First genetic analysis of blood haemoglobin levels and iron content in pork

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Introduction

Pork from modern pig genotypes has a lower iron content compared to pork that was produced in the past. Several studies (Dannenberger *et al*., 2007; Ruusunen *et al*., 2004) have shown that a decrease in muscle myoglobin (a source of iron) has inadvertently occurred in pork from lines of faster growing pigs. This myoglobin decrease is the result of a physiological change, specifically an increase in the ratio of white glycolytic fast twitch muscle fibres to red oxidative slow twitch muscle fibres. A decrease in muscle myoglobin results in paler pork. In addition consumers are aware that colour is a quality indicator of fresh pork and overly pale pork is often avoided by discriminating purchasers as it is associated with meat of dubious quality.

Pork haem pigment has been shown to be under genetic control with heritability estimates ranging between 0.17 and 0.39 in different studies (Larzul *et al*., 1997; Oksbjerg *et al*., 2004). This suggests that iron depletion in modern pork genotypes can be reversed through genetic selection. Genetic progress can best be achieved by direct measurement upon selection candidates. For meat quality traits such as iron content in pork this is logistically difficult as the animal is required to be slaughtered before muscle iron content can be effectively assayed. For this reason it would be beneficial to develop a measurement technique based upon a correlated trait that can be measured upon live animals. An alternative indirect measure for iron content in pork may be haemoglobin levels in blood.

Data are currently collected with an aim to investigate the genetic control of three iron traits, blood haemoglobin levels at five weeks (HAEM-5) and 22 weeks (HAEM-22) and iron content in pork (IRON-MOL) and to develop appropriate models to estimate variance components for these traits. An additional aim was to determine phenotypic correlations between the three iron traits and other production and carcase measures. First results are presented in these workshop notes describing the haemoglobin and iron content measures along with phenotypic correlations and heritability estimates.

Data and trait description

Blood haemoglobin level was recorded at a commercial piggery on 5,241 piglets of two breeds between September 2009 and September 2010. These piglets were approximately five weeks old (24 to 40 days). In addition 1,827 animals were measured for the same trait at approximately 22 weeks (134 to 157 days). Blood haemoglobin was measured using HemoCue diagnostics equipment (HemoCue).

Iron content in pork was available for 1,125 finisher boars of two breeds. Pork iron measurements were initially obtained by totally digesting duplicate muscle samples weighing approximately 500 mg in nitric/perchloric acid and then determining the iron concentration in the resulting sample via flame atomic absorption spectrometry. The sensitivity specification for this assay is 0.0014 mmol/kg. Duplicate samples were expected to be within 10% of each other, however, this criterion was not being met. Investigation revealed that steel used for cutting and handling samples were affecting results. Later samples were increased in weight to approximately 1000mg and were cut and handled using nickel/plastic/ceramic tools. Muscle iron records taken using steel implements were removed. All production traits, carcase, meat quality and remaining iron records were limited to ± 3 standard deviations from the mean (Table 1).

The same pigs measured for pork iron had several meat quality traits (pH at 45 minutes and at 24 hours and dorsal and ventral luminance, hue and chroma readings) as well as carcase P2 fat and loin muscle depth recorded. Muscle iron weight (IRON-WT) was derived by multiplying the molecular count for iron by the molecular weight of iron (55.85 mg/kg). Luminance, hue and chroma readings analysed were the average of dorsal and ventral readings.

Development of models

The GLM procedure (SAS, Inst., Inc., Cary, NC.) was used to determine suitable fixed effect models for estimating variance components for haemoglobin levels and pork iron content. Only significant effects ($P < 0.05$) were retained in final models. The final model for all iron traits included test date, and animal and permanent litter random effects. In addition, the final model for HAEM-22 included the animal's sex. Additional fixed effects for HAEM-5 were breed, number born alive and dead in the birth litter, average birth weight and age at recording. The only significant factor found for IRON-WT was test date. This is similar to HAEM-22 however unlike HAEM-22, IRON-WT was only measured on one sex (male).

Variance component estimates and final heritabilities for all traits were obtained from univariate model analyses using the ASReml software (Gilmour *et al.* 2006) utilising an animal model.

Results and discussion

1. Trait means

The mean iron content of samples exposed to steel tools during the first three weeks of recording was 0.073 mmol/kg compared to 0.050 mmol/kg for samples taken later with plastic, nickel or ceramic tools. This will be further explored in a comparative study.

Table 1. Basic statistics for recorded traits and covariables post data editing

	Units	N	Mean	SD	Minimum	Maximum
Traits						
HAEM-5	g/L	4971	106.6	16.15	60	145
HAEM-22	g/L	1570	104.8	13.9	70.0	135
IRON-MOL	mmol/kg	1306	0.050	0.008	0.028	0.076
IRON-WT	mg/kg	1306	2.8	0. 5	1.6	4.2
ADG	g/d	2917	612.3	69.7	420	833
BF	mm	2712	8.0	1.4	6.0	12.5
Loin depth	mm	1495	49.2	5.8	32.0	66.0
M-depth	mm	2916	43.1	5.2	28.0	59.0
pH 45m	units	1526	6.0	0.3	5.3	6.8
pH 24h	units	1487	5.6	0.1	5.3	6.0
COLL	units	1479	48.1	2.9	39.6	55.4
COLA	units	1471	5.6	1.0	2.2	8.5
COLB	units	1466	4.0	0.9	1.2	6.4
Covariables						
H-5 age	d	5187	32.3	3.0	23	40
H-22 age	d	1782	146	4.4	134	158
I-MOL age	d	1547	152	4.6	112	164
Birth weight	kg	5388	17.3	4.3	1.8	32.2
BNBA	n	5495	10.4	2.8	1.0	18.0
BNBD	n	5495	1.1	1.3	0.0	11.0

* Abbreviations: haemoglobin level at 5 weeks (HAEM-5), haemoglobin level at 22 weeks (HAEM-22), pork iron (IRON-MOL), iron weight (IRON-WT), average daily gain (ADG), backfat (BF), loin depth, muscle depth (M-depth), pH at 45 minutes (pH 45m), pH at 24 hours (pH 24h) , average luminescence reading (COLL), average hue reading (COLA) and average chroma reading (COLB) age at HAEM-5 collection (H-5 age), age at HAEM-22 collection (H-22 age), age at IRON-MOL collection (I-MOL age), average piglet birth weight (Birth weight), number born alive in birth litter (BNBA), number born dead in birth litter (BNBD),

2. Haemoglobin at 5 weeks

The model used to estimate HAEM-5 heritability included the birth weight and age of the piglet as well as the number of animals (alive and dead) in its birth litter. Regression coefficients for these linear covariables were all negative (Table 2) indicating that as piglets age, birth weight and total litter size increased the concentration of blood haemoglobin decreased. The size of the piglet could be a factor affecting blood haemoglobin content as all piglets are given an identical iron injection shortly post birth regardless of their weight. It is therefore likely that this would result in higher haemoglobin levels in smaller animals.

Phenotypic correlations between haemoglobin level at 5 weeks and the other iron measures were small and not significant at the 0.05 threshold (Table 3). The only significant correlation found was between haemoglobin level and average daily gain (P<0.0001). Haemoglobin levels at 5 weeks of age were lower for pigs with higher lifetime growth rates (-0.12).

Table 2. Regression coefficients for linear covariables used to model haemoglobin iron in 5 week old piglets (HAEM-5)

Variable	***Regression coefficient***	***standard error***
Number born alive in birth litter	-0.37	0.107
Number born dead in birth litter	-0.58	0.192
Average piglet weight at birth	-5.87	1.196
Age at HAEM-5 collection	-0.98	0.091

Table 3. Phenotypic correlations

	HAEM-22	***IRON-WT***	***ADG***	***BF***	***COLL***	***COLA***	***COLB***
HAEM-5	0.028	-0.04	**-0.12**	0.03	0.01	-0.01	-0.01
p-value	0.29	0.23	<.0001	0.17	0.73	0.80	0.86
n	1440	1039	2533	2361	1161	1158	1153
HAEM-22		**0.13**	**0.09**	**0.12**	-0.04	**0.15**	**0.09**
p-value		<.0001	0.0008	<0.0001	0.19	<.0001	0.004
n		924	1538	1439	1020	1015	1013
IRON-WT			-0.04	**0.09**	**-0.24**	**0.45**	**0.23**
p-value			0.11	0.002	<.0001	<.0001	<.0001
n			1292	1217	1299	1292	1287

*HAEM-5 = blood haemoglobin at 5 weeks, HAEM-22=blood haemoglobin at 22 weeks, IRON-WT=iron content in pork, ADG=average daily gain, BF=backfat, COLL=Minolta luminance value, COLA=Minolta hue value, COLB=Minolta chroma value

3. Haemoglobin at 22 weeks

Sex was significant for HAEM-22 and least squares means indicated that gilts had higher HAEM-22 (110.21±1.37 g/L) than entire boars (103.53±0.43 g/L). These findings are in agreement with the phenotypic correlations between HAEM-22 and backfat with sows consistently expressing higher backfat levels in comparison to boars.

The phenotypic correlation between HAEM-22 and IRON-MOL was positive (0.13, P<0.0001) (Table 3). This positive correlation gives some promise to the possibility that higher blood haemoglobin levels are significantly associated with higher pork iron content. Haemoglobin at 22 weeks was positively correlated with ADG (0.09, P=0.0008) indicating that increased growth rate is favourably associated with higher blood iron in pigs aged 22 weeks. A significant phenotypic correlation was also found between HAEM-22 and backfat (0.12, P<0.0001). This positive correlation is unfavourable as animals with lower backfat depth also have lower blood haemoglobin. The phenotypic correlations between HAEM-22 and hue and chroma measurements were also significant and positive. Hue relates to the colour of the sample and chroma relates to colour intensity, this result implies that higher blood haemoglobin levels at 22 weeks favourably influence the colour of pork.

4. Pork iron content

Iron content in pork was phenotypically correlated with HAEM-22 as well as backfat and the meat quality colour measures, luminance, hue and chroma (Table 3). The 0.09 (P=0.002) correlation with backfat gain was low but unfavourable. The negative correlation between IRON-MOL and luminance (-0.24, P<0.0001) indicates that pork with higher iron content is darker. Phenotypic correlations between IRON-MOL and pH at 45 minutes (-0.14) and 24 hours (0.14) were highly significant (P<0.0001). The correlations between IRON-MOL and hue (or colour) (0.111, P<0.0001) and chroma (colour intensity) (0.305, P<0.0001) were highly significant indicating that pork with higher iron content is redder and has a more vibrant or intense red. These results imply that increasing the iron content of pork muscle will result in pork that would be more visually desirable to consumers.

5. Heritabilities

Heritability estimations for HAEM-22 were markedly affected by the choice of boundaries imposed upon measurements. When the boundaries imposed were the usual ± 3 standard deviations of the mean, HAEM-22 was not heritable (Table 4). The range of heritability estimates obtained by altering boundaries for this trait indicates that measurement and/or typographic error can severely affect variance estimates for this trait.

A range of boundaries for both HAEM-5 and HAEM-22 were explored using a mixed model in SAS with sire fitted as a random effect. From this it was determined that the boundaries that optimised heritability without overly sacrificing phenotypic variance were from 60 to 145g/L for HAEM-5 and from 70 to 135g/L for HAEM-22 (Table 4). Heritability estimations were generated using these boundaries.

Table 4. The number of records available (n), sire (s) and residual variances (res) and heritability (h^2) estimations arising from the use of various boundaries for blood haemoglobin at 5weeks and at 22 weeks in SAS mixed models

	HAEM-5				HAEM-22			
Boundaries	***n***	***s***	***res***	h^2	***n***	***s***	***res***	h^2
nil	5095	2.71	344.37	0.031	1785	1.19	406.28	0.012
30 - 175	5049	3.27	288.25	0.045	1767	1.39	340.70	0.016
40 - 165	5021	2.55	269.50	0.037	1753	1.08	312.80	0.014
50 - 155	4966	2.68	237.56	0.045	1727	2.48	275.34	0.036
60 - 145	4881	2.39	206.03	0.046	1666	2.91	221.04	0.052
70 - 135	4641	2.01	163.24	0.049	1573	3.26	162.90	0.078
80 - 125	3989	1.79	103.99	0.068	1412	2.56	110.66	0.090

Heritability estimates for HAEM-5 (Table 5) differed between models. The estimated heritability for HAEM-5 was reduced from 0.19±0.03 to 0.04±0.02 when the permanent effect of the litter was included. The Log likelihood for the two random effect model (-5379.34) compared to the Log likelihood for the single random effect model (-5407.14) indicated that the two random effect model was statistically superior (Table 5). When regression coefficient and heritability results are taken into consideration it would appear that genetic variance for this trait is being masked by the iron injection given to piglets shortly after birth. Blood haemoglobin content for very young pigs may be a heritable trait provided that a blood sample were to be taken prior to piglets being given their iron injection.

The choice of boundaries was again investigated for IRON-WT via a mixed model in SAS with sire fitted as random. However, unlike HAEM-22 the choice of boundaries had little effect upon the estimated heritabilities for this trait and it was decided to use usual boundaries of ± 3 standard deviations from the mean. A Log likelihood test between the single random effect model (-4754.01) and the two random effect model (-4749.53) (Table 5) indicated again that the model including permanent environment of the litter as an additional random effect was superior. The final estimated heritability for HAEM-22 was 0.06±0.04. The large standard error associated with this result indicates that the final heritability for HAEM-22 can vary from this result substantially.

Table 5. Variance components, heritability (h^2) and permanent litter (c^2) estimates and Log likelihoods (Ll) for haemoglobin iron content at 5 weeks (HAEM-5), haemoglobin iron content at 22 weeks (HAEM-22), pork iron content (IRON-MOL)

	pvar*	avar*	evar*	pevar*	h^2	c^2	Ll
HAEM-5	216.5	41.08	175.5	-	0.19± 0.03	-	-5407.14
HAEM-5	210.4	8.594	179.3	22.44	0.04±0.02	0.11±0.02	-5379.34
HAEM-22	167.7	21.37	146.3	-	0.13±0.05	-	-4754.01
HAEM-22	167.1	10.02	140.5	6.94	0.06±0.04	0.10±0.03	-4749.53
IRON-WT	0.126	0.051	0.076		0.40±0.08		685.53
IRON-WT	0.124	0.034	0.078	0.012	0.28±0.08	0.10±0.04	688.83

pvar=phenotypic variance, avar= additive variance, evar=environmental variance, pevar=permanent effect of the litter variance

Log likelihood results for IRON-WT indicated that the most appropriate model included was the common litter effect. The estimated heritability for IRON-WT was 0.28 indicating that this trait is under genetic control.

Summary

Blood haemoglobin at 5 weeks was not a heritable trait of the piglet, however, a common litter effect was significant for this trait (0.11±0.02). Results here imply that any genetic difference in blood haemoglobin levels in five week old piglets is being masked by the iron injection given to piglets shortly after birth. It may be possible to determine genetic differences between very young pigs provided blood samples were to be taken prior to receiving iron injections.

Blood haemoglobin heritability estimation in 22 week old pigs was particularly sensitive to boundaries imposed upon data. Blood haemoglobin in 22 week old pigs was lowly heritable (0.06±0.04) and may alter significantly given the standard error for this heritability. Phenotypically this trait was positively correlated with backfat (0.12). This correlation is unfavourable as animals with lower backfat have lower blood haemoglobin at 22 weeks. Blood haemoglobin was, however, positively correlated with higher muscle iron (0.13). Breed differences were not evident for blood haemoglobin content at 22 weeks. However sex differences were significant. Gilts had 110.21 g/L compared to 103.53 g/L for boars. Sex and test date were the only significant explanatory variables for this trait.

Muscle iron was moderately heritable (0.28±0.08) and phenotypically was unfavourably correlated with backfat (0.09). Muscle iron was phenotypically correlated with the colour measurements luminance (-0.24) hue (0.45), chroma (0.23). The phenotypic correlation with luminescence indicates that pork with more iron is marginally darker.

In summary, these results indicate that blood haemoglobin in 22 week old pigs is related to iron content and colour in pork. In addition, a more visually desirable meat can be achieved through increasing the iron content of pork.

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