LATIONSHIP BETWEEN GENOTYPES FOR MALIGNANT HYPERTHERMIA DETERMINED BY THE RESTRICTION DONUCLEASE ASSAY AND PORK MEAT QUALITY.

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MMARY

notype for malignant hyperthermia was determined by a restriction endonuclease assay on 913 pork loins. Meat quality acteristics such as color, water holding capacity and ultimate pH (pHu) were found to be significantly affected by genotype. significant differences were found between Nn and nn meat but the meat from NN animals always exhibited significantly 0.001) superior quality (i.e. darker color, better water retention and higher pHu). When loins were classified into normal and E groups, a higher proportion of heterozygote individuals were found in the PSE meat quality category.

TRODUCTION

ine malignant hyperthermia (MH) is a genetic disease which affects calcium regulation in muscle and results in sudden deaths n pale, soft and exudative (PSE) meat. In swine, MH can be induced by halothane anesthesia. Because of the recessive nature the syndrome the test can only detect homozygous (nn) individuals. The alteration in calcium regulation in muscle has been ted to the ryanodine receptor (RYR), a Ca^{++} channel protein in muscle sarcoplasmic reticulum (Mickelson et al, 1988). In fication a C to T substitution at nucleotide 1843 in the cDNA of the RYR from carrier pigs has been associated with the MH ceptibility status of 182 pigs in six breeds (Fujii et al, 1991) and of 376 Landrace pigs which had been haplotyped and halothane ted (Otsu et al, 1991).

e objective of this work was to relate the PSE status of the meat to the genotype of commercial crossbred pigs. For the first e it was possible to characterize meat quality and then determine the genotype of the animal which produced it. Previous dies have all measured the meat quality from pigs of known genotypes and have shown that heterozygote individuals produced ect of intermediate quality between NN and nn individuals (Lundström et al, 1989; Murray et al, 1989; Sather et al, 1991).

TERIALS and METHODS

ee hundred and thirteen boned pork loins were sampled on a commercial cutting line on the basis of the L* value measured h a Colormet surface colorimeter at three ventral locations: posterior, L^*_1, middle, L^*_2 and anterior, L^*_3. For data analysis we fined L* avg as the mean of L^*_1, L^*_2, and L^*_3 and L* max as the maximum value of the three measurements. The Colormet s calibrated on a white plate (L* = 94.5, a = -1.0, b = 0.0). A 1 cm thick slice of longissimus dorsi (LD) was sampled at the anterior ation and used for water holding capacity (WHC) evaluation and pHu determination. The WHC was determined using a dified filter paper technique. Briefly, a 1.4 cm core from the slice of the LD was wrapped in four preweighed filter papers, mum packed and stored for 18 hrs at 1°C. The water retained by the filters was then expressed as a percentage relative to the t weight of the meat. The genotype was determined on a fat sample from the loin as described by Houde and Pommier (1992). efly, a fat sample (approximately 30 mg) was placed in a proteinase-K buffer, incubated for 1-2 hrs at 56°C, inactivated at 95°C d centrifuged. A 81 bp fragment containing the mutation was amplified by the polymerase chain reaction (PCR). The product s then cut by a Hha I restriction enzyme and separated on acrylamide gel electrophoresis in order to diagnose the syndrome.

SULTS and DISCUSSION

pulation description

e loins were selected to obtain a sample covering a wide range of colors but also to obtain a fairly important representation of le meat. The population distribution (Figure 1) demonstrated that most of the loins sampled were within the 50 to 60 range

of L* avg. Few loins were found which exhibited L* avg values lower than 40 or higher than 60 although extreme samples were systematically chosen. It must be emphasised that the distribution diagram does not reflect the natural distribution of color class found in commercial plants but merely results from our selection strategy. Loins within the the different categories exhibited characteristics (Table 1) reflecting the significant ($P < 0.001$) correlation coefficients found between L* avg and WHC or pHu (

Table 1. Meat characteristics of pork loins within the population distribution with respect to color (L* avg).

Meat characteristics	L* avg[1]					
	< 40	40-45	45-50	50-55	55-60	> 60
WHC	33.44	35.11	37.83	44.25	45.15	44.22
	(3.69)[2]	(4.32)	(6.06)	(5.32)	(4.40)	(5.46)
pHu	6.70	6.33	6.03	5.70	5.69	5.67
	(0.23)	(0.32)	(0.37)	(0.20)	(0.22)	(0.17)

[1] L* avg = $L_1^* + L_2^* + L_3^*/3$.

[2] Standard deviation.

and -0.69 respectively). For practical purposes, DFD loins could be found in the categories exhibiting L* avg values < 45 with extreme DFD loins appearing in the L* avg < 40 class. On the other hand PSE loins started to appear in the categories exhibiting L* avg values > 50 with extreme PSE cases showing up in the L* avg > 60 class. Within a color class the highest proportion of heterozygote individuals were found in the 50-55, 55-60 and L* avg > 60 classes. Homozygotes were more prominent in the 55-60 class.

Effect of genotype

The effect of genotype on the meat quality of the loins showed that color was paler for Nn and nn loins (Table 2). Water holding capacity was significantly better ($P<0.01$) for NN loins compared to Nn or nn loins and no significant differences were found between Nn or nn loins. The low occurrence of nn loins demonstrated that although the sample was biased towards the choice of pale loins the incidence of nn individuals in this sample was still small (2.4%). Interestingly pHu was greater for NN loins compared to Nn loins and nn loins were intermediate in pHu value. The results confirm previous studies as for the better quality of NN loins in terms of color and water holding capacity. However the statistical analysis does not segregate between Nn and nn loins and this may be due to the smaller sample size of nn loins.

Table 2. Effect of genotype on meat quality characteristics of loins from commercial crossbred pigs.

Meat characteristics	Genotype		
	NN	Nn	nn
n	693	198	22
L* avg[1]	$50.9^a(0.2)$[2]	$54.1^b(0.4)$	$56.6^b(1.2)$
WHC	$41.4^a(0.2)$	$43.8^b(0.4)$	$44.6^b(1.2)$
pHu	$5.90^a(0.01)$	$5.78^b(0.03)$	$5.84^{ab}(0.9)$

[a,b] Means with different superscripts are significantly different ($P<0.01$)

[1] L* avg = $L^*_1 + L^*_2 + L^*_3/3$

[2] Standard error of the mean

mal vs PSE meat

mentionned previously this sample was biased in favor of pale loins since the goal of the study was to determine the ratio of different genotypes within a color class defined as PSE. For this reason the loins were arbitrarily separated into a normal and SE group on the basis of the highest of the three L* readings on the loin and this reading was defined as L* max. The cut-off e was set at 53.5, a criteria defined by the Quebec industry for Japan exportation. Meat characteristics resulting from this eration (Table 3) showed that L* avg was, as expected, higher for PSE loins. More importantly WHC was significantly poorer PSE meat showing a greater proportion of free water accumulated in the filter paper. The pHu was also significantly lower PSE meat. The fact that 60% of sampled loins were found in the PSE population showed the bias toward selecting for pale s since on the cutting line the PSE incidence based on the color criteria of 53.5 was usually around 20 to 30% and never more an 50% on any given day for that particular plant.

e distribution of the genotypes within the two populations (Figure 2) revealed that in this data set, within the normal loin ulation, we found 88% of NN loins, 11% of Nn loins and 0.55% of nn loins. On the other hand, in the PSE loin population found 67% of NN loins, 28% of Nn loins and 3.6% of nn loins. A simplified approach would conclude that about 17% of PSE et is due to heterozygote individuals.

NCLUSION

ken together these observations confirm previous findings that the PSE condition does not result only from MH but that MH n exacerbate the problem. The elimination of the gene responsible for malignant hyperthermia would most certainly improve e quality of the meat and would reduce the strong interaction between genotype and environment to produce PSE meat. The mmercial raising of heterozygote pigs (Nn) for meat production would clearly increase the incidence of PSE meat. It would be mpting to conclude that by eliminating the heterozygote individuals we would get rid of 17% of PSE loins, but because of the eraction between genotype and environment this percentage would vary according to preslaughter management. Under harsh slaughter conditions 17% will be an underestimation of the contribution of Nn pigs to the PSE problem and under ideal ditions it will be an overestimation.

Table 3. Characteristics of loins separated into normal and PSE meat on the basis of a maximum L value of 53.5 on any of the three locations on the loins.

Meat characteristics	Meat class	
n	Normal (L* max < 53.5)[1]	PSE (L* max ≥ 53.5)
L* avg[2]	363	550
WHC	46.02[a](0.18)[3]	55.58[b](0.14)
pH_u	38.13[a](0.29)	44.59[b](0.24)
	6.12[a](0.02)	5.69[b](0.02)

Means with different superscripts are significantly different ($P<0.01$)
L* max defined as the maximum of L^*_1 or L_2 or L^*_3
L* avg = $L^*_1 + L^*_2 + L^*_3/3$
Standard error of the mean

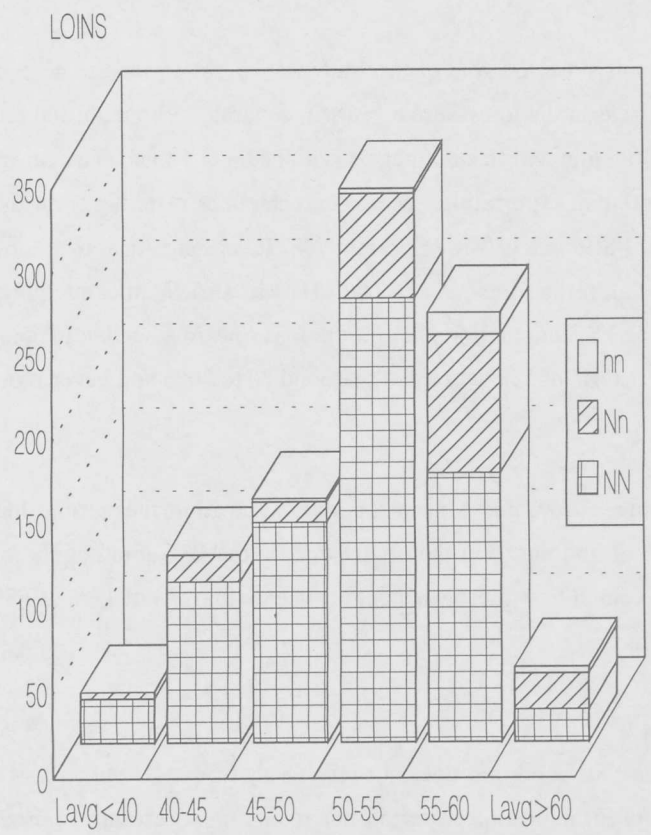

Figure 1. Distribution of loins of different genotypes across different color classes as defined by L* avg.

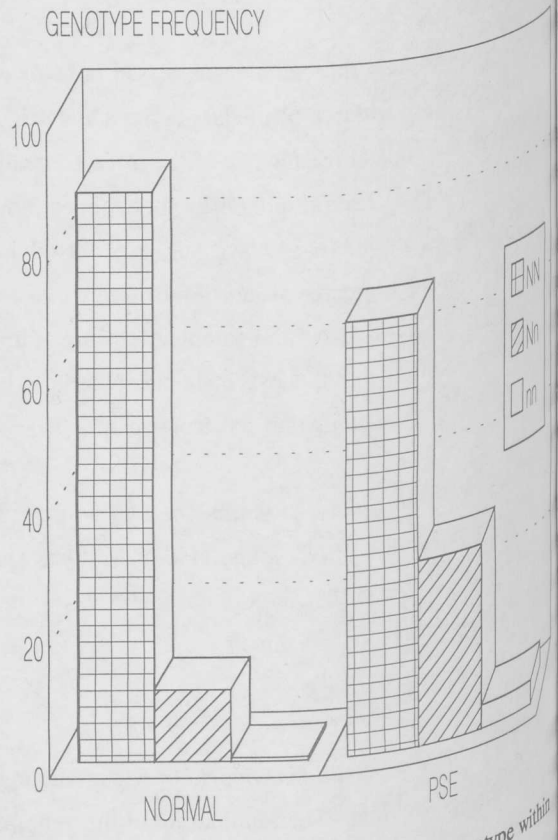

Figure 2. Percent distribution of each genotype within meat categories defined by L* max of 53.5.

REFERENCES

FUJII J., OTSU K., ZORZATO F., DE LEON S., KHANNA V.K., WEILER J.E., O'BRIEN P.J., MACLENNAN D.H. 1991 Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253, 448-451.

HOUDE, A., POMMIER, S.A. 1992 Use of PCR technology to detect a mutation associated with malignant hyperthermia different pig tissues. Meat Sci., (submitted).

LUNDSTRÖM K., GUSTAVSSON-ESSEN B., RUNDGREN M., LILJA EDFORS I., MALMFORS G. 1989 Effect of halothane genotype on muscle metabolism at slaughter and its relationship with meat quality: A within-litter comparison. Meat Sci., 25, 251-263.

MICKELSON J.R., GALLANT E.M., LITTERER L.A., JOHNSON K.M., REMPEL W.E., LOUIS C.F. 1988 Abnormal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. J. Biol. Chem. 263, 9310-9315.

MURRAY A.C., JONES S.D.M., SATHER A.P. 1989 The effect of preslaughter feed restriction and genotype for stress susceptibility on pork lean quality and composition. Can. J. Anim. Sci., 69, 83-91.

OTSU K., KHANNA V.K., ARCHIBALD A.L., MACLENNAN D.H. 1991 Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families. Genomics, 11:744-750.

SATHER A.P., MURRAY A.C., ZAWADSKI S.M., JOHNSON P. 1991 The effect of the halothane gene on pork production and meat quality of pigs reared under commercial conditions. Can. J. Anim. Sci., 71, 959-967.