Genetic parameters and breed differences for iron content in pork
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By

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Executive Summary

Meat is the main source of iron from livestock products for the human diet (Rooke et al. 2011). There are indications that levels of iron in pork have decreased over time (Dannenberger et al. 2007). Organisms regulate their iron homeostasis to avoid accumulation of iron to potentially toxic levels in tissue and opportunities to manipulate iron content in pork via dietary avenues are limited (Cottam et al. 2007; Rooke et al. 2011). However, considerable variation in iron content in pork has been observed (Rooke et al. 2011), which may be useful for genetic improvement strategies.

Cost effective measurements recorded on the live animal on farm prior to selection are beneficial for breeding programs. Haemoglobin levels in blood may be used as a selection criterion for iron content in pork, if it can be demonstrated that it is genetically associated with iron content in pork. In addition, the use of the HemoCue® device developed to measure haemoglobin levels at point-of-care in human medicine has been used successfully in veterinary and nutritional studies to measure haemoglobin levels in sows and piglets on farm. This technology provides opportunities for pig breeding programs to develop a simple on-farm measurement as a selection criterion for iron content in pork and pork quality. It was the aim of this study to obtain genetic parameters for haemoglobin levels in blood and iron content in pork and to estimate genetic correlations between these measures of iron and meat quality and performance traits.

Records for blood haemoglobin levels measured at five (N: 4974) and 21 (N: 2405) weeks of age in two sire lines were combined with data about iron content in pork and meat quality traits (N: 2255 records), and performance traits (N: about 60000). Iron measures were based on two replicates of samples from *m. longissimus dorsi*, which were prepared using ceramic knives in the laboratory. Iron levels of duplicate samples were expected to be within 10% of each other, which was only achieved with a sample weight of 1000 mg wet weight and after steel utensils had been replaced by ceramic knives. Additional investigations indicated that the use of steel knives increased the mean and variation in iron levels of pork compared with the use of ceramic knives. Genetic parameters were estimated based on an animal model using residual maximum likelihood procedures.

Iron content in pork was moderately heritable (0.34 ± 0.07) and genetic correlations with haemoglobin measures ranged from 0.39 ± 0.24 to 0.58 ± 0.13 indicating its potential use as a selection criterion for increasing iron levels in pork. However, heritabilities for haemoglobin were low, ranging from 0.04 ± 0.2 to 0.18 ± 0.04 and the on-farm haemoglobin measure may require refinement using experiences from other Australian studies that have used the HemoCue® device successfully on farm to measure haemoglobin levels in pigs (i.e. Payne, 2009).

Redness of pork, quantified by the a* value of the Minolta chromameter, had high genetic correlations with iron content (0.89 ± 0.04 and 0.91 ± 0.04) and moderate genetic correlations with haemoglobin levels (0.29 ± 0.22 to 0.55 ± 0.15). Iron content had significant genetic associations with L* value (-0.61 ± 0.14 to -0.54 ± 0.23), b* value (0.59 ± 0.14 dorsal, 0.86 ± 0.06 for average of dorsal and ventral b* value) and pH at 45 minutes post mortem (-0.42 ± 0.14). The high genetic correlations between colour measurements and iron content in pork provide further avenues for selection strategies to improve iron content in pork.

Current selection practices are not expected to affect iron content in pork, given that no significant genetic correlations between performance and haematological traits were found. Therefore, the exact causes of the observed reduction in iron levels of pork over time remain unknown.

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1. Introduction

Livestock products contribute a significant amount of trace elements and vitamins to the human diet, with meat being the main source of iron from livestock products (Rooke et al. 2010). It is generally well known that red meats have greater iron content than pork or chicken and there is some evidence that levels of iron in pork have decreased over time (Dannenberger *et al*. 2007). However, iron content in meat may vary considerably within species even exceeding variation between species as was highlighted by Rooke et al. (2010) who found a 10-fold ratio of the maximum to minimum iron content reported in research publications for ovine, bovine, porcine and chicken muscle. In regard to pork, iron content varied from 3 mg/kg to 30 mg/kg and this considerable level of variation may be exploited by genetic improvement strategies to increase iron content in pork.

Organisms regulate their iron homeostasis to avoid accumulation of iron to potentially toxic levels in tissue (Cottam et al., 2007; Rooke et al., 2011). Increasing the dietary levels of iron beyond the requirements for maintenance and growth does not lead to the transfer of iron to muscle but to the removal of excess iron in storage (in the liver) or increased excretion of iron. Therefore, iron content in pork is largely unresponsive to manipulations by dietary avenues.

Modern genotypes have been shown to have lower iron content in meat samples in comparison to pigs available 20 years earlier. For example, Oksbjerg et al. (2000) compared performance and meat quality characteristics of pigs representing an average sample of Danish Landrace in 1995 with pigs representing a sample available in 1975. The 1995 genotype had superior daily gain and feed conversion ratio accompanied by a lighter colour and lower myoglobin concentration of pork. The lower myoglobin content of pork in fast growing genotypes is believed to be due to higher proportion of glycolytic muscle fibre also called 'white' muscle fibre (Dannenberger et al., 2007; Ruusunen et al., 2004). Pork haem pigment was moderately heritable (0.39 ± 0.09) in the study by Larzul et al. (1997) but had a lower heritability of 0.17 ± 0.02 *in vivo* in the study by Oksbjerg et al. (2004).

Measurements on the live pig prior to selection are beneficial for breeding programs. An alternative indirect measure for iron content in pork may be haemoglobin content in blood. The HemoCue® Hb 201$^+$ device developed to measure haemoglobin levels at point-of-care in human medicine has been used successfully in veterinary studies (Auvigne et al. 2010, Van der Haeghe et al. 2010) to measure haemoglobin levels in sows and piglets on-farm. This technology provides opportunities for pig breeding to develop a simple on-farm measurement as a selection criterion for iron content in pork and pork quality.

It was the aim of this study to estimate genetic parameters for blood haemoglobin levels and iron content in pork and to estimate their genetic correlations with performance and meat quality traits.

2. Methodology

Description of Data

Blood haemoglobin levels were recorded on 4974 piglets from 2 terminal sire lines at 32.3 (± 3.0) days of age (HAEM5) between September 2009 and September 2010. A routine iron injection of 200 mg iron dextran was given to all piglets shortly after birth. Haemoglobin

levels were then measured on 2,405 pigs at approximately 21 weeks (134±161 days; HAEM21, HAEM21ex). Most of these pigs had both haemoglobin measures at five and 22 weeks of age (2,154 pigs). Blood haemoglobin was measured on farm by collecting blood via jugular venipuncture. Fresh blood samples were then stored in the fridge for a maximum of 30 hours and manually shaken prior to recording haemoglobin levels using the HemoCue Hb 201^{+} analyser (HemoCue® 2011). Limits imposed for haemoglobin at 5 and 21 weeks were 60 to 145g/L and 70 to 135g/L, respectively. These boundaries reduced residual variance without overly affecting additive genetic variance (Jones and Hermesch, 2010). In addition, a separate trait definition was used for haemoglobin levels at 22 weeks excluding records from the 22 July 2010 until 19 August 2010 (HAEM21ex) following initial analyses of these data.

Iron content in pork was recorded in the *m. longissimus dorsi* (IRON) for 2255 boars. Iron measurements were obtained by totally digesting duplicate muscle samples with a wet weight of approximately 1000 mg in concentrated nitric/perchloric acids to white fumes of perchloric. The digest was then cooled, water was added and total iron content was measured by flame atomic absorption spectrometry using an air/acetylene flame. Results of duplicate samples were expected to be within 10% of each other. This criterion was only achieved with a sample weight of 1000 mg wet weight and after steel utensils had been replaced by ceramic or plastic utensils.

Meat quality traits were recorded on the same boars tested for pork iron content. The tristimulus parameters L*, a* and b* of the Minolta Chroma Meter CR 400 (measurement area 8 mm) were recorded on the dorsal and ventral section of the *m. longissimus dorsi*. The L* value represents luminance on a scale of 0 to 100 where 0 is completely black and 100 is white, positive a* values represents red colours while negative a*values represent green colours and positive b* values represent yellowness and negative b* values represent blue colours. In addition, pH was measured on the *m. longissimus dorsi* at 45 minutes (pH45) and 24 hours (pH24) *post mortem*. Loin depth (LD) and fat depth (CP2) both recorded at the P2 site on the carcase, 65 mm from the midline of the carcase at the last thoracic rib (Greer *et al*. 1987) using PorkScan equipment (PorkScan Pty Ltd).

Performance data available from January 2004 until September 2010 for these two terminal sire lines were used for the genetic analyses of performance traits. Lifetime growth rate (ADG), backfat depth at the P2 site (BF) and loin muscle depth (MD) between the third and fourth last ribs at the same age were recorded on pigs at 150 ± 7.52 days of age. Backfat and muscle depth were recorded using real time ultrasound. Performance records exceeding 3 standard deviations from the mean were deleted.

The pedigree included 82795 animals from 537 sires and 4463 dams born from January 2004 until September 2010. The subset of pigs that had haemoglobin and iron traits recorded originated from 91 sires and 876 dams.

Statistical Analysis.

The following mixed linear animal models were used for the analyses of traits.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad [1]$$

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e} \quad [2]$$

where **y** represents the vector of observations with, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects of animals, **c** is the vector of common litter effects and **e** is the vector of residual effects. The terms **X**, **Z** and **W** are incidence matrices relating records to fixed, animal and common litter effects, respectively. The expectations of random effects were zero and the variances were assumed to be var(**a**)= **A**, var(**c**)= **I**, and var(**e**)= **I**, where **A** is the numerator relationship matrix among animals and **I** is the identity matrix. All remaining covariances were assumed to be zero. Variance components were estimated with ASReml (Gilmour *et al*. 2006) in univariate and bivariate analyses.

The GLM (SAS 1999) procedure was used to derive the fixed effect model for each trait. The fixed effect model for haemoglobin at 5 weeks included test date, breed as well as the linear covariables age of the piglet at recording and piglet birth weight and the total number of piglets born in the birth litter of each piglet. The models for HAEM21 and HAEM21ex included test date, breed and sex as fixed effects. In addition, piglet birth weight was fitted as a linear covariate for HAEMex. Test date, breed and the linear covariate of age at recording were significant fixed effects for iron in pork.

Fixed effects fitted for the tristimulus parameters L*, a* and b*, both pH measures and carcase fat and muscle depth were slaughter date and breed. In addition, the models for pH24 and carcase fat and muscle depth included hot standard carcase weight as a linear covariate.

The models for growth rate, backfat and muscle depth included test date, breed, sex and management group as fixed effects. Weight at test was fitted as a linear covariate for backfat and muscle depth.

3. Outcomes

Research results

The common litter effect was the main random effect (0.11 ± 0.02) (Table 1) for haemoglobin at five weeks of age, which had a low heritability of 0.04 ± 0.02. Traits of the piglet are usually affected by maternal genetic effects. It was not possible to estimate maternal genetic effects for this trait due to the lack of records across generations required for their estimation.

Haemoglobin at 21 weeks also had a low heritability of 0.09 ± 0.04 and a significant common litter effect estimate of 0.08 ± 0.03 using the complete data set. Additional analyses revealed that measurements taken from the 22nd of July until the 19th of August 2010 caused a shift of variance from additive genetic to common litter variance (Table 2). Excluding these records resulted in a moderately higher heritability of 0.18 ± 0.04 using the best model without the common litter effect, which was not significant (HAEM21ex in Table 1). Causes of this shift in variances are unknown. Data statistics did not differ

significantly between data subsets. However, there were slightly fewer piglets per litter in the excluded data subset (1.9 versus 2.2) due to a higher incidence of litters with one litter mate only. Further investigations showed that a higher incidence of litters with one litter mate only increased the estimate of the common litter effect at the expense of the heritability estimate in this data set, which had a limited pedigree depth for pigs with haemoglobin measures. Due to the limited duration of the trial there were no parents who had their own records for our main traits of interest (HAEM5, HAEM21and IRON). Similar issues were not found for iron content in pork, which was moderately heritable (0.34 ± 0.07) and less affected by common litter effect (0.06 ± 0.03) as may be expected for a meat quality trait.

Table 1 Number of records (N), means and standard deviations (SD), heritability estimates (h^2) and common litter effect (c^2) both with standard errors (se) along with phenotypic variance (σ^2_p) for haematological traits

Trait (unit, abbreviation)	N	Mean	SD	$h^2_{(se)}$	$c^2_{(se)}$	σ^2_p
Haemoglobin at 5 weeks (g/L, HAEM5)	4 974	106.6	16.2	$0.04_{(0.02)}$	$0.11_{(0.02)}$	206.4
Haemoglobin at 21 weeks (g/L, HAEM21)	2 405	105.4	13.4	$0.09_{(0.04)}$	$0.08_{(0.03)}$	167.1
Haemoglobin at 21 weeks (g/L, HAEM21ex)*	2 157	105.6	13.0	$0.18_{(0.04)}$	-	149.7
Iron in pork (mg/kg, IRON)	2 253	2.87	0.44	$0.34_{(0.07)}$	$0.06_{(0.03)}$	0.124

* records from July 22 to August 19 were excluded

Table 2 Number of records (N), heritability (h^2) and common litter effect (c^2) estimates for haemoglobin at 21 weeks using various data cut-off dates in 2010

Cut-off dates	N	h^2(se)	s.e.	c^2(se)	s.e.
2-Jul	920	0.15	0.08	0.04	0.05
8-Jul	970	0.22	0.09	0.02	0.04
15-Jul	1007	0.22	0.09	0.03	0.04
22-Jul	***1063***	***0.17***	***0.07***	***0.05***	***0.04***
29-Jul	***1109***	***0.11***	***0.06***	***0.08***	***0.04***
5-Aug	***1159***	***0.09***	***0.05***	***0.08***	***0.04***
12-Aug	***1213***	***0.07***	***0.05***	***0.11***	***0.04***
19-Aug	***1257***	***0.03***	***0.04***	***0.13***	***0.04***
26-Aug	1316	0.05	0.04	0.11	0.04
2-Sep	1383	0.06	0.05	0.11	0.04
9-Sep	1434	0.07	0.05	0.11	0.04
16-Sep	1485	0.06	0.04	0.10	0.04
23-Sep	1525	0.06	0.04	0.10	0.04

Negative b* values were observed for dorsal or ventral measurements, which were not apparent for the mean b* value leading to a higher mean accompanied by a lower variation for the average b* value (Table 3). This aspect was not observed for other colour measurements. Heritability estimates differed among the individual measurements of the Minolta chromameter. Estimates were lowest for L* values ranging from 0.03 ± 0.02 at the

dorsal site to 0.09 ± 0.03 at the ventral site and highest for a* values with estimates of 0.37 ± 0.05 and 0.33 ± 0.05 for the dorsal and ventral sites with the average being slightly higher (0.41 ± 0.06). Heritability was also slightly higher for the average b* value (0.26 ± 0.05) than heritability estimates for the two individual measurements at the dorsal (0.13 ± 0.04) and ventral (0.09 ± 0.03) sites. Potential genetic improvement for both pH measurements is limited by the extremely low variability in these traits despite low to moderate heritabilities.

Both carcase measurements had higher heritability estimates (CP2: 0.34 ± 0.05; LD: 0.40 ± 0.06) in comparison to the corresponding trait recorded on the live animal (BF: 0.27 ± 0.01; MD: 0.19 ± 0.01; Table 4). Growth rate was moderately heritable and was affected by the common litter effect as is usually observed.

Table 3 Number of records (N), means and standard deviations (SD), heritability estimates (h^2) with standard errors (se) along with phenotypic variance (σ^2_P) for meat quality traits

Trait	N	Mean	SD	$h^2_{(se)}$	σ^2_P
L* value Dorsal	2 417	47.21	3.33	$0.03_{(0.02)}$	7.69
L* value Ventral	2 419	48.11	3.05	$0.09_{(0.03)}$	7.71
L* value Average	2 419	47.65	2.91	$0.06_{(0.03)}$	6.19
a* value Dorsal	2 420	5.62	1.11	$0.37_{(0.05)}$	1.07
a* value Ventral	2 406	5.60	1.02	$0.33_{(0.05)}$	0.884
a* value Average	2 412	5.62	0.95	$0.41_{(0.06)}$	0.771
b* value Dorsal	2 420	2.33	1.04	$0.13_{(0.04)}$	0.815
b* value Ventral	2 418	2.23	1.08	$0.09_{(0.03)}$	0.821
b* value Average	2 419	3.92	0.85	$0.26_{(0.05)}$	0.556
pH 45 minutes *post mortem*	2 425	6.03	0.26	$0.23_{(0.05)}$	0.042
pH at 24 hours *post mortem*	2 436	5.64	0.14	$0.12_{(0.04)}$	0.009

Table 4 Number of records (N), means and standard deviations (SD), heritability estimates (h^2) and common litter effect (c^2) both with standard errors (se) along with phenotypic variance (σ^2_p) for carcase traits

Trait (unit, abbreviation)	N	Mean	SD	$h^2_{(se)}$	$c^2_{(se)}$	σ^2_p
Backfat (mm, BF)	58 719	9.33	2.09	$0.27_{(0.01)}$	$0.04_{(0.003)}$	2.57
Carcase P2 (mm, CP2)	2 423	7.04	1.37	$0.34_{(0.05)}$	-	1.49
Muscle depth (mm, MD)	59 753	44.3	5.90	$0.19_{(0.01)}$	-	21.5
Loin depth (mm, LD)	2 400	48.8	5.87	$0.40_{(0.06)}$	-	25.0
Average daily gain (g/d, ADG)	59 671	621	76.0	$0.22_{(0.01)}$	$0.10_{(0.004)}$	4910

Genetic correlations

Iron content in pork had high positive genetic correlations with both traits describing haemoglobin levels at 21 weeks of age (0.50 ± 0.19; 0.58 ± 0.13; Table 5). Genetic correlations between blood haemoglobin levels at five weeks of age and any one of the other three haematological traits were positive but not significantly different to zero given the magnitude of standard errors, which are increased due to the low heritability of haemoglobin at 5 weeks.

Table 5 Genetic (ra), permanent litter (rc), environmental (re) and phenotypic (rp) correlations and their standard errors (s.e.) between haemoglobin at 5 weeks (HAEM5), haemoglobin at 21 weeks (HAEM21, HAEM21ex) and iron in pork (IRON).

		HAEM5	s.e.	HAEM21	s.e.	HAEM21ex	s.e.
IRON	ra	0.39	0.24	0.50	0.19	0.58	0.13
	rc	0.10	0.21	0.34	0.29	-	-
	re	0.04	0.02	0.16	0.03	0.19	0.03
	rp	-0.02	0.04	0.07	0.04	0.06	0.05
HAEM5	ra	-	-	0.35	0.29	0.01	0.27
	rc	-	-	-0.16	0.17	-	-
	re	-	-	0.04	0.02	0.02	0.02
	rp	-	-	0.03	0.03	0.02	0.03

Genetic correlations between iron content in pork and tristimulus parameters L*, a* and b* were high for most trait combinations (Table 6). High iron content was genetically associated with darker meat (L* value) with genetic correlations ranging from -0.61 ± 0.14 to -0.54 ± 0.23. The magnitude of genetic correlations was even higher between iron content in pork and a* values with estimates of 0.89 ± 0.04 and 0.91 ± 0.04 at the dorsal and ventral site. It was not possible to estimate the genetic correlation between iron content in pork and the average of both a* value measurements. In addition, measures of blood haemoglobin levels at 21 weeks had positive genetic correlations with a* value measurements (range: 0.44 ± 0.13 to 0.55 ± 0.15). The average of both b* values had a high genetic correlation of 0.86 ± 0.06 with iron content in pork. In comparison, estimates of genetic correlations with iron levels were lower for b* values at the dorsal (0.59 ± 0.14) and ventral (0.28 ± 0.19) sites. Only the average b* value was genetically associated with haemoglobin levels at 21 weeks (0.47 ± 0.17 for HAEM21; 0.42 ± 0.15 for HAEM21ex). Iron levels in pork had negative genetic correlations with both pH measurements, which was significant for pH45 (-0.42 ± 0.14). In contrast, genetic associations between pH and haemoglobin measurements were stronger for pH24 (range: -0.57 ± 0.24 to -0.37 ± 0.20) in comparison to genetic correlations with pH45.

There were no significant genetic correlations between the haematological traits and any growth or carcase characteristic, although the direction between both fat measures (BF and CP2) were consistently favourable (Table 7).

Table 6 Genetic (ra), environmental (re) and phenotypic (rp) correlations and their standard errors (s.e.) between haemoglobin at 5 weeks (HAEM5), haemoglobin at 21 weeks (HAEM21, HAEM21ex) and iron in pork (IRON) and tristimulus parameters L*, a* and b* and pH at 45 minutes (pH45) and pH at 24 hours (pH24).

		HAEM5	s.e.	HAEM21	s.e.	HAEM21ex	s.e.	IRON	s.e.
L* value Dorsal	ra	0.25	0.40	0.08	0.35	-0.16	0.30	-0.54	0.23
	re	-0.01	0.03	-0.05	0.03	-0.05	0.03	-0.17	0.04
	rp	0.00	0.02	-0.04	0.02	-0.06	0.03	-0.19	0.02
L* value Ventral	ra	-0.25	0.30	-0.07	0.26	-0.16	0.22	-0.61	0.14
	re	0.00	0.03	-0.10	0.03	-0.08	0.04	-0.16	0.04
	rp	-0.01	0.02	-0.09	0.02	-0.09	0.03	-0.23	0.02
L* value Average	ra	-0.02	0.34	-0.02	0.29	-0.18	0.25	-0.59	0.16
	re	-0.01	0.03	-0.08	0.03	-0.07	0.03	-0.18	0.04
	rp	-0.01	0.02	-0.08	0.02	-0.08	0.03	-0.23	0.02
a* value Dorsal	ra	0.29	0.22	0.52	0.16	0.44	0.13	0.89	0.04
	re	0.01	0.04	0.06	0.04	0.07	0.05	0.35	0.05
	rp	0.04	0.02	0.14	0.02	0.16	0.03	0.56	0.16
a* value Ventral	ra	0.43	0.23	0.47	0.17	0.47	0.13	0.91	0.04
	re	-0.02	0.03	0.10	0.04	0.08	0.05	0.25	0.05
	rp	0.03	0.02	0.16	0.02	0.17	0.03	0.50	0.02
a* value Average	ra	0.36	0.22	0.55	0.15	0.49	0.13	ne	ne
	re	-0.01	0.04	0.08	0.04	0.07	0.05	ne	ne
	rp	0.04	0.02	0.17	0.02	0.18	0.03	ne	ne
b* value Dorsal	ra	0.24	0.27	0.23	0.23	0.16	0.19	0.59	0.14
	re	0.02	0.03	0.01	0.03	0.01	0.04	0.07	0.04
	rp	0.04	0.02	0.03	0.02	0.03	0.03	0.17	0.02
b* value Ventral	ra	0.33	0.30	0.00	0.27	0.04	0.22	0.28	0.19
	re	0.02	0.03	0.03	0.03	0.03	0.04	0.01	0.04
	rp	0.04	0.02	0.03	0.02	0.03	0.03	0.05	0.02
b* value Average	ra	0.39	0.24	0.47	0.17	0.42	0.15	0.86	0.06
	re	0.02	0.03	0.05	0.04	0.05	0.04	0.22	0.04
	rp	0.05	0.02	0.12	0.02	0.13	0.03	0.43	0.02
pH45	ra	-0.05	0.24	-0.18	0.21	-0.15	0.17	-0.42	0.14
	re	0.02	0.03	0.01	0.04	0.03	0.04	0.01	0.05
	rp	0.01	0.02	-0.02	0.02	-0.01	0.03	-0.10	0.02
pH24	ra	-0.50	0.29	-0.57	0.24	-0.37	0.20	-0.24	0.17
	re	0.08	0.03	0.06	0.03	0.06	0.04	0.01	0.04
	rp	0.03	0.02	0.00	0.02	0.00	0.03	-0.04	0.02

* ne=not estimable

Table 7 Genetic (ra), permanent litter (rc), environmental (re) and phenotypic (rp) correlations between haemoglobin at 5 weeks (HAEM5), haemoglobin at 21 weeks (HAEM21, HAEM21ex), iron in pork (IRON) and live backfat (BF), carcass backfat (CP2), live muscle depth (MD), carcass muscle depth (LD) and average daily gain (ADG).

		HAEM5	s.e.	HAEM21	s.e.	HAEM21ex	s.e.	IRON	s.e.
BF	ra	-0.01	0.21	-0.34	0.17	-0.22	0.14	-0.07	0.11
	rc	0.27	0.17	-0.4	0.26	-	-	0.19	0.21
	re	0.04	0.03	0.08	0.04	0.07	0.04	0.01	0.05
	rp	0.04	0.02	-0.02	0.03	0.00	0.03	0.00	0.03
CP2	ra	-0.04	0.22	-0.32	0.18	-0.13	0.15	-0.17	0.13
	re	0.08	0.03	0.09	0.04	0.05	0.05	0.04	0.05
	rp	0.06	0.02	0.01	0.03	0.01	0.03	-0.03	0.02
MD	ra	0.34	0.19	-0.03	0.17	-0.03	0.15	-0.16	0.11
	re	0	0.02	0.05	0.03	0.03	0.03	0.04	0.04
	rp	0.03	0.02	0.03	0.02	0.02	0.02	-0.01	0.02
LD	ra	0.38	0.22	0.02	0.18	-0.03	0.15	-0.26	0.12
	re	-0.05	0.04	0.02	0.04	0.03	0.05	0.02	0.06
	rp	0.01	0.02	0.02	0.03	0.02	0.03	-0.09	0.03
ADG	ra	-0.26	0.20	-0.10	0.17	0.11	0.14	0.17	0.12
	rc	-0.21	0.10	0.36	0.16	-	-	-0.10	0.18
	re	-0.08	0.02	0.09	0.03	0.12	0.03	0.02	0.04
	rp	-0.10	0.02	0.09	0.02	0.11	0.02	0.05	0.02

Discussion

Haemoglobin.

Blood haemoglobin levels are used to quantify the iron status of pigs. Haemoglobin levels above 100 g/L are considered adequate, while haemoglobin levels of 80 g/L or 70 g/L are generally considered borderline anaemia or anaemia (NRC, 1998). Mean haemoglobin levels at five and 21 weeks of age observed in this study were in the normal range and were similar to mean haemoglobin levels observed in a recent Australian study by Payne (2009). At 35 days of age, mean haemoglobin levels were 97, 105 and 112 g/L for piglets raised indoors that had received no creep feed, creep feed or an outdoor mix, respectively. The outdoor mix consisted of straw, sow feed and soil to resemble the substrates available to outdoor piglets. As in our study, these piglets had been given a routine iron dextran injection of 200 mg shortly after birth. However, the study by Payne (2011) demonstrates that it is possible to manipulate haemoglobin levels via husbandry strategies.

Haemoglobin heritabilities

There is a paucity of literature in regard to heritability estimates for haemoglobin. Our range of heritability estimates for haemoglobin measures was lower than the estimate of Bolormaa et al. (2010), who found high heritabilities ranging from 0.45 ± 0.12 to 0.51 ± 0.14 for haemoglobin levels recorded at three, five and 6.25 months of age in

approximately 600 sheep. Heritability of haemoglobin levels in humans ranged from 0.34 to 0.42 in geographically and genetically isolated Italian populations (Sala *et al*. 2009).

In summary, this comparison with other studies and the effect of excluding some recording dates on heritability estimates indicates that procedures to measure haemoglobin with the HaemoCue® equipment should be further evaluated. Blood was collected by jugular venipuncture and stored overnight prior to recording haemoglobin in accordance with measurement procedures outlined in the manual for human blood. Based on veterinary advice (Barb Frey, pers. comm.), pig blood samples stored in this way should be agitated for at least 10 minutes prior to haemoglobin measurement, which was not practiced in this study. Therefore, further investigations and procedural recommendations are required to improve the accuracy of the on-farm haemoglobin measurement in pigs, since this proven measurement technology does provide opportunities for pig breeding programs. The HemoCue Hb 201^+ analyser is designed to work in less than one minute on a small amount of blood (10 µL). This amount of blood can be collected at various ages of pigs and provides opportunities for the use of blood haemoglobin levels as a selection criterion in pig breeding programs.

Iron

The mean iron content in pork *m. longissimus dorsi* of 2.87 mg/kg observed in this study was slightly below the range of 3 to 30 mg/kg reported by Rooke et al. (2010). Pork cuts differ in regard to their iron content (Reichardt et al. 2002) and a comparison of mean iron content with other studies should only be based on iron content in *m. longissimus dorsi*. For this muscle, mean iron content was 4.18 mg/kg and 3.60 mg/kg in the studies by Reichardt et al. (2002) and Lombardi-Boccia (2002). Pork was ground in both studies and Lombardi-Boccia (2002) states that the food processor was equipped with stainless steel blades. The use of steel knives for the preparation of pork samples in the laboratory considerably increased the mean iron content in pork in this study (Appendix 2). Therefore, only studies that used ceramic knives to determine iron content in *m. longissimus dorsi* can be used for a valid comparison of the mean iron content found in this study. Dannenberger et al. (2007) used such an approach, however, sample size varied from 11 to 24 pigs per genotype and the range of means from 4.1 ± 0.15 to 5.0 ± 0.21 mg/kg fresh weight may have been affected by sampling effects.

The moderate heritability estimate for iron content in pork found in this study confirms previous heritability estimates for iron characteristics in pork of 0.39 ± 0.09 for haem pigment (Larzul *et al*. 1997) and 0.27 ± 0.09 for soluble myoglobin content (Newcom *et al*. 2004). Heritability estimates were somewhat lower for pigment in pork (Oksbjerg et al. 2004) and for total iron content in sheep meat (Mortimer *et al*. 2010) with estimates of 0.17 ± 0.02 and 0.12 ± 0.05, respectively.

Genetic correlations.

The three haemoglobin measures had moderate to high genetic correlations with iron content in pork indicating that haemoglobin may be used as a selection criterion for iron content in pork. Estimates were slightly higher, and significant, for haemoglobin traits at 21 weeks in comparison to the earlier haemoglobin measurement, which were supported by positive and significant phenotypic correlations with pork iron content. Haemoglobin at five weeks had no significant genetic or phenotypic associations with haemoglobin measures at 21 weeks indicating that haemoglobin shortly after weaning is genetically and physiologically a different trait to haemoglobin at 21 weeks. It is uncertain whether iron

dextran injections contributed to these low associations. Comparable estimates of genetic correlations were not found in the literature.

Among pork quality traits, a* value, a measure or redness, had the highest genetic correlations with iron content in pork accompanied by significant positive genetic correlations with haemoglobin measures at 21 weeks of age. Genetic correlations between IRON and L* values ranged from -0.54 ± 0.23 to -0.61 ± 0.14 indicating that pork with a higher iron content is darker. Magnitude of genetic correlations were slightly lower in Oksbjerg et al. (2004), who found estimates of genetic correlations of pigment in pork with a* value of 0.59 ± 0.04 and with L* value of -0.46 ± 0.06. At the phenotypic level, Lindahl et al. (2002) found that 86 and 90% of the variation in L* and a* value of pork was explained by pigment content and myoglobin forms, highlighting the strong association between these colour characteristics and measures of iron content in pork. The study by Newcom et al (2004) was based on 255 pigs and only residual correlations between myoglobin and pork quality traits were presented. Magnitude of residual correlation was highest for a* value (0.23) followed by L* value (-0.17) confirming the direction of genetic correlations in this study.

Positive b* value describes yellowness of pork. This trait had positive genetic correlations with iron content, which were variable ranging from 0.28 ± 0.19 to 0.86 ± 0.06 for alternative measures of b* values. Pigment content and fraction of metmyoglobin, which had been the main factors affecting L* and a* value, had no significant effect on b* value in the study by Lindhal et al. (2002). This colour measurement was, however, affected by internal reflectance, which may explain the stronger genetic correlation with iron content of pork for average b* value given that the average b* value only included positive b* values describing yellowness of pork. In comparison, the residual correlation presented by Newcom et al. (2004) between myoglobin content and b* value was -0.15. However, the direction of genetic correlations of individual colour traits with iron content of pork reported in this study corresponds to genetic associations found between the colour measurements L*, a* and b* by Gjerlaug-Enger et al. (2010) and Wyk et al. (2005). A lower L* value (darker meat) was genetically associated with a higher a* value (redder meat) and higher b* values (more blue colours).

In summary, all colour measures had moderate to high genetic correlations with iron content that were consistent with genetic relationships usually observed between these traits. Gjerlaug-Enger (2011) recommended to use a* value in pig breeding programs instead of L* value, since it had no major unfavourable genetic correlation with other meat quality traits and could be used as a selection criterion for iron content in pork. The high genetic correlations found in this study between a* value and iron content in pork support the recommendation by Gjerlaug-Enger (2011). In addition, the a* value has been found to be the most useful of the meat colour parameters for assessing pork quality at the phenotypic level (Karamucki *et al*. 2011).

Both pH measurements had moderate negative genetic correlations with iron content in pork. The direction of this genetic correlation does not correspond to genetic association between L* value and iron content in pork, given that genetic correlations between pH measurements and L* value are usually highly negative (Hermesch et al., 2000; Wijk et al., 2005, Gjerlaug-Enger et al. 2010). In comparison, the residual correlation between soluble myoglobin content and ultimate pH recorded 24 hours post mortem was zero in the study by Newcom et al. (2004). Variation in total haem pigment has been shown to influence colour of the meat Warris et al. (1990), and although variation in total haem pigment was

relatively small, this variation could be important in genetic improvement programs that use colour measurements to improve pork quality in regard to pale, soft, exudative or dark, firm, dry pork (Warris et al. (1990).

Genetic correlations between haematological traits and backfat, muscle depth and growth were generally not significant different from zero. Comparable estimates of genetic correlations were not found in the literature. A Danish comparison of faster growing pigs available in 1995 with slower growing pigs, representing a genotype of the 1970s, found lower haematin and myoglobin in pork for the faster growing pigs although no significant differences in meat haemoglobin were observed (Oksbjerg *et al*. 2000). It was suggested that correlated responses in muscle fibre types may have contributed to these differences. Growth rate and lean meat percentage were genetically positively associated with higher myofibre cross-sectional areas, which were genetically related to higher pigment (Larzul *et al*. 1997) indirectly supporting the lowly favourable genetic associations between iron content in pork and growth or backfat traits found in this study. Age at slaughter of pigs was positively correlated with concentration of haematin (Oksbjerg *et al*. 2000) and the lower haematin and myoglobin levels of the fast growing animals may have been a reflection of the younger slaughter age of these animals. Further, the two pig genotypes originated from an experimental test station (slow growing pigs) or Danish breeding herds (fast growing pigs). No information was provided about iron status of pigs at the beginning of the experiment and it is not known whether this confounding of pig genotype with herd of origin of piglets has affected results. For example, Payne (2009) found that different pre-weaning environments affected iron status of piglets at weaning with possible effects on subsequent performance of growing pigs.

4. Application of Research

Iron content in pork was moderately heritable and can be improved genetically.

A simple on-farm haemoglobin measure was heritable and genetically correlated with iron content as well as colour of pork (redness). This trait can be used as a selection criterion for iron content and pork colour, which has high benefits for the Australian pork industry, since the majority of breeding companies are unable to routinely retrieve information about characteristics of pork from commercial abattoirs. Heritabilities and genetic correlations between measures of iron status in blood and pork with other performance, carcase and meat quality traits are now available allowing breeders to include these traits in their breeding programs to improve iron levels in pork.

Colour redness was genetically correlated with iron content and haemoglobin and can be used as an additional selection criterion for iron content.

Iron levels in pork had no significant genetic correlations with performance traits indicating that current selection for productivity does not reduce iron content in pork.

Iron content in pork was lower than comparable mean values reported previously. However, only mean iron levels of the *m. longissimus dorsi* and from studies using ceramic knives are comparable. The use of stainless steel knives in the laboratory increased the mean and variation of iron levels in pork.

Impact of research

The rate of annual genetic improvement is expected to be around 10% of the additive genetic standard deviation, which is 0.02 mg/kg fresh weight for m. longissimus dorsi based on the mean and variation found in this study. However, annual genetic gain will be higher for other muscles with a higher mean and variability due to scaling effects, since a higher mean is usually associated with larger variation. Therefore, more additive genetic variation may be observed in other muscles with higher mean iron content.

Genetic correlations indicate that it is possible to improve iron content in pork and pork colour by using an on-farm selection criterion without any unfavourable consequences for selection for higher productivity. In addition, this simple blood test may be used to monitor iron status of sows, piglets and growing pigs with beneficial outcomes for improved health and disease resistant status of Australian pigs, since haemoglobin concentration of blood is a reliable indicator of the pig's iron status (Cottam et al, 2007). Iron is involved in various bodily functions including the transport of oxygen in the blood.

5. Conclusion

Iron content in pork is moderately heritable and can be improved via selection. The HemoCue Hb 201^{+} analyser provides rapid measures for blood haemoglobin levels on farm and may be used as a selection criterion for iron content in pork and redness of pork. Colour measurements had moderate to high genetic correlations with iron, providing further avenues for selection strategies to improve iron content in pork. Current selection practices are not expected to affect iron content in pork, given that no significant genetic correlations between performance and haematological traits were found.

Iron content in pork was lower than comparable mean values previously reported. However, only mean iron levels of the *m. longissimus dorsi* and from studies using ceramic knives are comparable, since the use of stainless steel knives increased the mean and variation of iron content in pork in this study.

6. Limitations/Risks

Breeders and producers have no experience in recording haemoglobin levels on farm. The measurement procedures outlined in the manual of the HemoCue® equipment for human blood are not always applicable to pig blood. For example, pig blood coagulates more easily and should be agitated for at least ten minutes with a commercial agitator if it has been stored prior to recording. This issue is not prevalent when using the HemoCue equipment at the point of blood collection. Therefore, the accuracy of haemoglobin measures should be evaluated on farm by taking repeated records initially before this measurement is routinely used in breeding programs or is used for monitoring of haemoglobin levels in sows, piglets or growing pigs for husbandry purposes.

7. Recommendations

Haemoglobin levels should be recorded in the growing pig prior to slaughter as a selection criterion for iron content in pork and pork colour.

The measurement procedures for haemoglobin using the HemoCue® equipment should be evaluated using repeated measurements. Haemoglobin levels may have to be recorded in the shed when blood is collected from pigs, since later recordings of haemoglobin levels using stored blood samples require thorough agitation of blood samples prior to measurements being taken.

Any research trial involving iron content in pork should use ceramic knives during the preparation of pork samples.

Genetic parameters should be obtained for haemoglobin levels in sows and their litters to explore the use of haemoglobin as a selection criterion for sow reproductive performance and piglet survival.

The iron status of pig populations should be evaluated by measuring sows prior to farrowing and piglets at birth prior to iron injections.

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Appendix 1: Improving accuracy of haemoglobin measurement

Following the AGBU Pig Genetics Workshop in October 2010, Barb Frey provided feedback on the Hemocue® measurement based on her experiences on measuring haemoglobin levels in sows. Barb Frey pointed out that 'blood samples MUST be agitated using a commercial agitator for AT LEAST 10 minutes, and ideally also be stored at room temperature'. However, the procedure outlined in the Hemocue® manual was followed at Rivalea, which involves a brief manual shake of blood samples prior to recording. In addition, staff commented on the difficulty to obtain haemoglobin measures from cold blood samples. A small trial was performed measuring haemoglobin on 62 pigs recorded on the 11th of November 2011 three times. The first measures (Haem1) was recorded on the day of slaughter, the second measure (Haem2) was taken after shaking the sample for 3 to 5 minutes manually and the third measure was taken the day after slaughter without shaking the sample (Haem3). Both Haem1 and Haem3 had been used in the existing Project.

Means for these three haemoglobin measures at 22 weeks of age increased continuously from Haem1 (mean: 98.2, sd: 16.3), to Haem2 (mean: 103.2, sd: 15.0) to Haem3 (mean: 106.3, sd: 12.8). Although standard deviations decreased from Haem1 to Haem3, coefficients of variation were still exceeding the level of 9 to 10% recommended as a quality control measure by Barb Frey for a sample of 35 sows. In addition, correlations between measures were very low ranging from 0.03 to 0.16 demonstrating measurement errors. These correlations increased to a range from 0.20 to 0.37 once records with values below 70 and above 135 were excluded as was applied in current genetic evaluations.

In conclusion, this small trial demonstrated the unreliability of the current haemoglobin measurement procedure, which was partly overcome in genetic analyses by excluding extreme measurements. However, this specific haemoglobin measurement will be more useful in pig breeding program if the accuracy can be improved by developing guidelines for recording procedures of pig blood on farm, which may involve taking repeated measures of haemoglobin with the Hemocue® equipment.

Appendix 2: Comparing the use of ceramic versus steel scalpels when measuring iron levels in pork

Introduction

Measurements of iron in *m. longissimus dorsi* were required to obtain genetic parameters for this trait and other economically important performance traits including haemoglobin levels in blood and pork quality characteristics. Measurements of iron levels in pork were based on duplicate samples which were expected to be within 10% of each other. This criterion was only achieved using a sample weight of 1000 mg wet weight and once steel utensils had been replaced by ceramic utensils. It was hypothesized that the use of steel scalpels contributed to measurement errors of iron content in pork samples.

Experimental design

Meat samples from the *m. longissimus dorsi* of 20 pigs were used. Only a small amount of pork (1000 mg) was required for measurement of iron content which was collected using either steel or ceramic scalpels. Samples were collected in close proximity to each other on either side of the midline of the pork chop. Two pork samples were collected with each utensil (ceramic or steel). All other procedures in the lab were the same for both scalpel types and iron content in pork was obtained by totally digesting duplicate muscle samples with a wet weight of approximately 1000 mg in concentrated nitric/perchloric acids to white fumes of perchloric. The digest was then cooled, water was added and total iron content was measured by flame atomic absorption spectrometry using an air/acetylene flame.

Results

Using steel scalpels increased mean iron content of both duplicates by 0.987 mg/kg (31%) from 3.150 mg/kg to 4.137 mg/kg wet weight in comparison to using ceramic scalpels (Table 1). In addition, standard deviation was 62 % higher for steel (0.727) than ceramic scalpels (0.448), which implies higher standard errors of the mean for steel scalpels. Some of this increase in variation is due to a higher mean for steel scalpels, however, coefficients of variation were also higher for iron measurements based on steel equipment indicating larger measurement errors in addition to increased variation due to scaling effects. The Pearson correlation between average iron content based on ceramic versus steel equipment was low (0.389).

There were no differences in means for both duplicate samples using ceramic scalpels (Table 1). In comparison, means differed by 0.192 mg/kg wet weight between duplicate measures using steel scalpels. The Pearson correlation was higher for duplicate measurements based on ceramic tools (0.97) in comparison to steel tools (0.84). This statistical characteristic quantifies the linear relationship between measurements. However, it does not detect any departure of measurements from the 45-degree line through the origin (Lin, 1989). Therefore, data are further illustrated by plotting values of the first duplicate against values of the second duplicate within each equipment class (Figure 1). The magnitude of the intercept from the regression of one duplicate on the second duplicate quantifies the deviation of the slope from the origin, which should be zero. The magnitude of this intercept was lower for measurements based on using ceramic scalpels (-0.1056) in comparison to steel scalpels (0.4666) in addition to the lower spread

of measurements around the 45-degree line. The square of the Pearson correlations between duplicate samples equals the coefficient of determination for the regression of one duplicate on the other duplicate within each utensil class. The coefficient of determination was 0.93 for ceramic and 0.71 for steel utensils.

Table 1 Mean, standard deviation (SD), coefficient of variation (CV), minimum (Min) and maximum (Max) for iron content in pork (mg/kg wet weight) using ceramic versus steel scalpels.

	Mean	SD	CV	Min	Max
	Ceramic scalpels				
Duplicate 1	3.150	0.437	0.139	2.51	3.80
Duplicate 2	3.150	0.467	0.148	2.57	3.85
Average	3.150	0.448	0.142	2.54	3.77
	Steel scalpels				
Duplicate 1	4.041	0.718	0.178	2.74	5.25
Duplicate 2	4.233	0.797	0.188	2.96	5.53
Average	4.137	0.727	0.176	2.96	5.39

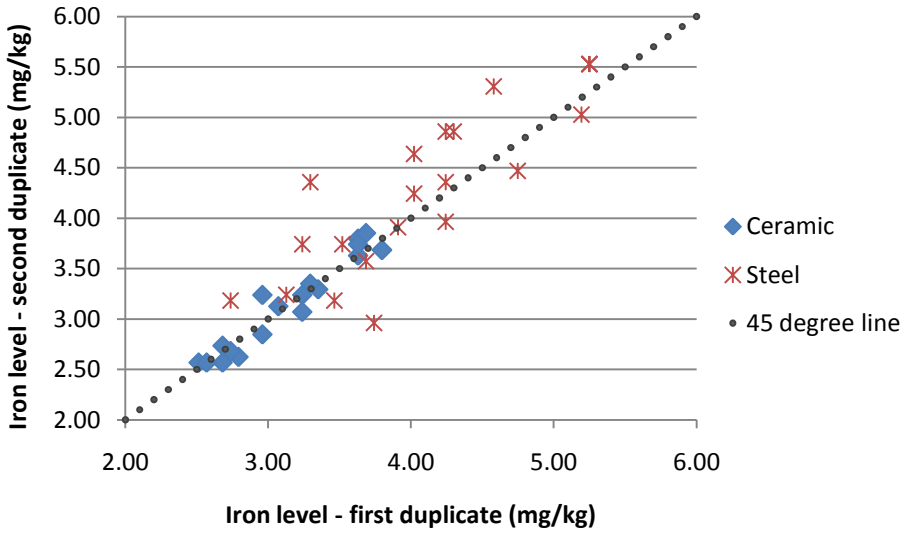

Figure 1. Plot of first against second duplicate using either ceramic or steel equipment.

Conclusion

Using steel scalpels in the lab to prepare pork samples for measuring iron content in pork increased the mean iron content by 0.987 mg/kg wet weight equivalent of 31% of the mean. In addition, variability was increased and a number of statistical characteristics demonstrated the lower reproducibility of measurements using steel equipment in comparison to using ceramic equipment. This trial should be repeated to confirm results, given their potential implications for a) human nutrition and b) measurement errors in other research trials.

Appendix 3: Breed and slaughter day affect carcass and pork quality

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(Paper submitted to APSA 2011)

In today's world breeding goals are changing to meet customer expectations by focusing more on meat quality traits rather than mainly on increased litter size and lean meat percentage along with weight gain and improving feed conversion. With consumers more focused on the heath attributes of food, novel measures of meat quality are being assessed to ensure that breeding programs can continue to improve pork quality. Many factors are known to influence meat quality, but a recent assessment of the impact of breed and slaughter date on meat quality including the iron content of pork has not been undertaken in Australia. As such, the aim of this study was to determine the effect of breed and slaughter day on a range of meat quality traits.

During 2010 and 2011 data were recorded on 2442 pigs from two terminal sire lines (Duroc (Db) and Large White based (LWb), PrimeGro™ Genetics). Pigs were housed in group pens at a commercial birth to bacon piggery. At 21 weeks of age, blood samples were obtained from all animals via jugular venipuncture (5ml collected in Vacutainer® K2E tubes) to measure hemoglobin content (HEM21) using a HemoCue® machine (HemoCue® Australia Pty Ltd, Wamberal, NSW). Pigs were subsequently slaughtered at a commercial abattoir at a hot carcass weight (HCW) of 69.9 (±7.95) kg and carcass fat depth was obtained at the P2 site, 65 mm from the midline of the carcase at the last thoracic rib. Twenty-four hours post slaughter a measure of pH was obtained on each carcass from the *longissimus dorsi (*LD*)* muscle. The LD muscle was collected and meat colour (L*, a*, b*) and muscle iron content were also measured. A sample of around 1000 mg wet weight from a 30 – 40mm cubed sample of the LD muscle was completely digested in concentrated nitric/perchloric acids to white fumes of perchloric. The digest was then cooled, water was added and total iron content was measured by flame atomic absorption spectrometry using an air/acetylene flame. A general linear model was used including the fixed effects of slaughter day (or collection day for HEM21) and breed (except L*) for all traits. Sex was significant for HEM21 and HCW was fitted as a linear covairable for fat depth and pH24.

The LWb line had a 0.36 higher value for colour a* (measure of redness in the meat) which correlated with a 1.10 g/l higher hemoglobin in the blood at 21 weeks of age and greater iron content in the muscle (0.07g/l). The Duroc based line displayed a 0.26mm P2 backfat depth increase and a 0.02 higher pH at 24 hours post slaughter. The results show that slaughter day had a much larger effect than breed on carcass and meat quality traits. Although breed is significant for most traits, slaughter day has between 0.08-0.53 greater proportion of variance over all traits tested. There was however no significant breed effect on colour L* or plasma HEM21. In conclusion, these results suggest that breed differences for these meat quality traits are small and that the effect of breed explains considerably less variance than does slaughter date. Breeding programs designed to meet customer expectations of meat quality may need to be supplemented with managerial manipulation of slaughter date contemporary groups so that these meat quality traits can best meet consumer expectations.

Table 1. Effect of breed and kill date on meat quality traits from two terminal lines

	N.	Mean (Db)	SE	Mean (LW)	SE	Variance proportion explained	
						Breed	Slaughter Day
HEM 21 (g/l)	2405	107.1	0.66	108.2	0.70	0.00**	-
Iron Content (g/l)	2367	2.87	0.01	2.94	0.01	0.00***	0.36***
P2 Carcass fat depth (mm)	2417	7.36	0.09	7.10	0.09	0.01***	0.09***
pH 24 hours	2430	5.64	0.00	5.62	0.00	0.01***	0.54***
Colour L*	2419	47.8	0.07	47.9	0.08	N.S	0.28***
Colour a*	2412	5.46	0.03	5.82	0.03	0.03***	0.16***
Colour b*	2419	3.82	0.02	4.08	0.02	0.02***	0.24***

*** P<0.001, **P<0.05

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