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PLASMA AND MUSCLE CORTISOL CONCENTRATIONS IN PIGS OF DIFFERENT HALOTHANE GENOTYPES AND THEIR RELATIONSHIP WITH PORK MEAT QUALITY

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INTRODUCTION

The processing quality of pork in Australia is extremely variable. In one survey, average incidences of pale, soft and exudative (PSE) pork and of dark, firm and dry (DFD) pork were 32 and 15% respectively (Trout *et al.*, 1991). A knowledge of the relative contributions of environmental (`stress') and genetic factors to these problems would assist efforts to reduce their incidence. It is generally accepted that PSE pork and DFD pork are `stress-related' and therefore cortisol measurements might be expected to be of value in investigations into these conditions. However, in one trail in which plasma cortisol values indicated a greater than normal amount of ante-mortem stress, the combined incidence of PSE and DFD was less than 3% (Gregory *et al.*, 1987).

It is logical to expect that there would be a closer relationship between muscle cortisol values and muscle properties than between plasma cortisol values and muscle properties. Thus, the measurement of cortisol in a liquid derived from muscle may assist in meat quality investigations. It was therefore decided to measure cortisol levels in muscle juice obtained after high-speed centrifugation.

This paper reports on meat quality measurements and plasma and muscle juice cortisol measurements on pork carcasses derived from pigs of three different halothane genotypes (NN=homozygous normal; Nn=heterozygous; and nn=homozygous halothane positive).

MATERIALS AND METHODS

Animals

Experiment I
The blood and muscle samples were obtained from 165 pigs. There were two lines of pigs in which the halothane allele (n) was segregating. The lines were a `lean' line selected for rapid growth and an unselected `fat' line. There were homozygous normal (NN), homozygous halothane positive (nn) and heterozygous (Nn) genotypes in both lines (McPhee *et al.*, 1992).

On eight separate occasions, a group of 18 to 24 animals were transported overnight by road (600km; 10 to 11 hours) to an abattoir where they were slaughtered approximately five to six hours after arrival. Feed was removed from the animals four hours before transportation to give a total time off feed before slaughter of approximately 20 hours. The animals were stunned electrically (210V AC) while in a V-restrainer and exsanguination commenced within 10 seconds of stunning.

Experiment II
The 48 animals in this experiment were from the same source and had the same genetic background as those in

Experiment I except that for the nn genotype, only one fat line ('fat') was represented. On two separate occasions, a group of 24 animals was transported to the same abattoir used in Experiment I. The journey to the abattoir lasted less than one hour followed by a lairage period of about two hours. Thus, in this experiment, approximately three hours elapsed between leaving the piggery and slaughter. The corresponding figure for the animals in Experiment I was 16 hours). Feed was removed from the animals 14 hours before transportation to give a total time off feed before slaughter of approximately 17 hours. In both experiments, the pigs were raised in individual pens but were intermingled for several hours weekly and prior to transport to the abattoir. This mixing familiarized pigs with each other so the agnostic encounters had fallen to a low level by the time they were consigned to the abattoir.

Sample collection and meat quality measurement

Plasma was obtained from blood samples collected at exsanguination. *longissimus dorsi* muscles were removed after overnight chilling of the carcass. Weighed muscle samples of three to four grams were centrifuged at 100,000g for 0.5 hours in stainless steel tubes (Bouton *et al.*, 1972). Both the expressed muscle juice and the blood plasma were frozen (-20°C) immediately after centrifugation and stored for subsequent cortisol analysis.

The plasma and muscle juice samples were assayed for cortisol using a radio-immunoassay method (Incstar Corporation, Stillwater, Minnesota, USA).

Ultimate pH (pH^u) and Minolta Chroma Meter, L, a, b values (Illuminant D65, 2° observer angle, Model CR-2000, Minolta Camera Co Ltd., Osaka, Japan) were measured on a section of the longissimus dorsi muscle between the 2^{nd} last rib and the $5^{th}/6^{th}$ lumbar vertebrae which was removed from the carcasses 24 hours post-mortem (Trout, 1992).

Statistical analyses

Experiment I

The data were analyzed by an analysis of variance procedure for unbalanced design (Harvey, 1985). The carcasses were subdivided into three groups: PSE (Minolta values>49.7 and pH^u<6.0), DFD (pH^u>6.0) and normal (all carcasses not in the previous two) (Trout, 1992).

Experiment II

The data was analyzed by a general linear model (GLM). Because of rank deficiency due to empty cells, a complete statistical analysis, incorporating all factors, was not possible. For the cortisol analysis, genotype was excluded, while for the PSE analysis, fat line was excluded. Separate analysis confirmed that these factors had no effect on the respective variates. The two carcasses classified as DFD were excluded from the statistical analysis and, thus, there were only two categories based on meat quality -- PSE and normal.

RESULTS AND DISCUSSION

The data in Table 1 are the least square means of plasma and muscle cortisol concentrations for the three carcass classifications (normal, PSE and DFD) for Experiment I. There were no significant differences between the three categories for plasma cortisol ($P>0.05$) but for muscle cortisol, DFD carcasses had significantly ($P<0.05$) greater concentration than the normal carcasses while the difference between normal and PSE carcasses was not statistically significant ($P>0.05$). In Experiment II, there were no statistically significant differences between the PSE and the normal groups for either plasma or muscle cortisol (mean plasma cortisol concentrations for the PSE and normal groups were 427±39 and 426±33 mmol/l respectively). In Experiment II, the homozygous nn group had a significantly ($P<0.05$) greater percentage of PSE carcasses than the homozygous NN group.

In both experiments, genotype had no significant effect on plasma or muscle juice cortisol concentrations.

Cortisol and meat quality

PSE is regarded as a `stress-related' condition. However, in both experiments in this study, plasma or muscle cortisol concentrations in samples from PSE carcasses did not differ significantly from those in samples from normal carcasses. In contrast, DFD carcasses had elevated muscle cortisol concentrations in comparison with normal carcasses (Table 1). If it is accepted that elevated cortisol concentrations are an indication of stress, then the results suggest that pre-slaughter stress is a contributing factor to the production of DFD pork, but it may not be a major factor in the production of PSE pork. Alternatively, it is possible that the stress contributing to the production of PSE may have occurred immediately before slaughter and thus not be indicated by increased cortisol concentrations. The measurement of adrenocorticotrophic hormone (ACTH), which initiates the release of cortisol, may be of value in confirming or refuting this hypothesis. In both experiments, plasma cortisol concentrations were generally greater than those reported by others (Shaw and Tume, 1992).

Cortisol and genotypes

In both experiments, mean muscle and plasma cortisol levels for the three genotypes did not differ significantly. Nyberg *et al.* (1988) found that in some cases, there was a significant interaction between halothane genotype and treatment, but concluded that there was no simple relationship between plasma cortisol and halothane genotype.

Incidence of PSE and DFD

The low incidence of DFD pork in Experiment II is presumably due to the short time the animals spent in transport and lairage. The results presented in this paper are in agreement with previous findings that the halothane gene has a deleterious effect on meat quality with homozygous (nn) individuals having a high incidence of PSE (Murray and Jones, 1992; Pommier and Houde, 1992).

CONCLUSION

Halothane positive homozygous (nn) pigs produced a greater percentage of PSE carcasses than did homozygous (NN) pigs. However, the incidence of DFD was not affected by genotype. Muscle cortisol concentrations in carcasses classified as DFD were significantly greater than those from normal carcasses. Plasma and muscle cortisol concentrations in carcasses classified as PSE did not differ significantly from those of normal carcasses. If it is accepted that elevated cortisol concentrations are an indication of stress, then these results lend support to the conventional hypothesis that pre-slaughter stress is a contributing factor to the production of DFD pork. Similarly, if the relationship between cortisol concentrations and stress is accepted, then the results suggest that pre-slaughter stress may not be the major factor in the production of PSE pork. The results support the hypothesis that genetic factors are important in the production of this condition. This information should form the basis of education and extension campaigns to reduce the incidence of DFD and PSE pork.

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Table 1. Least square means (±SE) of plasma and muscle cortisol concentrations (nmol/l) for carcasses classified as PSE, normal or DFD (Experiment I).

	PSE	Normal	DFD
Plasma	459 ± 41	389 ± 19	420 ± 26
Muscle	81 ± 13[ab]	69 ± 6[a]	100 ± 7[b]
n	22	85	58

Row means with the same, or no, superscript are not significantly different (P>0.05).

Table 2. Percentage of pigs (±SE) of each halothane genotype whose carcasses exhibited either PSE or DFD (Experiments I and II).

	NN	Nn	nn
Experiment I:			
PSE	5.8 ± 3.8	14.7 ± 3.9	20.5 ± 7.8
DFD	40.9 ± 5.5	34.5 ± 5.6	32.5 ± 11.1
Number	71	68	26
Experiment II:			
PSE	26.3 ±10.3[a]	63.1 ±11.4[b]	100.0b[c]
DFD	5.0 ± 4.9	5.0 ± 4.9	0
Number	20	20	8

Row means with the same, or no, superscript, are not significantly different (P>0.05).