Prediction of meat quality from biopsy in live pigs with different *RYR1* genotypes

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Abstract. To predict meat quality after slaughter, biopsy samples were taken from *musculus longissimus lumborum et thoracis* of live pigs at approximately 40 kg and 80 kg of weight. The obtained values from biopsies for pH_1 and EC_{50} (electric conductivity) were compared with measurements after slaughter at a weight of approximately 110 kg. *RYR1* genotypes were determined from blood samples using PCR-RFLP. Mating of *Nn* sows with two *nn* boars resulted in 72 *Nn* and 40 *nn* offspring. Significant differences between the two genotypes were found for pH_1 and EC_{50} values for the three weights. The coefficients of correlation for the *Nn* genotype of the *RYR1* gene between the values after slaughter and both the first and the second biopsy for pH_1 and EC_{50} were very low (r = 0.06, r = 0.14; and r = 0.26, r = 0.26; $P \leq 0.05$). For the *nn* genotype were r = –0.23, r = –0.15; and r = –0.25, r = –0.11 respectively. The values of pH_1 and EC_{50} were highly correlated (r = –0.52 to –0.84; $P \leq 0.001$) both within biopsies and after slaughter.

Key words: biopsy, correlation, meat quality, pigs, *RYR1* gene.

Introduction

The major effects of *RYR1* genotypes (*N* and *n* alleles) on carcass quality traits in pigs have been reported repeatedly (SELLIER 1995, 1998). The prediction of meat quality from live observation in pigs is very important because of economic losses associated with porcine stress syndrome and PSE (pale, soft, exudative) meat linked to *nn* and *Nn* genotypes of the *RYR1* gene; the more so because PSE meat occurs in *NN* genotypes as well (SIMPSON, WEBB 1989, HARDGE, SCHOLZ 1994, CHEAH et al. 1994, 1997, 1998, KUCIEL et al. 1996, PRZYBYLSKI et al. 1996).

The predictive value of LD (*musculus longissimus dorsi*) biopsy samples has been tested comparing measurements of *NN* live pigs weighing from 60 to 65 kg with values after slaughter at 80-85 kg, at least three weeks later (CHEAH et al. 1994). A correlation of 0.74 (P<0.001) was observed between F values (an indicator of waterholding capacity – WHC) and pH_1 one hour after slaughter. According to CHEAH et. al. (1995) the predictive biopsy test offers an opportunity to select *Nn* pigs at about 65 kg with superior WHC thereby avoiding the production of pigs prone to developing PSE meat. LAHUČKÝ et. al. (1997) found a correlation of 0.65 between pH_1 from biopsy samples at live weights from 80 to 90 kg (approximately 14 days before slaughter) and pH_1 values one hour after slaughter. The sensitivity of the biopsy test based on WHC values was evaluated using pigs of three *RYR1* genotypes at live weights of 60-75 kg (CHEAH et al. 1997, 1998). The results demonstrated the usefulness and reliability of muscle biopsy test based on WHC three weeks before slaughter to predict meat quality (pH_1 and drip loss) one hour after slaughter.

This paper reports the results of an experiment aimed at determining whether biopsy at approximately 40 and 80 kg of live weight can be used for prediction of meat quality after slaughter in pigs with *Nn* and *nn RYR1* genotypes. The biopsy samples and meat quality were both characterised with pH_1 and EC_{50} (electric conductivity) values.

Material and methods

Animals

Altogether 112 pigs were obtained from Large White × Landrace maternal line sows that had been mated with one Large White boar from a paternal line and one Large White × Piétrain sire. The *RYR1* genotypes of parents and offspring were determined from blood samples using PCR-RFLP (NEBOLA et al. 1994). The mating of *Nn* sows with two *nn* boars resulted in 72 *Nn* and 40 *nn* offspring.

Experimental procedures

To predict meat quality after slaughter, biopsy samples (600-900 mg) were taken using the spring biopsy technique according to the procedure described by KOVÁČ et al. (1992). The technique eliminates stress, which is important because it gives unbiased results. The first and the second biopsy samples were taken on the right side of the body, at the last rib, from the *musculus longissimus lumborum et thoracis* (*MLLT*) of pigs weighing approximately 40 kg and later at approximately 80 kg.

The pH_1 values were measured in biopsy samples after incubation at 39° C for 50 min with a digital pH-meter Gryf 209S and electric conductivity EC_{50} with a digital conductometer Biotech PMV-21 in mS (milliSiemens) according to

KOVÁČ et al. (1992). The obtained values were compared with results of measurements carried out 50 min after slaughter at a body weight of approximately 110 kg. Animals were slaughtered following electrical stunning in a local commercial slaughterhouse two hours after arrival. After slaughter, the carcasses were kept at room temperature for 50 min before chilling at +1°C.

Statistical analysis

The statistical analyses were carried out using the GLM (SAS 1988) procedure to estimate the least squares means for a simultaneous determination of effects of the *RYR1* genotype, sex, hybrid combination (effect of sires) and month of measurement on the variation of the traits under study. The following model with fixed effects was assumed:

$$y_{ijklm} = m + RYR_i + S_j + H_k + M_l + e_{ijklm}$$

where: y_{ijklm} = the *ijklm*-th observation, m = general mean, RYR_i = effect of the *i*-th *RYR1* genotypes ($i = 1,2$), S_j = effect of the *j*-th sex ($j = 1,2$), H_k = effect of the *k*-th hybrid combination ($k = 1,2$) (effect of sires), M_l = effect of the *l*-th month of measurement ($l = 1,2,3,4$), e_{ijklm} = residual effect.

The least squares means (LSM) and standard errors (SE) were computed for the *Nn* and *nn* genotypes. Coefficients of linear Pearson's correlation were calculated for raw data on measurements carried out during the first and the second biopsy and after slaughter, for *Nn* and *nn RYR1* genotypes.

Results

The offspring differed in their *RYR1* genotypes. The statistical analysis for pH_1 showed at 40 and 80 kg and after slaughter a highly significant effect ($P < 0.0001$) of *RYR1* genotypes and a significant effect ($P < 0.05$) of the month of measurement. Similar results were found for EC_{50}. Least squares means and standard er-

Table 1. Least square means (LSM) and standard errors (SE) of pH_1 and EC_{50} (electric conductivity)values for the genotypes of the *RYR1* gene at the first and second biopsy and after slaughter

Time of analysis	Geno-types	n	pH₁		EC₅₀	
			LSM	SE	LSM	SE
First biopsy	*Nn*	72	5.94[a]	0.04	6.33[a]	0.26
	nn	40	5.66[a]	0.04	8.52[a]	0.30
Second biopsy	*Nn*	68	6.10[a]	0.03	5.05[a]	0.24
	nn	38	5.87[a]	0.04	6.49[a]	0.30
After slaughter	*Nn*	68	6.08[a]	0.05	6.19[a]	0.30
	nn	38	5.70[a]	0.06	8.41[a]	0.39

Values with the same exponents show significant differences in columns: [a] - $P \leq 0.05$.

Table 2. Correlation coefficients between pH_1 and EC_{50} (electric conductivity) from samples of *musculus lumborum et thoracis* taken at the first and second biopsy and after slaughter for *Nn* and *nn* genotypes

Parame-ter	Time of analysis	Geno-type	pH_1		EC_{50}		
			2nd biopsy	After slaughter	1st biopsy	2nd biopsy	After slaughter
pH_1	1st biopsy	*Nn*	0.35** ± 0.11	0.06 ± 0.12	−0.62*** ± 0.09	−0.44*** ± 0.11	−0.16 ± 0.12
		nn	−0.10 ± 0.16	−0.23 ± 0.16	−0.64*** ± 0.12	−0.07 ± 0.16	0.18 ± 0.16
	2nd biopsy	*Nn*		0.14 ± 0.12	−0.50*** ± 0.10	−0.54*** ± 0.10	−0.19 ± 0.12
		nn		−0.15 ± 0.16	0.07 ± 0.16	−0.52*** ± 0.14	0.23* ± 0.16
	After slaughter	*Nn*			−0.07 ± 0.12	−0.31*** ± 0.11	−0.59*** ± 0.10
		nn			0.27 ± 0.16	−0.01 ± 0.16	−0.84*** ± 0.08
EC_{50}	1st biopsy	*Nn*				0.24* ± 0.12	0.26* ± 0.12
		nn				−0.06 ± 0.16	−0.25 ± 0.16
	2nd biopsy	*Nn*					0.26* ± 0.12
		nn					−0.11 ± 0.16

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

rors for the first and the second biopsy, as well as after slaughter for different *RYR1* genotypes are presented in Table 1. LSM's for pH_1 were always significantly lower and for EC_{50} always significantly higher in *nn* genotypes than in *Nn* genotypes for the three weights.

The correlation coefficients used to predict meat quality after slaughter on the basis of biopsy samples for the *Nn* and *nn* genotypes (n = 112) are presented in Table 2 because of the highly significant effect of *RYR1* genotypes from statistical analysis. The same statistical analysis showed a significant effect of "month of measurement". Howewer, the use of this factor in correlation analysis was problematic due to the reduced number of measurements in four seasons of groups.

For *Nn* genotypes the correlations between samples at first and second biopsy were r = 0.35 ($P \le 0.01$) for pH_1 values and r = 0.24 ($P \le 0.05$) for EC_{50}. For differences in pH_1 values between the first biopsy and after slaughter, and between the second biopsy and after slaughter, correlations were non-significant and very low (r = 0.06 and r = 0.14, respectively). For EC_{50}, these were low but statistically significant (r = 0.26 and r = 0.26). The correlations between pH_1 and EC_{50} values in the same measurements for *Nn* genotype were r = –0.62 ; r = –0.54 and r = –0.59, respectively ($P \le 0.001$) .

For *nn* genotypes the correlations between values at first and second biopsies were non-significant and negative for both pH_1 (r = –0.10) and EC_{50} (r = –0.06). The correlations between pH_1 values at first or second biopsies, and those obtained after slaughter were low, non-significant and negative (r = –0.23 and r = –0.15, respectively). The corresponding correlations for EC_{50} were r = –0.25 and r = –0.11. The correlations between pH_1 and EC_{50} values at the first biopsy, at the second biopsy and after slaughter for *nn* genotypes were greater (r = –0,64; r = –0.52 and r = –0.84) and highly significant.

Discussion

Significant differences for pH_1 and EC_{50} in *Nn* and *nn* genotypes of the *RYR1* gene, have been described by CHEAH et al. (1994,1997), URBAN et al. (1996), from biopsies and after slaughter sampling in other populations, and agree with our results. The pH_1 and EC_{50} values determined one hour after slaughter are the most important pig meat quality traits and were regarded and accepted for PSE meat characterisation.

Post-mortem changes are characterised by a decrease in pH. This parameter has a marked influence on the degree of protein denaturation during the onset of rigor mortis and when it is too fast, there is a danger of the occurrence of a serious defect described as PSE (pale, soft, exudative) meat.

The aim of the present study was to further investigate the predictive value of earlier biopsies (at approximately 40 and 80 kg) for meat quality characterised by

pH_1 and EC_{50} values after slaughter at approximately 110 kg. The biopsy test could be employed to select the best stress-resistant pigs for breeding.

The correlations between pH_1 and EC_{50} from biopsies carried out immediately before slaughter and after slaughter have already been known (ŠPRYSL et al. 1986, STUPKA et al. 1993). LAHUČKÝ et al. (1997) found a correlation of 0.65 between pH_1 from biopsy (14 days before slaughter) and pH_1 after slaughter.

The results of this experiment indicated that the correlations for pH_1 values from both the first and second biopsy and those after slaughter were non-significant for pH_1 within *Nn* genotypes (r = 0.06 and r = 0.14) and within *nn* genotypes were even found to be negative (r = –0.23 and r = –0.15). The corresponding values within the *Nn* genotype for EC_{50} were significant (r = 0.26 and r = 0.26) but again very low. For the *nn* genotype they were negative (r = –0.25 and r = –0.11).

CHEAH et al. (1994, 1995, 1997) identified the variations in meat quality in live pigs of all three *RYR1* genotypes using biopsy samples of *M. longissimus dorsi* at live weights of 60-70 kg, approximately three weeks before slaughter. They demonstrated the usefulness and reliability of muscle biopsy test based on the water-holding capacity (WHC) from biopsy and pH_1 meat one hour after slaughter. High correlations (P < 0.001) were observed between biopsy fluid values, as an indicator of WHC, and drip loss (r = 0.63) from post-mortem muscle (CHEAH et al. 1998).

Regarding meat quality traits, the halothane gene has no effect on the ultimate pH but induces a very large reduction in pH_1, the difference between *NN* and *nn* pigs amounting to about 2.5 standard deviations of this trait (SELLIER 1995). As reported by DE VRIES et al. (1994) pH_1 and ultimate pH are not significantly correlated (r = 0.15).

The results of our study, based on pH_1 and EC_{50} values, showed a low predictive reliability for biopsies of the *MLLT* at approximately 40 kg and 80 kg of live weight for values after slaughter, and were not suitable for prediction of meat quality after slaughter.

Conclusions

The results of this study indicated that the prediction reliability for pH_1 and EC_5 from biopsies at approximately 40 kg and 80 kg of live weight for these traits after slaughter were very poor. The correlations for the *Nn* genotype of the *RYR1* locus between post-mortem values and both the first and the second biopsy for pH_1 and EC_{50} values were very low (r = 0.06; r = 0.14 and r = 0.26 and r = 0.26, respectively). The correlations for *nn* genotype were r = –0.23; r = –0.15 and r = –0.25; r = 0.11 respectively. The values of correlations between pH_1 and EC_{50} both biopsies and after slaughter were high (r = –0.52 to –0.84).

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