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

Marina Solé[1], Sara Negro[1], Alberto Membrillo[2], Francisco Peña[3], Valeriano Domenech[3], Juan Carlos Verona[4], Maria Rubí[4], Mercedes Valera[1], and Antonio Molina[2]

[1]Departamento de Ciencias-Agroforestales, Universidad de Sevilla, Seville, 41013, Spain
[2]Departamento de Genética, Universidad de Córdoba, Córdoba, 14071, Spain
[3]Departamento de Producción Animal, Universidad de Córdoba, Córdoba, 14071, Spain
[4]Inddeco S.L. Ganso Ibérico de Dehesa, Madrid, 28200, Spain

Correspondence to: Marina Solé (ge2sobem@uco.es)

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 post-mortem tenderness process of meat 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 and related economic traits in goose raised in the *dehesa* ecosystem. For the analysis, 50 geese of 3 different subpopulations (20 Embden *Anser anser*; 20 Toulouse *Anser anser*; 10 F1 cross) were studied. The experimental protocols were followed complying with principles of animal welfare. 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 thigh muscle width ($P = 0.020$) and the b* 10-day meat colour parameter ($P = 0.024$) for the global goose population. The association of this gene with meat tenderness (Warner–Bratzler shear force) 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 programmes.

1 Introduction

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., 2016). 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 (FAO, 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 and 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 (FAO, 2004), and the Embden and Toulouse breeds have been popular as stock for generating these hybrid crosses for meat production: for instance, the Embden × Toulouse cross offspring grow rapidly and have good fleshing qualities (Jacob and Pescatore, 2013).

The genetic improvement of goose characteristics reared in free-range conditions using classical phenotype recording is extremely complicated. The application of genetic selection and the use of molecular technologies have played a key role in improving the productivity in animal breeding programmes. 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). 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 during post-mortem tenderization and is responsible for meat tenderness (Avilés et al., 2009). Several SNPs in CAPN1 gene have been previously associated with tenderness in different livestock species (e.g. Cattle: Avilés et al., 2013; Casas et al., 2005; Costello et al., 2007 or Chickens: Felício et al., 2013; Shu et al., 2015; Zhang et al., 2008); however, it has not been previously described in *Anser anser*. Moreover, the genotype CC of c.2554T → C polymorphism of CAPN1 gene has been previously associated with an increase in live body weight (Felício et al., 2013) and the AA genotype of the c.3535G → A was associated with smaller WBSF values, lower water loss rates, and higher intramuscular force contents in chickens (Shu et al., 2015).

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 al., 2016). In this case, a genetic selection of the animals together with the improvement of production systems and processing practices is recommended in order to improve meat tenderness.

Therefore, the main objective of this study was to identify polymorphisms in the CAPN1 gene in *Anser anser* and to perform an association analysis with carcass and meat quality traits in different goose breeds raised in organic *dehesa*.

2   Material and methods

2.1   Animals and sampling

For the molecular characterization of the goose population, a limited sample of 50 geese of 3 different genotypes or subpopulations (half in each sex type: 20 Embden *Anser anser*; 20 Toulouse *Anser anser*; 10 F1 cross) were analysed, derived from a previous study (Solé et al., 2016). 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, thigh muscle width, thigh muscle length) and meat quality traits (Warner–Bratzler shear force; WBSF, kg cm^{-2}), cooking and drip loss, pH and colorimetric values of the breast muscle (*L**, lightness, a measure of the light reflected (100 = white; 0 = black); a*,

redness index, measures positive red and negative green; b*, yellowness index, measures positive yellow, negative blue) measured 72 h, 5 days and 10 days after slaughter. A detailed description of the methodology used can be found in Solé et al. (2016). The experimental protocols using meat geese in this study were followed complying with principles of animal welfare according to Spanish rules and regulations (EU Council Directive 86/609/EEC).

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 was performed using standard protocols in Eppendorf Thermal Cycler (Eppendorf$^{®}$, Germany). The thermal profiling consisted of a hot starting step at 94 °C for 4 min, followed by 35 cycles of 30 s at 95 °C, 30 s at the annealing temperature of 58 °C, 4 min at 72 °C and a final extension step of 10 min at 72 °C. The PCR products were analysed with Sequencher™ v.4.6 software.

2.2   Statistical analysis

Allelic and genotypic frequencies were performed in each goose subpopulation and in the global population. The Hardy–Weinberg equilibrium (HWE) was tested ($\alpha = 0.05$) with Genepop software (Version 3.4) (Rouseet, 2008). 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 logistic regression procedure (Fagerland et al., 2008) was performed to determinate the association of each genotype (they were coded as 0, 1, and 2 for the number of risk alleles carried by individual subjects) in carcass and meat quality traits. To obtain the receiver operating characteristic (ROC) curve (Brethour, 2000) on a multi-marker basis, the values of the traits were transformed using two response levels, high (1) and low (0) values, according to their contribution to carcass and meat quality. This showed the predictive power of this molecular multi-marker tool through the percentage of area under the curve (AUC) in ROC space, equivalent to the probability that the classifier will rank a randomly chosen positive instance higher than a randomly chosen negative instance (Brethour, 2000). Sensitivity against 1-specificity is plotted, with the area under the curve often being used as a measure of discriminatory performance (1 perfect prediction; 0.5 random guess).

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

Furthermore, when the association was statistically significant, the percentage of variance absorbed for the gene marker was measured for each goose sex type, subpopulation and in the global population using the general linear model (PROC GLM) and following the Gianola et al. (2009) methodology. The parameter SGoF+ (Carvajal-Rodriguez and Uña Álvarez, 2011) was used to control the false discovery rate (FDR) of the p values at a significance level of 5 % and a q(FDR) of 5 %. Statistical analysis was performed using SAS 9.2 (Statistical Analysis System, Inc., Cary, USA).

3   Results and discussion

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*). 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). All the frequencies conformed to HWE ($P = 0.474$). 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.

The association between CAPN1 and meat tenderness (WBSF) has been previously described in several species such as poultry and cattle (Page et al., 2002; Mazzucco et al., 2010; Felício et al., 2013), but no significant association has been detected in the global goose population
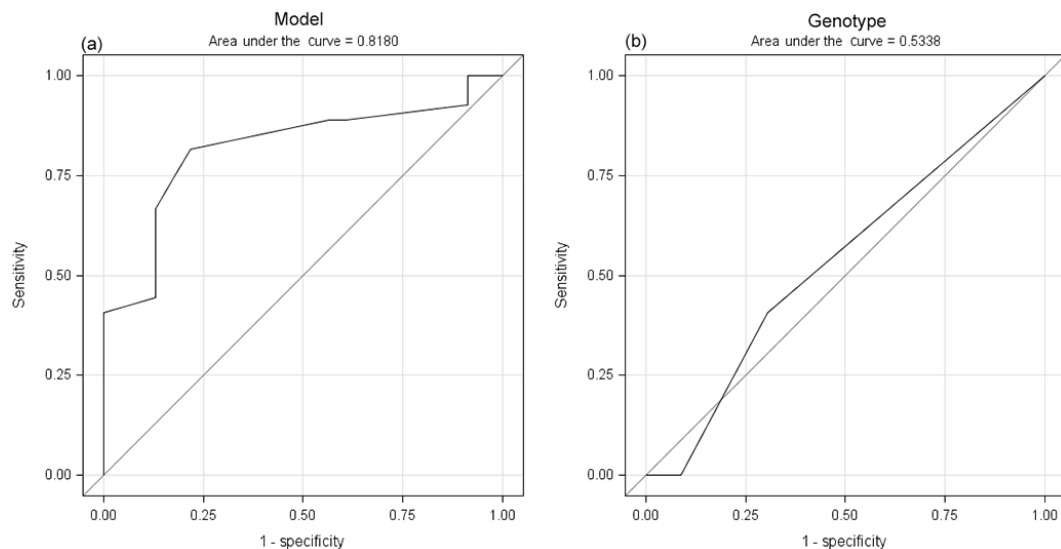
studied. The association analyses between g.68G → A SNP with carcass and meat quality traits using different fixed effects (breed, sex and genotype variables) are shown in Table 2. The association in the Embden male individuals could not be analysed because 100 % of individuals presented GG genotypes. 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 thigh muscle width ($P = 0.0197$) and the percentage of variance explained by the gene marker, V_a, was 10.97 %. According to the meat quality traits, the b* 10-day parameter (meat colour, $P = 0.024$; $V_a = 5.47\%$) was statistically significant. The ROC curves corresponding to statistically significant genetic association between the genotypes and these significant parameters are presented in Fig. S1a and S1b in the Supplement, respectively. The percentages of area under the curve in ROC space (prediction accuracy of the models with selected markers) referring to thigh muscle width and b* 10-day parameter (meat colour) were 0.54 and 0.60, respectively. These values increased to 0.77 and 0.75, respectively, when all the factors are included in the model for the global population.

Besides, a significant association has also been found with liver weigh ($P = 0.045$; $V_a = 4.16\%$), cooking loss ($P = 0.006$; $V_a = 47.14\%$), pH at 3 days ($P = 0.008$; $V_a = 53.95\%$), pH at 10 days ($P = 0.008$; $V_a = 44.10\%$) and L^* 10-day ($P = 0.009$; $V_a = 75.17\%$) parameters in the global Embden subpopulation. For the Embden female individuals, a significant association has been found with meat quality traits such as cooking loss ($P = 0.038$;

Table 2. Association analysis between g.68G → A (CAPN1 gene) with carcass characteristics and meat quality traits using a logistic regression procedure and general linear model in Toulouse and Embden subpopulations.

P values	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**[a]	0.4092	0.3842	0.5865	0.1792
Thigh muscle width	**0.0197**[a]	0.7819	0.8104	0.0225[a]	0.0537	0.2404
Thigh muscle length	0.0256[a]	0.7209	0.8091	0.0149[a]	0.0635	0.1625
Meat quality						
WBSF (kg cm^{-2})	0.2578	1.0000	1.0000	0.1289	0.9414	**0.0434**[a]
Cooking loss	0.9065	**0.0064**[b]	**0.0383**[a]	0.1885	0.1587	0.9207
Drip loss	0.3071	0.3442	0.4256	0.1118	0.2856	0.3615
pH 72 h	0.5951	**0.0077**[b]	0.9959	0.2417	0.6353	0.3138
L* 72 h	0.6477	0.3685	0.3718	0.4349	0.1892	0.8349
a* 72 h	0.4709	0.2716	0.4845	0.9795	0.0537	0.1967
b* 72 h	0.4099	0.6091	0.6133	0.5827	0.8357	0.6774
pH 5 days	0.4785	0.0115[b]	**0.0219**[a]	0.1858	0.1457	0.7381
L* 5 days	0.8460	0.5798	0.3174	0.8088	0.4340	0.9363
a* 5 days	0.6399	0.9848	1.0000	0.5770	0.5867	0.9353
b* 5 days	0.1910	0.0103[b]	**0.0390**[a]	0.8230	0.7024	0.8194
pH 10 days	0.4112	**0.0078**[b]	0.9959	0.8013	0.8357	0.6684
L* 10 days	0.1691	**0.0089**[b]	0.9959	0.4618	0.9441	0.1641
a* 10 days	0.7910	0.3212	0.3172	0.4627	0.9441	0.1205
b* 10 days	**0.0242**[a]	0.0427[a]	0.0383[a]	0.5010	0.9294	0.3457

* Association with male individuals could not be analysed for Embden breed. [a] $P < 0.05$. [b] $P < 0.01$. Bold values correspond to significant p values after applying SGoF+ correction for multiple testing.

Figure 1. Receiver operating characteristic (ROC) curve analysis for the gene and the model predictive ability evaluation of meat tenderness (WBSF) in the global goose population.

$V_a = 74.98\%$) and pH at 5 days ($P = 0.022$; $V_a = 60.48\%$). The Fig. S2 shows the ROC curves corresponding to the predictive level of the model and the significant parameters (cooking loss, 0.90 and pH 5 days, 0.90), where the model shows a greater predictive ability. These findings suggested a biological implication of the CAPN1 gene in the metabolic pathway of post-mortem meat process in geese, as have been stated in other species before (Costello et al., 2007; Mazzucco et al., 2010; Shu et al., 2015).

However, a significant association has been found in the case of female individuals of the Toulouse subpopulation ($P = 0.043$; $V_a = 37.86\%$) for WBSF, as it has been previously mentioned that the females of this breed are mainly used for egg and foie gras production, therefore more selected for meat qualities than rate of growth (Jacob and Pescatore, 2013). The ROC curve analyses corresponding to the association between the genotype and WBSF in the global goose population and in the Embden and Toulouse subpopulations are shown in Figs. 1 and S3a–d, respectively. For the global goose population, the predictive power of the SNP marker is reduced (0.53), but the model including all the fixed effects (breed, sex, and genotype) shows high predictive ability (0.82). In the case of the Toulouse breed, the predictive power of the SNP (0.75) is higher compared to the Embden subpopulation (0.59). Finally, the complete model for the two subpopulations presents highly predictive ability (0.92 and 0.94, respectively).

4 Conclusions

This research describes for the first time part of the polymorphic coding region of CAPN1 gene in *Anser anser* and suggests that the novel g.68G → A SNP polymorphism of this gene is associated with some carcass and meat quality traits such as thigh muscle width and b* 10-day (meat colour) parameters in the goose subpopulations raised in *dehesa* ecosystem. The association of this SNP marker with post-mortem meat quality traits suggests that this gene participates in the metabolic pathway in goose. This opens the opportunity for selection to improve meat tenderness in goose breeds. Due to the limitations of the sample size, 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 whether this association is maintained.

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