An In Vivo Predictive Test for Meat Quality in Pigs

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SUMMARY: An *in vivo* predictive test for meat quality in pigs was devised. Meat quality was assessed on the 12,000g supernatant ("Fluid Volume") by measurements of pH, water-holding capacity (WHC) and Ca^{2+} released after incubation of 0.5g *M. longissimus dorsi* (LD) biopsy and/or immediate (<3mins) post-mortem samples with an equal volume of 150mM KCl at 39°C for 45 mins.

A significant difference ($P<0.001$) in the "Fluid Volume", pH and Ca^{2+} released was observed between halothane +ve and halothane -ve pigs. A high correlation [$r = -0.85 \pm 0.19$ ($P<0.001$)] between pH and "Fluid Volume" was observed. Pigs which exhibit a "Fluid Volume" less than 0.45g/0.5g wet wt LD produce normal pork and those with values greater than this produce PSE pork. Measurements of "Fluid Volume" and pH using small "Shot Biopsy" LD samples can identify PSE-prone pigs and can be used to select pigs with a potential to produce pork of good WHC.

INTRODUCTION: Various methods of predicting meat quality in pigs have been devised using both non-invasive and invasive techniques (CHEAH, 1991) in an attempt to reduce or eliminate the porcine stress syndrome (PSS) and thereby prevent the formation of pale, soft and exudative (PSE) pork (SYBESMA and EIKELENBOOM, 1978; CHEAH and CHEAH, 1979; ALLEN et al., 1980). A combination of different parameters such as pH at either 45 mins or 60 mins post-mortem, and colour and WHC at 24 hrs post-mortem are frequently employed to characterize PSE pork (CHEAH et al ., 1984)

Halothane testing is widely used to reduce the incidence of PSS, but this test is incapable of eliminating PSS as halothane cannot detect the heterozygotes (Nn). Various procedures have been developed to identify the heterozygotes in order to eliminate the halothane gene and PSE. A non-invasive test using a combination of the blood enzymes Phi and Pgd, and the plasma protein, postalbumin-2, has been suggested to be able to identify the heterozygotes (OSKAM, 1987). Heterozygotes could also be differentiated from the homozygotes (NN and nn) by their isolated LD muscle mitochondrial phospholipase A_2 activity but this invasive biochemical test is unsuitable for general application (CHEAH, 1991). The ryanodine receptor gene has recently been postulated to be the candidate for predisposition to human (MACLENNAN et al., 1990) and porcine (LOUIS et al., 1990) malignant hyperthermia (MH). When fully evaluated this may provide a sensitive non-invasive procedure for detecting the heterozygotes.

This paper describes a new *in vivo* procedure for predicting meat quality in pigs using small "Shot Biopsy" samples of LD muscles. A combination of measurements of pH and "Fluid Volume", an indicator of WHC, with and without the estimation of Ca^{2+} released in the 12,000g supernatant after incubation of 0.5g biopsy LD muscle with an equal volume of 150mM KCl at 39°C for 45 mins is used to characterize the pork as either normal or PSE.

MATERIALS and METHODS: Only genetically selected stress-susceptible (halothane +ve) and stress-resistant (halothane -ve) pigs were used for our studies. "Shot Biopsy" samples were taken from LD muscles (LAHUCKY, 1987) from genetically selected 70-80kg pigs bred at the Research Institute of Animal Production, Nitra, Czechoslovakia. LD samples (0.5g), biopsy and immediate post-mortem (<3mins), were incubated with an equal volume of 150mM KCl in Eppendorf micro-centrifuge tubes for 45 mins at 39°C. After incubation, the LD samples were finely minced with scissors for 2 mins in ice and centrifuged at 12,000g for 2 mins. The 12,000g supernatant or "Fluid Volume" was carefully removed with a Hamilton syringe (250ul) and the pH and amount of Ca^{2+} released were determined in the "Fluid Volume". Ca^{2+} was determined by atomic absorption at 422.7nm in the presence of 1% (w/v) lanthanum (CHEAH et al., 1984), and the "Fluid Volume", an indicator of the WHC of LD muscle, is expressed as the amount of fluid per 0.5g LD muscle. The pH values of the "Fluid Volume" and the 12,000g muscle residue were measured with a combined micro-electrode. Colour was assessed in LD muscles removed from the carcasses at 24 hrs post-mortem (CHEAH et al., 1984).

RESULTS and DISCUSSION: The relationship between "Fluid Volume", Ca^{2+} released and pH of immediate post-mortem (<3mins) LD muscle samples of British Landrace (B/L) after incubation for 45 mins at 39°C is shown in Table 1. Halothane +ve pigs showed significantly ($P<0.001$) higher values in "Fluid Volume" and Ca^{2+} released, and lower pH than corresponding values for halothane -ve pigs. The Ca^{2+} released by LD muscles of halothane -ve B/L pigs closely resembled values from our previous studies (CHEAH et al., 1986) using 10g LD muscles obtained at 45 mins post-mortem from the carcasses, but the values for halothane +ve B/L pigs were approximately 50% lower. The difference in the amount of Ca^{2+} released in the halothane +ve pigs is due to different experimental conditions. However, with both conditions, the amount of Ca^{2+} released is significantly different ($P<0.001$) between the halothane +ve and halothane -ve B/L pigs.

Table 1. Relationship between "Fluid Volume", Ca^{2+} released, pH and meat quality in (<3mins) post-mortem LD muscle samples after incubation with an equal volume of 150mM KCl at 39°C for 45 mins.

Pigs (B/L)	"Fluid Volume" (g/0.5g wet wt LD)	Ca^{2+} Released (ug/g wet wt LD)	pH (45' PM)	Meat Quality
+ve	0.59 ± 0.03 (n = 6)	4.74 ± 1.19 (n = 6)	5.74 ± 0.08 (n = 6)	PSE
-ve	0.35 ± 0.03 (n = 8)	1.19 ± 0.49 (n = 8)	6.77 ± 0.17 (n = 8)	NORMAL
Student's t-test	P<0.001	P<0.001	P<0.001	

The *in vitro* data (Table 1) suggest that halothane +ve and -ve B/L pigs and meat quality can be differentiated by our simple "Fluid Volume" test in conjunction with pH measurements. This procedure was then applied to live pigs using "Shot Biopsy" LD muscle samples for evaluation as a potential *in vivo* diagnostic test for assessing meat quality.

Table 2 summarizes the relationship between "Fluid Volume", pH and halothane sensitivity using "Shot Biopsy" and immediate post-mortem (<3mins) LD samples from Landrace x Duroc pigs.

Table 2. Comparative studies between "Shot Biopsy" and immediate (<3mins) post-mortem LD muscle samples after incubation with an equal volume of 150mM KCl at 39°C for 45 mins.

(Landrace x Duroc)	"Shot Biopsy"			Post-Mortem (<3mins)		
	pH (Muscle)	pH (Sup^n)	"Fluid Volume"	pH (Muscle)	pH (Sup^n)	"Fluid Volume"
-ve	6.34 ± 0.33 (n = 7)	6.51 ± 0.26 (n = 7)	0.376 ± 0.02 (n = 7)	6.43 ± 0.10 (n = 7)	6.51 ± 0.23 (n = 7)	0.383 ± 0.02 (n = 7)
+ve	5.73 ± 0.19 (n = 5)	5.82 ± 0.14 (n = 5)	0.530 ± 0.09 (n = 5)	5.64 ± 0.15 (n = 5)	5.78 ± 0.15 (n = 5)	0.470 ± 0.04 (n = 5)
t-test	P<0.01	P<0.001	P<0.01	P<0.001	P<0.001	P<0.05

In both "Shot Biopsy" and post-mortem samples, halothane +ve pigs showed significantly higher "Fluid Volume" than halothane -ve pigs, and their pH values in the 12,000g supernatant (Sup^n) and muscle residue are significantly lower than those of halothane -ve pigs. There are no significant differences in the pH values between the supernatant and muscle residue within each type of pigs. Within each group of pigs, there was also no significant difference in the values of "Fluid Volume" and pH between "Shot Biopsy" and immediate post-mortem LD muscle samples.

The data presented in Table 2 support the *in vivo* "Shot Biopsy" procedure for obtaining small samples of LD muscle as suitable for evaluating pig meat quality. Further evaluation using 32 halothane +ve and 42 halothane -ve Landrace x Duroc pigs also showed that the halothane +ve pigs have significantly higher (P<0.001) "Fluid Volume" and lower pH values (P<0.001) after incubation at 39°C for 45 mins. Halothane +ve pigs showed a "Fluid Volume" (g/0.5g wet wt LD) of 0.55 ± 0.04 (n = 32) and pH values of 5.87 ± 0.17 (n = 32) (fluid) and 5.83 ± 0.17 (n = 32) (muscle residue) as against corresponding values of 0.39 ± 0.03 (n = 42), 6.50 ± 0.16 (n = 42) (fluid) and 6.37 ±0.25 (n = 42) (muscle residue) for the halothane -ve pigs.

A high correlation [r = - 0.85 ± 0.19 (P<0.001)] between pH and "Fluid Volume" was observed in the "Shot Biopsy" LD muscle samples of both halothane +ve and -ve lines after incubation for 45 mins at 39°C (Figure 1). The data suggest that pigs which exhibit "Fluid Volume" values of less than 0.45g/0.5g wet wt LD will potentially produce normal pork, and those greater than this will produce PSE pork.

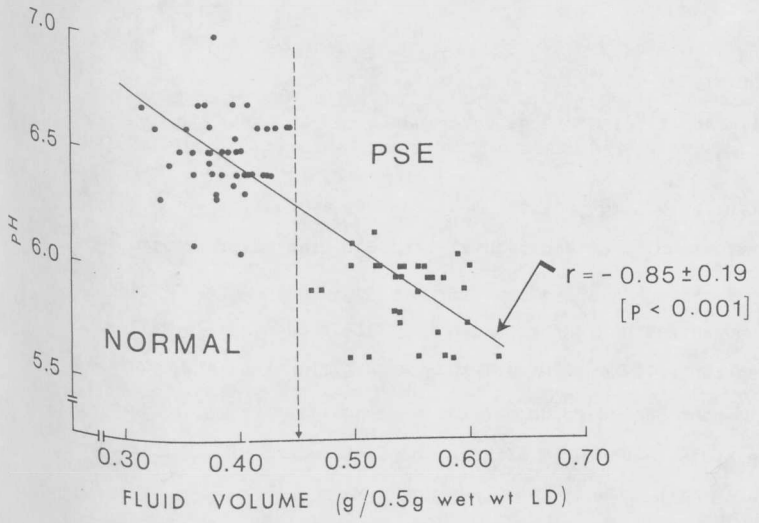

Figure 1. Relationship between "Fluid Volume" and pH of "Shot Biopsy" LD muscle samples after incubation with an equal volume of 150mM KCl at 39°C for 45 mins.

■, +ve (n = 32)

●, -ve (n = 42)

CONCLUSIONS: An in vivo predictive test for meat quality in pigs was devised using small (0.5g) "Shot Biopsy" LD muscle samples. The test can be used to select pigs with a potential to produce pork of good WHC and can be applied by the pig industries to identify potentially PSE-prone pigs.

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