EFFECT OF RN$^-$ AND CALPASTATIN (*CAST*) GENE AS RELATED TO MEAT QUALITY OF STRESS RESISTANT FATTENERS

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Background

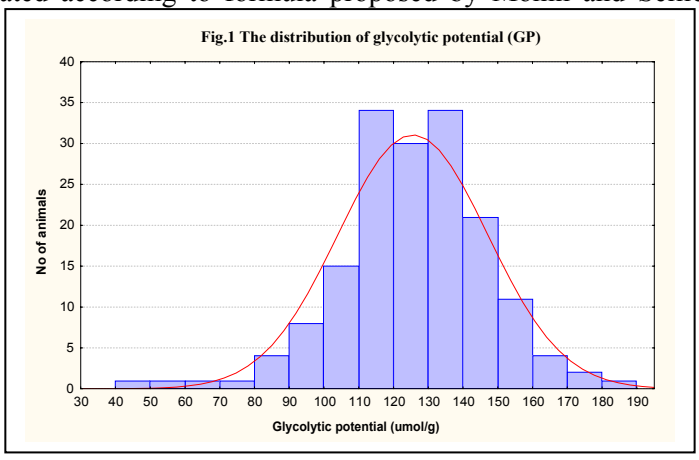
Pork quality traits are composite, being affected by major genes (RYR1 RN) and the candidates e.g. mapped on 2 chromosome calpastatine gene – an endogenous inhibitor of calpain (Koćwin-Podsiadła et al. 1995, Przybylski et al.1995, Sellier 1998, Sensky et al. 1999).

Objectives

The present study was conducted to estimate the polymorphism of calpastatin (*CAST*) gene identified with *Hinf*I, *Msp*I and *Rsa*I restriction endonucleases, and the effect of RN$^-$ gene (determined on the basis of glycolytic potential), on meat quality traits.

Materials and methods

The investigations were carried out on 169 fatteners of three genetic groups: Landrace (41), Landrace x Duroc (67) and Landrace x Yorkshire (61), obtained from F_0 animals, imported from Denmark. The animals were kept under the same environmental conditions and fed a full bath feed. The animals were slaughtered at the live weight about 110 kg (hot carcass weight 85 kg), 4-5 hours after transportation, using the electrical stunning method and recumbent bleeding out. Immediately after slaughter blood samples were collected in EDTA-coated tubes for subsequent DNA analysis for the RYR1 and *CAST* genotype. The RYR1 genotypes were established according to Fuji et al. (1991). The polymorphism of *CAST* gene was identified with *Hinf*I, *Msp*I and *Rsa*I restriction endonucleases, according to Ernst et al. (1998). At 45 min *post mortem* the samples from *Longissimus lumborum* (LL) muscle were collected into tubes with 0,5 M PCA for determination of glycogen (Darympale and Hamm, 1973) and lactate (Bergmayer, 1974). On the basis of them, the glycolytic potential (GP) was calculated according to formula proposed by Monin and Sellier (1985). The RN genotypes were identified on the basis of glycolytic potential (GP) and its bimodal distribution: rn^+rn^+ (GP≤130µmol/g) RN$^-$/? (GP>130µmol/g) (Fig.1). The pH was measured directly in the tissue of *Longissimus lumborum* muscle, using a pH-meter Master produced by Dramiński (Poland). The electrical conductivity was evaluated using a LF-Star apparatus, produced by Matthaus (Germany). The lightness (L*) of the muscle tissue was measured 24 hours *post mortem* using a Minolta CR-310 apparatus (Japan) in CIE L*a*b* colour system. The drip loss at 48, 96 and 144 hours after slaughter was evaluated in accordance with the method of Prange et al. (1977). The shear force was determined in (144h) on cooked meat slices, using an Instron 1140 apparatus with Warner-Bratzler device. The data was analysed, using a two-way analysis of variance in a non-orthogonal scheme. The significance of differences between means of groups for the investigated traits, was calculated using Tukey's test.

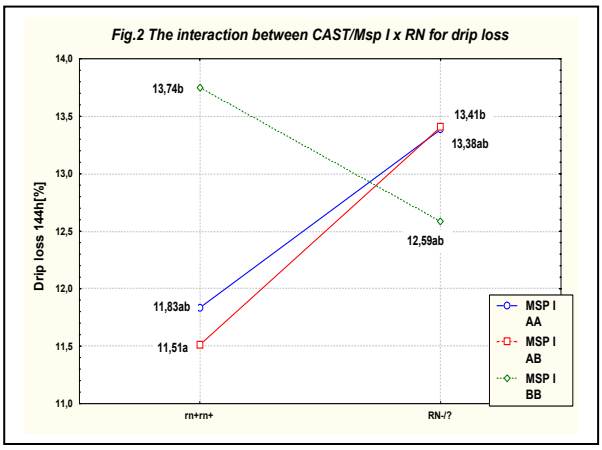


Results and discussion

The high muscle glycolytic potential (GP>130µmol/g) has an effect on pH decline (at 24, 48 and 96 hours after slaughter), and higher meat lightness [Tab.1]. The low pH_u is connected with a higher drip loss from pork meat (Kauffman et al. 1993, Bertram et al. 2000). The deeper glycogenolysis (as the effect of RN$^-$ allele), affected fluid loss from fresh meat during *post mortem* storage, giving higher (about 0,7 percent points) drip loss in 48 hours after slaughter. Josell et al. (2003) suggested, that high amount of glycogen, the most important substrate for ATP regeneration, might contribute to hinder muscle contraction in RN$^-$ carriers, while Deng et al. (2002) showed, that the low water-holding capacity of meat from RN$^-$ carriers, was caused by high degree of denaturation of proteins. The tenderness (expressed by shear force) has been shown to be related to RN gene, which is in agreement with studies by different authors (Lundstrom et al. 1994, Enfalt et al. 1997, Josell et al. 2003).

In table 1 showed the effect of calpastatin gene (regardless RN$^-$ gene) on analysed meat quality traits. The AA genotype at the *CAST/Hinf*I locus affected the initial pH, given (in comparison to animals with AB and BB genotypes) lower at about 0,15 units pH_{35} and 0,22-0,25 units pH_3. In contradiction to this experiment, in the investigations of Koćwin-Podsiadła et al. (2003), carried out on fatteners differentiated by RYR1 gene, the faster pH_{35} decline, in group of fatteners with BB genotype at *CAST/Hinf*I locus, was noted. There was no effect of GP (RN gene) on drip loss measured in 96 and 144 hours *post mortem* but the influence of *CAST/Hinf*I gene on fluid loss, was noted. It should suggest that *CAST/Hinf*I genotype is connected with a later drip loss from fresh meat during storage. The AA animals compared with AB and BB porkers at the *CAST/Hinf*I locus given about 4 pp. higher drip loss measured in 96 and 144 hours *post mortem*. During the storage from 48 to 144 hours after slaughter, a highest fluid loss has been shown from fresh meat of AA genotype porkers at the *CAST/Hinf*I locus (8, 5 and 6 pp. respectively for AA, AB and BB genotype at the *CAST/Hinf*I locus).

In contrast, the BB genotype porkers at *CAST/Rsa*I locus (compared with AA and AB animals) affected fluid loss, given about 2 pp. higher drip loss measured in 144 hours *post mortem*. During the storage from 48 to 144 hours after slaughter, a highest fluid loss has been shown from fresh meat of BB genotype porkers at the *CAST/Rsa*I locus (8, 6 and 5 pp. respectively for BB, AA and AB genotype at the *CAST/Rsa*I locus).



It should the mention that both genotypes with highest drip loss (AA at *CAST/Hinf*I and BB at *CAST/Rsa*I) had a lowest percentage share in analysed population of fatteners (8,88 and 6,51% respectively).

The AA genotype at the *CAST/Msp*I affected the glycolytic changes in *Longissimus lumborum* muscle, given about 0,05 units higher pH at 24 and 144 hours after slaughter. The effect of calpastatin gene (*CAST/Msp*I and *CAST/Rsa*I) on meat quality traits, in the investigations of Emnet et al. (2000), was not found.

In this experiment, the interaction was found between RN gene and *CAST/Msp*I for a drip loss measured in 144 hours after slaughter (Fig.2). The AA genotype at the *CAST/Msp*I locus was differed by RN gene, given 1,9 pp. lower drip loss (144h) in non-carriers of RN gene. Among non-carriers of RN$^-$ gene, were two groups related *CAST/Msp*I genotype and differed by drip loss measured in 144 hours *post mortem*. The AB animals compared with BB porkers at the *CAST/Msp*I given 2,23 pp. lower drip loss from *Longissimus lumborum* muscle.

Conclusions

Meat from RN$^-$ carriers, compared to meat from non-carriers had higher glycogen content, lower ultimate pH and higher meat lightness (L*) and drip loss (48h).

The highest fluid loss from fresh meat during storage in a group of porkers with AA genotype at the *CAST/Hinf*I and BB genotype at the *CAST/Rsa*I locus, was noted. The frequency of mentioned above genotypes with highest drip loss was lowest in analysed population and reached 8,88 and 6,51% respectively.

In this experiment, we also found the interaction between RN gene and *CAST/Msp*I for a drip loss evaluated in 144 hours after slaughter. The non-carriers of RN$^-$ gene, but with AB genotype at the *CAST/Msp*I locucs

porkers compared with BB genotype animals at the same locus of *CAST* and RN, given 2,23 pp. lower drip loss (144h after slaughter) during storage of fresh *Longissimus lumborum* muscle samples.

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Table 1. The effect of RN^- and calpastatine (*CAST*) gene on analysed meat quality traits.

	*CAST/Hinf*I				*CAST/Msp*I				*CAST/Rsa*I				rn^+rn^+	$RN^-/?$	Sign
	AA n=15	AB n=70	BB n=84	Sign	AA n=31	AB n=73	BB n=65	Sign	AA n=71	AB n=87	BB n=11	Sign	n=95	n=74	
GP [µmol/g]	124,52 ±12,21	127,13 ±25,70	126,36 ±26,22	NS	122,23 ±25,86	126,72 ±21,11	128,33 ±28,47	NS	126,59 ±27,51	127,39 ±23,72	119,10 ±16,59	NS	111,47A ±16,19	145,84B ±20,60	**
Glycogen [µmol/g]	44,65 ±8,32	46,35 ±14,87	46,06 ±13,67	NS	43,42 ±15,40	46,00 ±13,56	47,37 ±14,22	NS	47,21 ±13,96	45,71 ±13,70	41,30 ±12,79	NS	38,15A ±	56,20B ±10,50	**
Lactate [µmol/g]	35,22 ±11,02	35,09 ±10,37	34,22 ±10,62	NS	35,40 ±9,97	35,34 ±10,45	33,56 ±10,85	NS	32,83 ±9,54	35,94 ±10,75	36,51 ±13,31	NS	35,14 ±10,61	34,07 ±10,39	NS
pH_{35}	6,49a ±0,11	6,22b ±0,17	6,58b ±0,16	*	6,59 ±0,20	6,60 ±0,16	6,57 ±0,19	NS	6,59 ±0,17	6,59 ±0,18	6,52 ±0,23	NS	6,56a ±0,19	6,62b ±0,16	*
pH_3	6,01a ±0,21	6,26b ±0,21	6,23b ±0,25	*	6,19 ±0,26	6,23 ±0,24	6,23 ±0,23	NS	6,22b ±0,25	6,24b ±0,22	6,01a ±0,32	*	6,19 ±0,26	6,26 ±0,21	NS
pH_{24}	5,56 ±0,08	5,56 ±0,12	5,54 ±0,09	NS	5,61B ±0,11	5,52A ±0,10	5,55A ±0,08	**	5,55 ±0,09	5,55 ±0,12	5,58 ±0,08	NS	5,57B ±0,11	5,52A ±0,09	**
pH_{48}	5,37 ±0,06	5,41 ±0,11	5,40 ±0,08	NS	5,43 ±0,11	5,40 ±0,09	5,39 ±0,08	NS	5,40 ±0,09	5,41 ±0,10	5,41 ±0,09	NS	5,42b ±0,10	5,38a ±0,08	*
pH_{96}	5,37 ±0,08	5,39 ±0,11	5,40 ±0,09	NS	5,42 ±0,09	5,40 ±0,11	5,38 ±0,08	NS	5,39 ±0,08	5,40 ±0,11	5,42 ±0,07	NS	5,43B ±0,09	5,36A ±0,08	**
pH_{144}	5,55 ±0,09	5,49 ±0,12	5,49 ±0,10	NS	5,55b ±0,13	5,49a ±0,11	5,48a ±0,10	*	5,50 ±0,10	5,48 ±0,12	5,59 ±0,02	NS	5,52 ±0,12	5,46 ±0,09	NS
EC_{24} [mS/cm]	3,42 ±1,08	3,49 ±1,18	3,90 ±1,18	NS	3,61ab ±1,20	3,47a ±1,02	3,97b ±1,30	*	3,67 ±1,07	3,73 ±1,27	3,44 ±1,20	NS	3,79 ±1,16	3,50 ±1,19	NS
L*	53,47 ±3,03	54,69 ±3,20	54,70 ±2,86	NS	53,97 ±2,92	54,74 ±3,14	54,71 ±2,95	NS	54,38 ±2,79	54,89 ±3,27	53,54 ±2,15	NS	53,90a ±2,98	55,48b ±2,87	*
Drip loss 48h [%]	8,02 ±2,88	7,08 ±2,14	6,73 ±2,18	NS	6,95 ±2,15	7,19 ±2,49	6,80 ±2,02	NS	6,88 ±2,37	6,97 ±2,08	7,89 ±2,74	NS	6,69a ±2,18	7,38b ±2,29	*
Drip loss 96h [%]	14,06B ±4,79	10,89A ±2,54	10,79A ±2,73	**	11,42 ±3,48	11,05 ±3,28	10,99 ±2,37	NS	11,01 ±2,94	10,96 ±2,84	13,07 ±4,38	NS	11,03 ±3,13	11,18 ±2,81	NS
Drip loss 144 [%]	16,19b ±1,69	12,48a ±2,59	12,75a ±3,01	*	12,38 ±2,90	12,41 ±2,90	13,19 ±2,79	NS	13,02a ±3,10	12,34a ±2,61	15,98b ±1,63	*	12,46 ±2,88	13,04 ±2,82	NS
Shear force [N/cm^2]	45,41 ±9,94	62,36 ±15,06	56,26 ±13,26	NS	60,32 ±11,42	60,59 ±12,77	54,43 ±15,67	NS	59,97 ±14,79	55,77 ±13,71	56,11 ±17,76	NS	63,22B ±13,62	47,24A ±8,35	**

Results are given as means ± SD; **, A, B signigicant at P≤0,01; *, a, b signigicant at P≤0,05