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PAPER

Dry-cured ham *Kraški pršut* seasoning losses as affected by PRKAG3 and CAST polymorphisms

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Abstract

Association between polymorphisms on *PRKAG3* (*Ile199Val*) and CAST (*Lys249Arg and Ser638Arg*) genes and dry ham seasoning losses was studied. A total of 724 green hams (same pig crossbreed, same pig producer) were selected, genotyped (PCR-RFLP) and processed according to the rules of *consortium* for dry-cured ham *Kraški pršut*. Weight losses after each processing phase were recorded. We observed significant effect of interaction between gene polymorphism and dry ham producer on seasoning losses, indicating that the effect of studied genes differ in relation to manufacturing practice or product type, despite narrow *consortium* constraints. The analysis was thus made separately for each producer; in case of producer B, *PRKAG3* affected salting, resting and overall losses (*Val/Val* higher than *Ile/Ile or Ile/Val*) but in case of producer A, the effect of PRKAG3 was significant only for salting losses (*Ile/Val* lower than *Ile/Ile or Val/Val*). Effects of *CAST* polymorphisms were significant only in case of producer A; *CAST249 Arg/Arg* hams showed the highest first salting, drying, and overall seasoning losses, whereas *CAST638 Arg/Arg* hams had the highest drying, ripening, and overall seasoning losses. In conclusion, *PRKAG3* and *CAST* polymorphisms were associated with seasoning losses, important from economic viewpoint, but also for salt intake and product quality.

Introduction

A dynamics of ham dehydration and salt intake is important for activities of endogenous enzymes (Buscailhon and Monin, 1994; Toldra and Flores, 1998) and consequently for the characteristics of the final product. There are two key factors determining production yields and quality of dry ham, the preservation technique and raw material properties (Toldra, 2002). In general, according to Russo and Nanni Costa (1995), higher seasoning losses are associated with leaner hams or low quality (PSE) meat so there is an antagonism between leanness, seasoning yield and quality of dry ham. Recent reviews (Garnier *et al*., 2003; Rosenvold and Anderson, 2003; Mancini and Hunt, 2005; Barbut *et al*., 2008) have documented the association between certain genetic polymorphisms and carcass properties or meat quality i.e. water holding capacity, pH, colour, fatness and ham weight. Among the investigated genes, *PRKAG3* and *CAST* were considered as promising. *PRKAG3* gene encodes a specific isoform of γ subunit of the adenosine monophosphate dependent protein kinase (AMPK), an enzyme with the key role in cell energy metabolism regulation. Five non-synonymous substitutions in the *PRKAG3* gene have been demonstrated (Milan *et al*., 2000). Besides the well known *RN*$^-$ mutation (*Arg200Gln* substitution), causing the so called acid meat (Sellier and Monin, 1994), the *Ile199Val* polymorphism has been proven to affect carcass leanness, meat colour properties, muscle glycogen content, muscle pH and water holding capacity (Ciobanu *et al*., 2001; Lindahl *et al*., 2004 a, b; Enfält *et al*., 2006; Otto *et al*., 2007; Ramos *et al*., 2008; Škrlep *et al*., 2009). The second gene (*CAST*) encodes for calpastatin, a physiological inhibitor of calpain enzymes (Goll *et al*., 2003) that are responsible for early *post mortem* muscle proteolysis (Koohmaraie and Geesink, 2006) and meat tenderization (Koohmaraie, 1992). Various polymorphisms on *CAST* gene have been associated to pork texture, backfat thickness, meat colour, leanness, pH value and also dry-cured ham weight, water content and colour (Emnett *et al*., 2000; Kurył *et al*., 2003; Ciobanu *et al*., 2004; Stalder *et al*., 2005; Krzęcio *et al*., 2005; 2008). Although there is some evidence of *PRKAG3* or *CAST* effect on pork quality, the information regarding the influence of these polymorphisms in regard to dry curing process is lacking. An association between *PRKAG3* or *CAST* genetic polymorphisms and green ham properties was observed in our previous study (Škrlep *et al*., 2010), which could influence dry ham processing. For that reason we were interested in the evaluation of the effect of three polymorphisms, *PRKAG3 Ile199Val*, *CAST Arg249Lys*, and *CAST Ser638Arg* on the dynamics of dry ham seasoning losses taking into consideration two different producers (processing techniques).

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Materials and methods

Animals and slaughter

The hams included in the present study (n=724) were harvested from commercial pig fatteners (198±9 days old) originating from one pig producer and one crossing (maternal line Landrace×Large White and paternal line Duroc×Hampshire). Pigs from this herd were previously demonstrated to be free of *RYR1* and *RN*$^-$ mutations (Škrlep *et al*., 2009). Pigs were slaughtered in one commercial abattoir in ten batches within ten weeks period (from October to January) according to the routine slaughter procedure i.e. CO_2 stunning, vertical exsanguination, vapour scalding, dehairing and evisceration followed by veterinary inspection and carcass classification. Carcasses were

cooled (by storage at 0-2°C) overnight. The following day the hams were cut off the carcass between 6th and 7th lumbar *vertebra* and sent to the producer of dry ham. Two producers of dry hams participated in the present study and received hams in alternating weeks giving in total five batches of hams per each producer.

Genotype determination

Small pieces of skin tissue were taken from each animal for genotyping. After the final selection of hams, the samples were genotyped using PCR-RFLP method according to Ciobanu *et al*. (2001; 2004) for *PRKAG3 Ile199Val* and for *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms.

Green ham evaluation

Upon arrival to the producer (second day after the slaughter) the hams were trimmed into a prescribed shape and selected according to *consortium* rules for dry ham *Kraški pršut* i.e. for green ham weight (≥9.5 kg), subcutaneous fat thickness below *caput ossis femoris* (≥10 mm) and acceptable visual appearance (absence of skin lesions, good muscle cohesion, without soft, pale, exudative aspect). The measurements of pH value 48 hours *post-mortem* were taken with pH Meter MP120 (Mettler-Toledo GmbH, Schwarzenbach, Switzerland) in *semimembranosus* muscle (SM). An average of measurements (pH SM) on two locations was calculated; first measurement was taken on the caudal edge of the open surface of SM muscle and the second on the inner edge of SM muscle next to the *caput ossis femoris*.

Ham processing

Seasoning losses were registered after each processing step (first salting, second salting, resting, drying, and ripening). They were calculated as percentage of ham weight prior to each processing step. Overall seasoning losses were expressed as percentage of trimmed ham weight. According to the *consortium* rules for dry ham *Kraški pršut* only sea salt is allowed as the conservation additive. Duration and steps of the seasoning are presented in Figure 1. Green hams were first put to salting for 2-3 weeks at 2-4°C with two salting stages; the first salting (salting 1) lasted 7 days and the second salting (salting 2) 7 or 14 days, depending on the processor. After the salting had been completed, the hams were left to rest for about 9-10 weeks at 4-6°C and 70-85% of relative humidity. Following this equilibration period, the hams were submitted to drying at 14-20°C and 60-80% relative air humidity. When hams (lot average) attained a required weight loss (25%), the open surface of the hams was coated with a mixture of fat, flour and spices to permit ripening while preventing further major desiccation. According to the *consortium* rules for dry ham *Kraški pršut* a minimum of 33% weight losses (lot average) and 12 months of age is required. In this experiment, the hams were boned and prepared for sale after 60 weeks of processing.

Statistical analysis

Analysis of variance was performed using procedure GLM of statistical package SAS (2001). The model consisted of the fixed effects of dry-cured ham producer, batch within dry-cured ham producer, marker genotype, and interaction between marker genotype and dry-cured ham producer. No significant interaction between marker genotype and dry-cured ham producer was observed in case of raw material traits. Due to the detected significant effect of interaction marker genotype×dry-cured ham producer on seasoning losses, the effect of individual gene was analysed within dry-cured ham producer (SLICE option). In case of significant effects, least squares means were compared (LSMEANS, PDIFF and Tukey-Kramer option). Additionally, *CAST* haplotypes were compared. Haplotypes inferred between *CAST249* and *CAST638* were analysed considering animals having 0, 1 or 2 copies of the haplotype in question using equivalent model to the one applied for single marker tests. Here also, the significant effect of the interaction between dry-cured ham producer and two haplotypes (*Arg249/Arg638* and *Arg249/Ser638*) was detected so the results were presented within dry-cured ham producer.

Results and discussion

Differences between dry-cured ham producers

Due to the absence of any significant interaction between marker genotype and dry-cured ham producer on raw material traits, these are presented only in relation to the producer. Moreover, the influence of studied genetic polymorphisms on raw material quality has been presented and discussed in our previous article (Škrlep *et al*., 2010). In spite of the fact that two producers respected the same general consortium rules regarding raw material quality, processing duration and constraints on seasoning loss, we could note differences between them (Table 1).

Similar ham weight before trimming and similar ham fat thickness are indicative that both producers received equivalent raw material. However, after the trimming of the hams into the prescribed shape and in-house selection of hams for further processing, we could note lower trimmed ham weight (*i.e.* higher trimming loss) and higher ham pH for producer B. These results reveal that producers differed in trimming routine and in the severity of ham selection according to ham aspect (indirectly according to ham pH). Moreover, despite practically the same processing duration and slightly heavier trimmed ham weight, producer A demonstrated 0.7% points (P=0.006) higher seasoning losses. Considering green ham properties the explanatory factor for this difference could be pH. Namely, higher seasoning losses have been associated with lower pH value (Arnau *et al*., 1987) or PSE

Table 1. Raw material properties and seasoning losses according to dry-cured ham producer.

Trait	LSM±SE		P
	Producer A	Producer B	
Raw material°			
Ham, kg	15.0±0.1	15.0±0.1	0.845
Trimmed ham, kg	11.1±0.1	10.8±0.1	0.007
Ham fat, mm	13.6±0.2	13.4±0.2	0.578
SM pH	5.71±0.01	5.80±0.01	0.000
Seasoning loss#, %			
Salting 1	2.3±0.03	2.3±0.02	0.279
Salting 2	1.9±0.02	1.6±0.02	0.000
Resting	18.0±0.08	17.1±0.08	0.000
Drying	7.9±0.05	6.3±0.05	0.000
Ripening	12.0±0.11	13.8±0.11	0.000
Overall	36.3±0.17	35.6±0.17	0.006

°Ham was weighed when cut off the carcass and after the trimming into a prescribed shape. Ham fat thickness was measured on a trimmed ham below *caput ossis femoris*. The value of pH is an average of two measurements taken at two different sites in *m. semimembranosus*. #Seasoning loss is expressed as % of ham weight prior to each processing step. Overall seasoning loss is expressed as % of trimmed ham weight. SM, semimembranosus muscle.

meat (Maggi and Oddi, 1988; Griot *et al*., 1998). It can also be further supported with our observation during the experiment, that producer B was stricter in selection according to ham aspect and muscle cohesion (Čandek-Potokar *et al*., 2007), thus very likely rejecting more hams with lower pH. Although overall processing duration was similar for both producers, differences in duration of individual processing steps could be observed (Figure 1). Thus producer A, compared to producer B, practices longer salting, resting and drying, but shorter ripening period. As a result, the rate of ham dehydration is different for two producers (Figure 2); we could observe that producer A maintained lower dehydration rate compared to producer B.

In case of producer A we observed higher second salting, resting and drying losses (Table 1), due to longer duration of these steps. Only the ripening losses were higher in case of producer B, in agreement with longer ripening period. Ham seasoning losses are not important just from the economic point of view, but also for dry ham properties. The extent of weight loss at the end of seasoning is a key factor for water content, an attribute that is important for sensory properties of dry ham such as saltiness and texture (Ruiz-Ramirez *et al*., 2005) and also differentiates the products of the same type. For example, in the case of Italian *prosciutto*, final processing losses can vary from 20 to 30% (Russo and Nanni Costa, 1995) with the average values about 27% (Nanni Costa *et al*., 1993), while in the case of Slovenian *Kraški pršut* seasoning losses reported are higher and in the range of 34-37% (Čandek-Potokar *et al*., 2002). Since the Slovenian consortium of *Kraški pršut* producers prescribes minimal extent of dehydration losses (33%) and minimal processing duration (12 months), the options for product differentiation between the producers are limited. As observed in the present study, the way that producers manage to give certain uniqueness to their products is trough water loss dynamics, which can be expected to influence the activity of endogenous enzymes and consequently sensory quality (Toldra, 2006).

Effect of *PRKAG3*

Significant interaction between *Ile199Val* polymorphism and producer were found for seasoning losses for the initial seasoning steps (salting, resting) and consequently at the end of seasoning (*data not shown*). This result implies that the genotype of *PRKAG3* could be affected by processing technique and intensity of raw material selection. Therefore, the effect of the *PRKAG3* genotype was observed separately for each producer (Table 2).

PRKAG3 effect was more pronounced in producer B, which did a more severe trimming and had higher SM pH. In case of producer A, the effect of *PRKAG3* genotype was significant only for the first salting phase, with *Ile/Val* hams exhibiting lower salting losses as *Ile/Ile* or *Val/Val*. As a result, we could observe a bit lower (P<0.10) overall seasoning losses for *Ile/Val* hams. In case of producer B, the *PRKAG3* genotype affected significantly ham weight losses during initial steps (first salting and resting), and consequently accumulated losses at the end of seasoning, with *Val/Val* hams exhibiting the highest losses after salting, resting and consequently overall losses. It is noteworthy that both producers received equivalent material (insignificant interaction between *PRKAG3* genotype and producer on raw material), so the effect of *PRKAG3* genotype may be related to its effect on green ham traits (fat thickness, meat quality) presented and discussed in our previous study (Škrlep *et al*., 2010). Namely, in the mentioned study, *Ile/Val* pigs had hams with the thickest fat, while *Val/Val* pigs exhibited the lowest meat quality. Contrary to our results, in the few comparable studies on US country dry hams (Stalder *et al*., 2005; Ramos *et al*., 2008) no significant effect of *PRKAG3* genotype was observed on processing yields. However, in agreement with our results, a bit lower yield was reported by Stalder *et al*. (2005) for *Val/Val* genotype. Regarding the effect of *PRKAG3* genotype on pork quality, the available literature indicates a favourable effect of the allele 199Ile (or unfavourable of allele 199Val) on pH or drip loss (Ciobanu *et al*., 2001; Lindahl *et al*., 2004a; Otto *et al*., 2007; Škrlep *et al*., 2009) and the association of allele *Ile* with higher fatness (Enfält *et al*., 2006) which can help in explaining why higher seasoning losses were observed for *Val/Val* hams. Namely, research reports demonstrate that higher seasoning losses are associated with lower fat thickness (Čandek-Potokar *et al*., 2002; Bosi and Russo, 2004), lower pH value (Arnau *et al*., 1987) or PSE meat (Maggi and Oddi, 1988; Griot *et al*., 1998). However, in the range of normal pH values, this relationship is not very strong

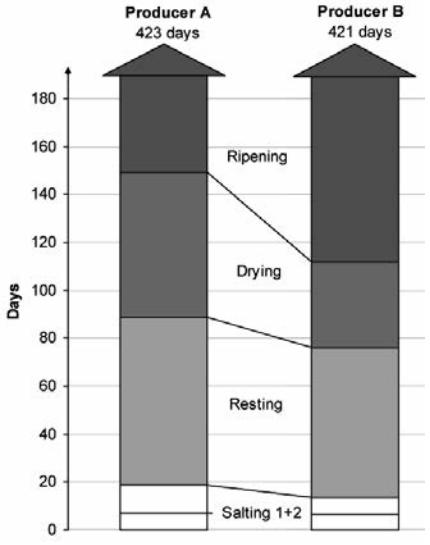

Figure 1. Seasoning phases and duration according to dry ham producer.

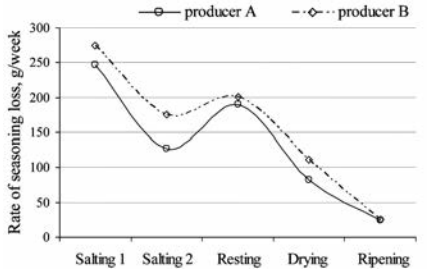

Figure 2. Rate of ham seasoning losses (g/week) according to phase and producer.

Table 2. Ham seasoning losses as affected by *PRKAG3 Ile199Val* polymorphism.

	LSM ± SE for *PRKAG3* effect sliced by producer							
	Producer A				Producer B			
	Ile/Ile	*Ile/Val*	*Val/Val*	P	*Ile/Ile*	*Ile/Val*	*Val/Val*	P
Number	49	196	107		45	198	129	
Salting 1	2.3±0.05^{b}	2.2±0.03^{a}	2.3±0.03^{b}	0.003	2.3±0.05^{a}	2.3±0.02a	2.4±0.03^{b}	0.003
Salting 2	1.9±0.04	1.9±0.02	1.9±0.03	0.314	1.5±0.04	1.6±0.02	1.6±0.03	0.044
Resting	18.1±0.16	17.9±0.08	18.1±0.11	0.178	16.8±0.16^{a}	17.0±0.08^{a}	17.5±0.10^{b}	0.000
Drying	8.0±0.10	7.9±0.05	7.9±0.07	0.462	6.2±0.10	6.3±0.05	6.4±0.06	0.184
Ripening	12.1±0.22	11.8±0.12	12.0±0.14	0.156	13.8±0.22	13.8±0.11	14.0±0.14	0.364
Overall	36.6±0.34	35.9±0.18	36.4±0.23	0.090	35.3±0.34^{a}	35.5±0.17^{a}	36.2±0.22^{b}	0.010

Seasoning loss is expressed as % of ham weight prior to each processing step; overall seasoning loss is expressed as % of trimmed ham weight; LSM, least squares means; ^{a,b}with different superscripts are significantly different (P<0.05).

(Čandek-Potokar *et al*., 2002; Ramos *et al*., 2007). Our results also indicate that the effect of *PRKAG3* genotype on seasoning losses was producer dependent, and was more pronounced in case of higher rate of dehydration (e.g. first steps in processing, in case of producer B).

Effect of *CAST*

Here again, no interaction between dry-cured ham producer and *CAST* gene on green ham traits was detected, whereas significant interactions between genotype (and haplotype) and dry ham producer were observed for seasoning losses. Due to detected significant interactions, which imply that *CAST* effect on seasoning losses is likely to depend on product type or manufacturing practice, the effects of *CAST* polymorphisms (and haplotypes) were investigated separately for each producer. Moreover, in the present study two *CAST* polymorphisms *Lys249Arg* (Table 3) and *Ser638Arg* (Table 4) were examined and results presented separately. However, it is noteworthy that they are not independent. As shown in our previous article (Škrlep *et al*., 2010), certain genotype combination was never identified i.e. *249Lys/638Ser*, which is consistent with the results of Ciobanu *et al*. (2004) reporting only three haplotypes on *CAST* gene (*249Lys/638Arg*, *249Arg/638Arg*, *249Arg/638Ser*).

Looking at *CAST249* (Table 3), we could observe significant differences between polymorphic variants in case of producer A (first salting, drying and overall seasoning losses), but not in case of producer B. The effect of *CAST638* (Table 4) was also more pronounced in case of producer A (resting, drying, ripening phase) compared to producer B (second salting phase only). Comparison of genotypes at *CAST249* shows the highest seasoning losses for *Arg/Arg* hams in case of producer A, but no differences in case of producer B. Regarding *CAST638*, the highest seasoning losses were observed for *Arg/Arg* hams in case of producer A, and no differences in case of producer B. According to these results, the haplotype *249Arg/638Arg* would be the least acceptable (expected to give the lowest seasoning yields) which was also confirmed by haplotype analysis (Tables 5 and 6). Haplotype analysis also indicated a tendency for lower seasoning losses associated with *249Arg/638Ser* haplotype.

The effect of *CAST* is difficult to comment since there is a lack of comparable literature. The extent of seasoning loss in dry-cured ham production is related mainly to fat thickness and to pH (Russo and Nanni Costa, 1995; Čandek-Potokar and Škrlep, 2011). In the present study the key factor, ham thickness was not

Table 3. Ham seasoning losses as affected by *CAST Lys249Arg* polymorphism.

	CAST249 effect sliced by producer							
	Producer A LSM ± SE				Producer B LSM ± SE			
	Lys/Lys	*Lys/Arg*	*Arg/Arg*	P	*Lys/Lys*	*Lys/Arg*	*Arg/Arg*	P
Number	49	188	114		80	183	109	
Salting 1	2.3±0.06^{ab}	2.2±0.03^{a}	2.4±0.03^{b}	0.011	2.3±0.05	2.4±0.03	2.3±0.03	0.272
Salting 2	1.9±0.05	1.9±0.03	1.9±0.03	0.173	1.5±0.04	1.5±0.03	1.6±0.03	0.067
Resting	17.9±0.18	18.0±0.10	18.2±0.11	0.231	17.13±0.15	17.1±0.11	17.1±0.10	0.909
Drying	7.8±0.11^{a}	7.8±0.06a	8.2±0.07^{b}	0.001	6.3±0.10	6.3±0.07	6.3±0.06	0.899
Ripening	11.7±0.25	11.9±0.13	12.3±0.15	0.064	13.8±0.21	13.7±0.15	14.0±0.14	0.538
Overall	35.9±0.39^{ab}	36.1±0.21a	36.9±0.23^{b}	0.019	35.6±0.33	35.6±0.23	35.8±0.22	0.79

Seasoning loss is expressed as % of ham weight prior to each processing step; overall seasoning loss is expressed as % of trimmed ham weight; LSM, least squares means; ^{a,b}with different superscripts are significantly different (P<0.05).

Table 4. Ham seasoning losses as affected by *CAST Ser638Arg* polymorphism.

	CAST638 effect sliced by producer							
	Producer A				Producer B			
	Ser/Ser	*Arg/Ser*	*Arg/Arg*	P	*Ser/Ser*	*Arg/Ser*	*Arg/Arg*	P
Number	53	171	124		53	150	168	
Salting 1	2.2±0.06	2.3±0.03	2.3±0.03	0.283	2.4±0.05	2.3±0.03	2.3±0.03	0.130
Salting 2	1.8±0.05	1.9±0.03	1.9±0.03	0.196	1.5±0.05a	1.6±0.03^{b}	1.6±0.02^{ab}	0.003
Resting	17.8±0.18^{a}	18.0±0.11^{a}	18.4±0.10^{b}	0.005	17.1±0.17	17.2±0.10	17.0±0.09	0.484
Drying	7.7±0.11^{a}	8.0±0.07^{b}	8.1±0.06^{b}	0.004	6.3±0.11	6.4±0.06	6.3±0.06	0.271
Ripening	11.5±0.25^{a}	12.0±0.15^{ab}	12.4±0.14^{b}	0.013	13.9±0.24	13.9±0.15	13.7±0.12	0.722
Overall	35.5±0.38^{a}	36.3±0.23^{ab}	37.0±0.21^{b}	0.003	35.7±0.38	35.8±0.23	35.5±0.19	0.517

Seasoning loss is expressed as % of ham weight prior to each processing step; overall seasoning loss is expressed as % of trimmed ham weight; LSM, least squares means; ^{a,b}with different superscripts are significantly different (P<0.05).

Table 5. Effect of *CAST* haplotypes on green ham traits or seasoning losses.

	Haplotype classes	LSM ± SE		
Trait	0	1	2	P
249Lys/638Arg				
N	222	371	125	
SM pH	5.77±0.01	5.76±0.01	5.81±0.02	0.024
Trimmed ham, kg	11.0±0.06	11.0±0.04	10.8±0.08	0.049
249Arg/638Arg				
N	461	230	27	
Drying, %	7.1±0.03	7.2±0.04	7.4±0.13	0.063
Overall, %	36.0 ±0.12	36.2±0.15	36.9±0.43	0.097
249Arg/638Ser				
N	295	322	102	
SM pH	5.79±0.01	5.76±0.01	5.76±0.02	0.037

The interaction between haplotype and producer was insignificant (P>0.10); only variables with P<0.10 are reported; haplotype classes 0, 1 or 2 denote number of copies of the haplotype in question; SM, semimembranosus muscle.

Table 6. Effect of *CAST* haplotypes on seasoning loss sliced° by dry-cured ham producer.

	Producer A Haplotype class LSM ± SE				Producer B Haplotype class LSM ± SE			
Trait	0	1	2	P	0	1	2	P
249Arg/638Arg								
N	223	115	10		239	115	17	
Resting, %	18.0±0.08^{a}	18.2±0.10^{ab}	18.8±0.32^{b}	0.028	17.2±0.08	17.1±0.10	17.0±0.25	0.857
Drying, %	7.9±0.05^{a}	8.1±0.06^{b}	8.4±0.20^{b}	0.007	6.3±0.05	6.3±0.06	6.4±0.15	0.882
Ripening, %	11.9±0.10^{a}	12.3±0.13^{b}	12.5±0.43^{b}	0.032	13.9±0.11	13.7±0.13	14.3±0.33	0.162
Overall, %	36.2±0.16^{a}	36.8±0.21^{b}	37.7±0.68^{b}	0.012	35.8±0.17	35.5±0.21	36.1±0.52	0.524
249Arg/638Ser								
N	125	171	52		170	151	50	
Overall,%	36.8±0.20	36.3±0.18	36.1±0.31	0.108	35.6±0.18	35.9±0.20	35.9±0.32	0.420

°Haplotype effect was sliced by producer in case of interaction between haplotype and producer (P<0.10); haplotype classes 0, 1 or 2 denote number of copies of the haplotype in question; LSM, least squares means; ^{a,b}with different superscripts are significantly different (P<0.05).

affected by *CAST*, while slightly lower pH associated with haplotype *249Arg/638Ser* could be expected to increase (not decrease) seasoning losses. Since CAST is a gene, encoding the calpastatin, a physiological inhibitor of calpain enzymes (Goll *et al*., 2003) responsible for early *post mortem* muscle proteolysis (Koohmaraie and Geesink, 2006) and meat tenderization (Koohmaraie, 1992), there may be a certain connection with proteolysis. According to some hypotheses (Morrison *et al*., 1998; Melody *et al*., 2004; Huff-Lonergan and Lonergan, 2005), the proteolysis of key muscle proteins minimizes the loss of water holding capacity caused by lateral shrinkage of myofibrils *post mortem*. Recent research reports (Koćwin-Podsiadła *et al*., 2003; Ciobanu *et al*., 2004; Otto *et al*., 2007; Krzęcio *et al*., 2005, 2008) demonstrated possible effect of *CAST* polymorphisms on pH value or drip loss, factors related to processing losses (Arnau *et al*., 1987). However, their results are difficult to compare with ours, due to the fact that they imply on different (intron) polymorphisms (Koćwin-Podsiadła *et al*., 2003; Krzęcio *et al*., 2005; 2008). There is a lack of literature dealing with *CAST* polymorphisms, especially in relation to dry-cured ham. In the only comparable study (Stalder *et al*., 2005) examining the *CAST Ser638Arg*, a significant effect on dry-cured muscle moisture content and a tendency ($P<0.10$) for total processing yield and salt content were reported. Contrary to our results, in that study *Arg/Arg* hams had the highest processing yield and consequently moisture.

Conclusions

Ham seasoning losses were affected by *PRKAG3* and *CAST* polymorphisms and dry-cured ham producer despite narrow constraints imposed by *consortium* rules. In addition, significant interaction between dry-cured ham producer and genotype indicates that the manifestation of genotype in regard to dry ham processing is likely to depend on manufacturing technique or product type.

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