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INVESTIGATING PRKAG3 POLYMORPHISMS FOR TRAITS OF INTEREST IN DRY HAM PRODUCTION

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ABSTRACT

Genetic markers affecting proteolysis could prove useful for selection of pigs suitable for dry ham production with lower salt content and/or prolonged maturation. One of the candidate genes to affect muscle metabolism, and consequently meat and dry-cured ham quality, is the I199V polymorphism of PRKAG3 gene which is currently studied within the 6th FP project TRUEFOOD (FOOD-CT-2006-016264) in three countries (France, Spain and Slovenia). Presented results involve 569 Slovenian Duroc×Hampshire sired commercial pigs slaughtered in 10 batches from which 723 hams were harvested and processed into dry hams "*kraški pršut*". Pigs were genotyped (PCR-RFLP) and traits of interest measured (backfat thickness, meat%, carcass weight, ham weight, ham subcutaneous fat thickness, pH in *semimembranosus* muscle, colour, processing losses). Genotype frequencies on codon 199 of PRKAG3 gene were 12.7%, 55.5% and 31.8% for I/I, I/V and V/V, respectively. Results indicate an effect of PRKAG3 gene polymorphism on carcass traits (carcass weight, ham weight, ham subcutaneous fat thickness), meat quality (*m.gluteus medius* colour score and Minolta L*) and processing losses (weight losses at the end of salting, resting and drying phase).

Key words: pigs / meat quality / processing losses / dry ham / PRKAG3 / genotype frequencies / carcass traits

RAZISKOVANJE POLIMORFIZMOV GENA PRKAG3 ZA LASTNOSTI POMEMBNE PRI PROIZVODNJI PRŠUTA

IZVLEČEK

Genski markerji, povezani s proteolizo v mišičnini, bi lahko precej pripomogli k selekciji prašičev, namenjenih za predelavo v pršut, z manjšo vsebnostjo soli in/ali podaljšanim zorenjem. V projekt 6. OP TRUEFOOD (FOOD-CT-2006-016264) je vključena tudi raziskava vpliva kandidatnih genov. Eden od preučevanih polimorfizmov je I199V na genu PRKAG3. V raziskavi sodelujejo tri države (Francija, Španija in Slovenija), predstavljamo pa slovenske rezultate na materialu, ki vključuje 569 križancev, potomcev očetovske linije durok×hempšir, zaklanih v 10 serijah. Prašiči so bili genotipizirani s pomočjo PCR-RFLP metode. Odbranih je bilo 723 stegen, ki so bila poslana v predelavo v "kraški pršut". Izmerili smo zanimive lastnosti (debelina hrbtne slanine, mesnatost, masa trupa, masa stegna, debelina slanine na stegnu, pH v *m. semimembranosus*, barva in izgube mase med predelavo). Frekvence genotipov na kodonu 199 gena PRKAG3 so bile 12,7 % za I/I, 55,5 % za I/V in 31,8 % za V/V genotip. Analize rezultatov kažejo na možen vpliv polimorfizmov PRKAG3 gena na lastnosti klavnega trupa (masa toplih trupov, masa stegna in debelina stegenske slanine), kakovosti mesa (ocena barve in vrednost L* v *m.gluteus medius*) ter izgube med predelavo (izgube po soljenju, podaljšanem soljenju in sušenju).

Ključne besede: prašiči / meso / kakovost / predelava mesa / izgube / pršut / PRKAG3 / frekvence genotipov / klavne lastnosti

INTRODUCTION

One of the main health threats in particular for cardiovascular diseases in the contemporary human diet is the increased salt ingestion due to its excessive use in food processing. High content of salt is still present in many traditional dry hams (e.g. Spanish "*Jamon Serrano*", French "*Jambon de Bayonne*" and Slovenian "*kraški pršut*"). One of the reasons is that salt plays an important role as an enzymatic inhibitor (especially for proteolytic enzymes) and as such prevents the occurrence of quality defects related to excessive proteolysis (Toldra *et al*., 1992). Reducing salt content may cause defects in texture (excessive softness, pastiness), aroma, taste and colour (Buscailhon and Monin, 1994). Proteolysis is strongly affected by the properties of raw material (fat, water content and meat pH, Morales *et al*., 2007). Therefore it has been suggested that the selection of pigs (based on genetic polymorphisms) would be of interest so as to obtain appropriate raw material for salt reduction and/or prolonged maturation. Among the investigated genes known to affect meat quality in pigs the PRKAG3 is a good candidate. The gene encodes a specific isoform of γ subunit of the adenosine monophosphate dependant protein kinase (AMPK), an enzyme with the key role in cell energy metabolism regulation. Five nonsynonymous substitutions (T30N, G52S, L53P, I199V and R200Q) have been detected in the PRKAG3 gene (Milan *et al*., 2000). In addition to R200Q substitution also known as RN^- mutation, well known for causing a defect named "acid meat" (Sellier and Monin, 1994), the I199V substitution (Ile →Val on the codon 199) turned out to be the most interesting. It was shown to affect carcass leanness, muscle glycogen content, ultimate muscle pH and water holding capacity (Ciobanu *et al*., 2001; Enfält *et al*., 2006; Lindahl *et al*., 2004a). The allele that seems to be favourable for meat quality is the 199I. Low incidence of I/I genotype (< 10%) have been reported for the majority of modern breeds and crosses, with the exception of Berkshire breed (Ciobanu *et al*., 2001; Josell *et al*., 2003; Huang *et al*., 2004; Lindahl *et al*., 2004 a, b; Stalder *et al*., 2005; Otto *et al*., 2007). The aim of the present study was firstly to screen the PRKAG3 genotype frequencies in one Slovenian commercial crossing and secondly to evaluate the effect of I199V polymorphisms on the aptitude of raw material (green hams) for dry ham processing.

MATERIAL AND METHODS

The material consists of 569 approximately 6.5 months (200 days) old pigs, commercial crosses originating from one herd and line i.e. landrace (LN) × large white (LW) as maternal and DU (duroc) × hampshire (HA) as paternal line. Pigs from this herd were previously demonstrated to be free of RYR1 and RN^- mutation (Škrlep *et al*., 2008 in press). All animals were slaughtered in the same commercial abattoir according to the routine procedure (i.e. CO_2 stunning, vertical exsanguination, vapour scalding, dehairing and evisceration, followed by veterinary inspection and carcass classification). The carcasses were cooled (by storage at 0–2 °C) until the internal temperature dropped below 5 °C.

For genotype determination, small pieces of ear laps were taken from each animal at the end of the slaughter line and frozen until the analysis. All animals were genotyped for PRKAG3 I199V polymorphisms according to Milan *et al.* (2000). Backfat thickness (Fat-DM5), muscle thickness (Muscle-DM5), warm carcass weight and meat% were measured according to the approved method for carcass classification (OJ EU L324/87 10.12.2005). The following day, the hams were selected against visible lesions (skin lacerations, haemorrhages, hematomes and extensive veining) on the surface of the hams (Šegula *et al*., 2007), and sent to two processors of dry ham "kraški pršut", where they were cut into the prescribed shape, and further selected according to weight (≥ 9.5 kg), subcutaneous fat thickness (≥ 10 mm) and meat quality consistent with the rules for "kraški pršut". Subcutaneous fat under *caput femoris*, pH in *m.*

semimembranosus (MP120 Mettler Toledo pH Meter) and Minolta L* value (Minolta Chroma Meter CR-300) on *m. gluteus medius* were measured. Colour on *m. gluteus medius* was also assessed using Japanese 1–6 colour scale (Nakai *et al.*, 1976). The hams were processed according to the specifications for "kraški pršut". i.e. two salting phases, resting phase, drying phase and maturation phase (in total 60 weeks). Weight losses after each processing phase were recorded.

Analysis of variance was performed using a statistical package SAS (SAS Inst., Inc., Cary, NC) and procedure GLM. The model included the fixed effects of the processor, PRKAG3 gene and slaughter batch within processor. In case of significant genotype effect ($P < 0.05$) least square means were compared using PDIFF option, adjust = Tukey.

RESULTS AND DISCUSSION

In the present study, the observed frequencies of PRKAG3 codon 199 genotypes (Table 1) were 12.7% (I/I), 55.5% (I/V) and 31.8% (V/V). Low incidence of I/I genotype is coherent with the available literature reports for the majority of modern breeds or crosses (Ciobanu *et al.*, 2001; Josell *et al.*, 2003; Huang *et al.*, 2004; Lindahl *et al.*, 2004 a, b; Stalder *et al.*, 2005; Otto *et al.*, 2007). Also, the observed frequencies for the I199V genotypes are similar to the ones obtained in our previous study on Slovenian pigs (9.8% I/I, 51.1% I/V and 39.1% V/V, Škrlep *et al.*, 2008 in press) The only breed for which the literature reports higher frequency of I/I genotype (74%) is the Berkshire breed (Ciobanu *et al.*, 2001), also known for its good meat quality, especially appropriate for cured products (Breeds of livestock, 2008)). The results presented in Table 1 show, that I199V genotypes at PRKAG3 gene significantly affected green ham weight and fat thickness over *m. gluteus medius* at carcass split line and below *caput femoris* bone. There was also notable effect ($P < 0.1$) for leanness, carcass weight, meat colour and Minolta L* value. Pigs of I/I genotype had lower carcass and green ham weights than I/V or V/V pigs. In agreement with our previous study (Škrlep *et al.*, 2008 in press) the heterozygous pigs (I/V) exhibited the thickest subcutaneous fat at two anatomical locations. Overall, there is not much available literature about the effect of I199V PRKAG3 polymorphism on carcass composition (especially for I/I genotype). The related studies of Josell *et al.* (2003), Lindahl *et al.* (2004a, b) and Enfält *et al.* (2006) pooled the results of I/I and I/V genotype into one group, due to very low frequencies of I/I. Even so, according to Enfält *et al.* (2006) the presence of allele 199I is related to lower lean meat content. Our study showed no significant effect on the ultimate pH, which is against our expectations, since the available literature indicates an effect of this gene on muscle pH, i.e. higher pH for the I/I in comparison to V/V pigs (Ciobanu *et al.,* 2001; Otto *et a*l., 2007). On the other hand, notable effect ($P < 0.1$) of I199V polymorphism was observed on colour; both Minolta L* and subjective colour note. Pigs with I/I genotype had darker LD muscle colour than V/V, which corroborates with other literature data (Ciobanu *et al.,* 2001; Lindahl *et al.,* 2004b, Otto *et al.*, 2007).

The results presented in Table 2 show, that I199V polymorphism in PRKAG3 gene affected significantly ham processing weight losses during first salting and resting phase and as a consequence total weight losses. Again it was the heterozygote genotype that exhibited the lowest processing losses (significantly different from V/V, but not from I/I), in agreement with the thickest fat, which represents the obstacle for salt diffusion and water removal. In the only comparable study available in the literature (on American country-style dry-cured hams), Stalder *et al.* (2005), did not report any significant differences for processing yields although the values they obtained show similar trend as in our case i.e. the I/V genotype exhibited the highest processing yield or the lowest processing weight loss compared to other two genotypes.

Table 1. Least squares means (standard errors) of I199V genotypes for carcass and ham properties

	I/I	I/V	V/V	P
Number of pigs	72	316	181	
Muscle-DM5, mm	74.2 (0.6)	74.7 (0.3)	75.0 (0.4)	0.42 ^{ns}
Fat-DM5, mm	15.4 (0.4) ^{ab}	15.9 (0.2) ^{a}	15.0 (0.3) ^{b}	0.03*
Meat, %	57.9 (0.4)	57.6 (0.2)	58.2 (0.2)	0.09 †
Carcass weight, kg	96.4 (0.4)	98.3 (0.4)	98.3 (0.5)	0.06 †
Number of hams	94	394	235	
Green ham, kg	10.8 (0.08) ^{a}	11.0 (0.04) ^{b}	11.1 (0.05) ^{b}	0.005**
Ham fat, mm	12.9 (0.3) ^{a}	13.8 (0.1) ^{b}	13.3 (0.2) ^{a}	0.008**
pH_u *(m. semimembranosus)*	5.70 (0.02)	5.69 (0.01)	5.70 (0.01)	0.83 ^{ns}
Colour (1–6)	3.7 (0.06)	3.6 (0.03)	3.5 (0.04)	0.07 †
Minolta L*	47.6 (0.3)	48.3 (0.2)	48.6 (0.2)	0.08 †

^{a,b,c} Least squares means followed by a different letter are significantly ($P < 0.05$) different; P = level of significance, $P > 0.1$ = statistically insignificant (ns); $P < 0.1$ = tendency (†); statistically significant: $P < 0,05$ (*), $P < 0.01$(**), $P < 0.001$(***); Muscle-DM5 = muscle thickness according to DM5 method; Fat-DM5 = subcutaneous fat thickness according to DM5 method; Meat = % of meat in the carcass according to DM5 method; Carcass weight = hot carcass weight; Green ham = weight of the ham prior to processing; Ham fat = subcutaneous fat thickness below *caput ossis femoris*; pH_u = ultimate pH value of *m. semimembranosus*; colour (1–6) = colour of *m. gluteus medius* according to Japanese scale (Nakai *et al*., 1976); Minolta L* = CIE L parameter measured with Minolta chromameter on *m.gluteus medius*.

Table 2. Least squares means (standard errors) of I199V genotypes for ham weight processing losses

	I/I	I/V	V/V	P
Number of hams	94	394	235	
Salting 7d, %	2.30 (0.03) ^{ab}	2.26 (0.02) ^{a}	2.37 (0.02) ^{b}	0.0005***
Salting 7–14d, %	1.77 (0.03)	1.75 (0.01)	1.75 (0.02)	0.79 ^{ns}
End of salting 14d, %	4.02 (0.05) ^{ab}	3.97 (0.02) ^{a}	4.08 (0.03) ^{b}	0.02*
Resting, %	17.64 (0.11) ^{ab}	17.43 (0.06) ^{a}	17.78 (0.07) ^{b}	0.0006***
End of resting, %	20.95 (0.13) ^{ab}	20.70 (0.07) ^{a}	21.13 (0.09) ^{b}	0.0005***
Drying, %	7.18 (0.07)	7.14 (0.04)	7.17 (0.05)	0.79 ^{ns}
End of drying, %	26.61 (0.16) ^{ab}	26.36 (0.08) ^{a}	26.80 (0.10) ^{b}	0.003**
Maturation, %	13.11 (0.15)	12.48 (0.07)	12.98 (0.10)	0.23 ^{ns}
End of maturation, %	36.25 (0.23) ^{ab}	35.81 (0.11) ^{a}	36.27 (0.15) ^{b}	0.02*

^{a,b} Least squares means followed by a different letter are significantly ($P < 0.05$) different; P = level of significance, $P > 0.1$ = statistically nonsignificant (ns), $P < 0.1$ = tendency (†), statistically significant: $P < 0,05$ (*), $P < 0.01$(**), $P < 0.001$(***); Salting 7d = weight loss after 7 days of salting; Salting 7–14d = weight loss between 7 and 14 days of salting; End of salting = weight loss at the end of salting period; Resting = weight loss in the resting period; End of resting = weight loss from the beginning of the processing to the end of resting; Drying = weight loss in the drying period; End of drying = weight loss from the beginning of the processing to the end of drying period; Maturation = weight loss in the maturation period; End of maturatiom = weight loss from the beginning of the processing to the end of maturation period.

CONCLUSIONS

Our study indicates an effect of I199V polymorphism in PRKAG3 gene on green ham properties and dynamics of ham weight losses, all susceptible to influence salt intake and diffusion. As the endogenous proteolytic activity is directly (pH, Aw, salt concentration) or indirectly (ham fatness, marbling and weight) affected by green ham properties, we expect to be able to observe the effect of the PRKAG3 polymorphism also on the properties of processed dry hams.

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