DETERMINATION OF RN⁻ PHENOTYPE IN PIGS EARLY POST-MORTEM USING VISUAL AND NEAR-INFRARED SPECTROSCOPY

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Key words: pork, RN⁻ gene, glycolytic potential, near-infrared

Background

The specific characteristics of meat from the Hampshire breed of pig, including high glycogen content and low ultimate pH and technological yield (Monin & Sellier, 1985; Lundström *et al.*, 1996), have been associated with the dominant RN⁻ gene (Naveau, 1986). In Sweden, pure-bred Hampshire or Hampshire x Yorkshire is often used as terminal sire in the three-way crosses used for pig meat production. For industrial production of cured and cooked hams, there is a demand to be able to identify the carriers of the RN⁻ gene among pigs on the slaughter-line as early post-mortem as possible. In a recent investigation, Josell *et al.* (2000) showed, on commercial pigs in a slaughterhouse, that by using NIR together with classification with neural networks, it is possible to separate RN⁻ carriers from non-carriers 30 minutes post-mortem. The NIR-measurements were performed with a fibre optic insertion probe. The mathematical model used for discrimination was very complex and it was feared that the method was not very robust. In order to acquire spectra under well-controlled conditions, at-line measurement is needed. Near-infrared spectroscopy has traditionally been used for the determination of chemical composition (water, fat and protein content) in meat (Williams & Norris, 1987). Since there is a difference in protein and glycogen content in carriers and non-carriers of the RN⁻ gene, it may be this difference that is detected with NIR.

Objectives

The aim of this investigation was to confirm that it is possible to detect carriers of the RN⁻ gene early post-mortem and to predict protein and glycogen content in muscle using NIR spectroscopy, both on-line and at-line.

Methods

The pigs were selected from commercial herds producing crossbred pigs for slaughter and using purebred Hampshire or Hampshire x Yorkshire boars and Swedish Landrace x Yorkshire sows. On the slaughter line, carcasses with a pH above 6.0 measured 30 minutes post-mortem in *M. Longissimus dorsi* (LD) were selected. NIR-measurements were performed on 150 carcasses using two NIR-Systems 6500 spectrometers (Foss NIR-Systems). One of the spectrometers was equipped with a fibre optic insertion probe for measurements inside the intact muscles on the slaughter line. The other instrument was equipped with a reflectance transport module for at-line measurements. The fibre optic probe was positioned in LD in such a way that the spectra were measured perpendicular to the muscle fibres. Three spectra were acquired from each carcass over a 3 minute period. After each carcass had been measured with the fibre optic probe, the LD muscle was removed from the carcass and immediately taken to the laboratory where a sample was extracted for scanning in the at-line NIR-instrument. The lapsed time between the probe measurement and the at-line measurement was less than 3 minutes. A sample of meat from each carcass, quickly frozen using liquid nitrogen, was subsequently analysed for glycogen, lactate, glucose and glucose-6-phosphate (Dalrymple & Hamm, 1973) and the glycolytic potential (GP) was calculated (GP = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]) (Monin & Sellier, 1985). The remainder of each loin was packed in vacuum bags and subsequently analysed for water and protein. Carcasses that had been measured were stored overnight in the chilling room (+4°C), and at 24 h post-mortem pH_{24h} and fibre optic probe (FOP_{24h}) reflection levels were measured. The results were statistically evaluated with SYSTAT (Wilkinson, Leland version 7.0) using independent t-test and the Unscrambler (version 6.2, CAMO, Trondheim, Norway) using PLS, cross validation and jack-knife validation.

Results and discussion

The distribution of the glycolytic potential (GP) in pre-rigor *M. Longissimus dorsi* (LD) for the entire material (n=150) is shown in Figure 1. Carriers and non-carriers of the RN⁻ gene can be classified on the basis of distribution of the GP in post-mortem muscle using the valley between the bimodal peaks as the threshold (Fernandez *et al.*, 1992). As can be seen in the Figure, the distribution of the samples in this investigation was bimodal, with few samples between 185 and 210 µmol/g. The distribution was not as bimodal as previously reported (Fernandez *et al.*, 1992 and Josell *et al.*, 2000). In this study, the GP is measured in pre-rigor instead of post-rigor muscle, which may contribute to a somewhat different GP since GP is the sum of glycogen, glucose, glucose-6-phosphate and lactate whereas other intermediate substances from the breakdown of glycogen and glycolysis are not considered. As can be seen seen in Figure 1, the threshold between carriers and non-carriers of the RN⁻ gene lay somewhere between 185 and 210, and 98 animals were considered to be carriers with a GP above 210 µmol/g, and 44 animals non-carriers with a GP below 185 µmol/g. Eight animals with a GP between 185-210 µmol/g were excluded. The frequencies of RN⁻ carriers (69%) and non-carriers (31%) reflect the frequency of RN⁻ carriers in the Swedish slaughter-pig population.

In Table 1, data is shown on the quality traits for the carriers and non-carriers of the RN⁻ gene. The carriers of the RN⁻ gene had a significantly lower pH_{24h} of 5.38, compared with 5.57 for the non-carriers. The FOP_{24h} value was significantly higher in the meat from the carriers than from the non-carriers. Glucose-6-phosphate, glycogen and GP were significantly higher in carriers of the RN⁻ gene than in non-carriers. Glucose and lactate concentration, on the other hand, did not differ between the two RN⁻ genotypes. The protein content was significantly lower and the water content significantly higher in carriers than in non-carriers of the RN⁻ gene.

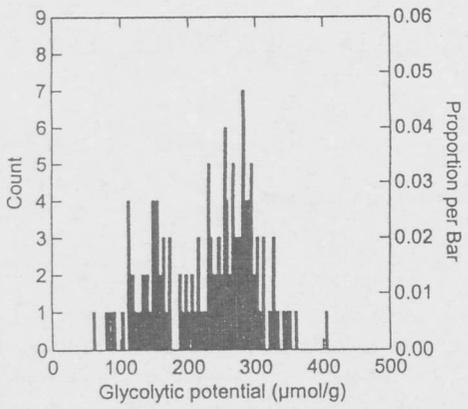

Figure 1. Distribution of the glycolytic potential in the *M. Longissimus dorsi* (n=150).

Table 1. Quality traits (mean ± standard deviation) according to RN⁻ genotype in *M. longissimus dorsi*.

	RN⁻ (n=98)		m⁺m⁺ (n=44)	
pH_{30min}	6.60	± 0.18	6.57	± 0.15
pH_{24h}	5.38[a]	± 0.07	5.57[b]	± 0.22
FOP_{24h}	34.0[a]	± 6.2	29.4[b]	± 7.3
Drip (%)	4.4[a]	± 1.4	3.2[b]	± 1.4
Glycogen (mmol/kg)	114.0[a]	± 16.6	46.5[b]	± 13.6
Glucose (mmol/kg)	1.7	± 0.8	1.8	± 0.7
G-6-P (mmol/kg)	2.9[a]	± 1.9	1.7[b]	± 1.4
Lactate (mmol/kg)	37.2	± 9.1	37.3	± 12.0
Glycolytic potential	274.4[a]	± 36.0	137.3[b]	± 26.6
Water	75.6[a]	± 0.6	74.9[b]	± 0.6
Protein	21.5[a]	± 0.6	23.2[b]	± 0.7

Significant differences between genotypes: a, b: $p \leq 0.001$.

In order to separate RN⁻ carriers from non-carriers using NIR, two evaluation methods were used. In the first method, a PLS model to predict the GP was created, and then a threshold value at 210 GP units was inserted. Carcasses with a predicted GP above 210 were classified as RN⁻ carrier, and the rest were designated as non-carriers. The second way to discriminate the two genotypes was to make a prediction model based directly on prior knowledge of which genotype each carcass belongs to, thus assigning a value of 0 and 1 to each carcass dependent on genotype, and then making a model that can predict this 0-1 variable. The NIR-measurements on-line with the fibre optic probe gave an overall classification error of 15.5% using the threshold method and 16.1% using the discrimination method. The at-line NIR-measurements improved classification and gave an overall misclassification of 5.6% using the threshold method and 6.3% using the discrimination method. A higher percentage of non-carriers was misclassified than carriers of the RN⁻ gene, as regards both at- and on-line measurements.

The GP and content of protein, water and glycogen were predicted with a model from PLS regression based on NIR-spectra. Insignificant wavelengths (based on jack-knife validation) were removed from the regression model. The results of the prediction of water, protein, glycogen and GP, using the at-line NIR-measurements, are shown in Table 2. The prediction model based on the at-line measurements used fewer PLS factors and gave a better prediction of the quality traits than the on-line measurements. As can be seen in the Table, the quality traits were possible to predict with high correlation coefficients (0.81-0.89). The results indicate that NIR can measure both glycogen and protein, which is important in order to discriminate between carriers and non-carriers of the RN⁻ gene. It is not possible to distinguish whether protein contributes more than glycogen when detecting RN⁻ carriers, since these compounds correlate.

Table 2. Prediction of quality traits using PLS.

	Glycogen	GP	Water	Protein
Average value	113.6	272.4	75.39	22.07
std	41.5	84.7	0.69	0.985
R	0.806	0.885	0.823	0.887
RMSEP	24.73	39.4	0.397	0.436
PLS Factors	6	10	7	6

Conclusions

NIR-measurements can be used to differentiate between RN⁻ carriers and non-carriers early post-mortem. Acquiring spectra under well-controlled conditions, using at-line measurement, provided better accuracy than on-line measurement using fibre optic probe. The overall misclassification of carriers and non-carriers of the RN⁻ gene was approximately 6% using the at-line instrument. Glycogen and protein could be measured using NIR.

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