0.70	0.30
Toulouse	*Male*	0.80	0.10	0.10	0.85	0.15
	Female	0.60	0.30	0.10	0.75	0.25
	Overall	0.70	0.20	0.10	0.80	0.20
GLOBAL	*Male*	0.84	0.12	0.04	0.90	0.10
	Female	0.44	0.52	0.04	0.70	0.30
	Overall	0.64	0.32	0.04	0.80	0.20

Table 2 Association analysis between g.68G→A (CAPN1 gene) with carcass characteristics and meat quality traits using a General Linear Model in Toulouse and Embden subpopulations.

	Global	**Embden**		**Toulouse**		
	Genotype	***Global***	***Female^***	***Global***	***Male***	***Female***
Carcass characteristics						
Carcass weight	0.6020	0.1877	0.2386	0.1536	0.1002	0.6992
Carcass length	0.4093	0.8193	0.8192	0.0568	0.0681	0.5045
Carcass width	0.5641	0.4044	0.3873	0.0668	0.1335	0.3686
Breast muscle weight	0.5409	0.3295	0.2216	0.1398	0.1919	0.3971
Liver weight	0.5348	0.0449*	0.4092	0.3842	0.5865	0.1792
Thigh muscle width	0.0197*	0.7819	0.8104	0.0225*	0.0537	0.2404
Thigh muscle length	0.0256*	0.7209	0.8091	0.0149*	0.0635	0.1625

Meat quality traits						
WBSF (kg/cm2)	0.2578	1.0000	1.0000	0.1289	0.9414	0.0434*
Cooking loss	0.9065	0.0064**	0.0383*	0.1885	0.1587	0.9207
Drip loss	0.3071	0.3442	0.4256	0.1118	0.2856	0.3615
pH 72hours	0.5951	0.0077**	0.9959	0.2417	0.6353	0.3138
L* 72 hours	0.6477	0.3685	0.3718	0.4349	0.1892	0.8349
a* 72 hours	0.4709	0.2716	0.4845	0.9795	0.0537	0.1967
b* 72 hours	0.4099	0.6091	0.6133	0.5827	0.8357	0.6774
pH 5days	0.4785	0.0115**	0.0219*	0.1858	0.1457	0.7381
L* 5days	0.8460	0.5798	0.3174	0.8088	0.4340	0.9363
a* 5days	0.6399	0.9848	1.0000	0.5770	0.5867	0.9353
b* 5days	0.1910	0.0103**	0.0390*	0.8230	0.7024	0.8194
pH 10days	0.4112	0.0078**	0.9959	0.8013	0.8357	0.6684
L* 10days	0.1691	0.0089**	0.9959	0.4618	0.9441	0.1641
a* 10days	0.7910	0.3212	0.3172	0.4627	0.9441	0.1205
b* 10days	0.0242*	0.0427*	0.0383*	0.5010	0.9294	0.3457

^ *Association with male individuals could not be analysed for Toulouse breed. * P-value < 0.05; ** P-value < 0.01*

The association between CAPN1 and meat tenderness (WBSF) has been previously described in several species such as poultry and cattle (Felício *et al*., 2013; Page *et al*., 2002), but no significant association has been detected in the global goose population studied. The association analyses between g.68G→ASNP with carcass and meat quality traits using different fixed effects (breed, sex and genotype variables) for the global goose population and the parental subpopulations are shown in Table 2. The association in the Toulouse male individuals could not be analysed because the 100% of individuals presented AA genotypes. Regardless of the well-documented fact that the analysed CAPN1 gene is related mainly to the process of meat maturation in mammalian species, the existence of linkage disequilibrium with other carcass and meat characteristics in chickens cannot be excluded (Zhang *et al*., 2008; Felício *et al*., 2013). Thus, for the global goose population, a significant association was found between the CAPN1 SNP with the carcass characteristics of tight muscle parameters (width, p = 0.0197; length, p = 0.026). According to the meat quality traits, the parameter b* 10days (meat color, p = 0.024) was statistically significant. Besides, a significant association has also been found with tight muscle parameters (p = 0.023 for width; p = 0.015 for length) in the global Toulouse subpopulation. For the Embden female individuals, a significant association has been found with meat quality traits such as cooking loss (p = 0.038), pH at 5days (p = 0.022), and meat color: b* 5days (p = 0.039) and b* 10days (p = 0.038). However, a significant association has been found in the case of female individuals of the Toulouse subpopulation (p = 0.043; Table 2).

In conclusion, this research suggests that the novel g.68G→A SNP polymorphism of the CAPN1 gene is associated with some carcass and meat quality traits such as tight muscle width and length and b* 10days (meat color) parameters in the goose subpopulations raised in *dehesa* ecosystem. The association of this snp marker with meat tenderness (WBSF) was statistically significant in the case of female individuals of the Toulouse breed. This opens the opportunity for selection to improve meat tenderness in goose breeds. It is therefore important to carry out future analyses into the association of these trait effects and CAPN1 gene in a larger animal sample to prove if this association is maintained.

Acknowledgements: S. Negro was in receipt of a grant from the Unión de Entidades Españolas de Ciencia Animal (UEECA) for attending the 2016 EAAP meeting in Belfast.

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