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Review

Modelling quality variations in commercial Ontario pork production

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ABSTRACT

This study explores the interactions of sensory and nutritional environment with genotype occurring in current commercial pork production in Ontario, Canada, which may interact to result in poor quality meat. The study focussed on identifying factors and signalling mechanisms that contribute to poor meat quality, in order to develop strategies to reduce the incidence of unacceptable product quality. In the first phase of the work reported here, animal behaviour and muscle metabolism studies were related to meat colour, tenderness and water-holding capacity measurements from commercially-produced pigs killed in a commercial packing plant. A partial least squares analysis was used to determine the most important of the principal production variables, peri-mortem biochemical measures and post-mortem carcass condition variables studied, in terms of their influence on water-holding, toughness and colour (L^*-value). Variations between producer and kill day at the slaughterhouse were very strong contributors to variability in these three meat quality parameters, followed by pH variations. A second phase of the study is currently underway to characterize patterns of gene expression related to extremes of end-product quality and to reduce quality variations by nutritional and behavioural management strategies.

Contents

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1. Introduction

Canada produced 23.4 million pigs for domestic slaughter and live export in 2006 (Canadian Meat Council, 2008; Canadian Pork Council, 2008). Export of pork meat in 2006 was over 1.03 million tonnes, with exports to the USA and Japan accounting for more than half this figure. With such a strong dependence on export, Canada needs to remain competitive in the global marketplace and therefore must focus on producing a quality product that meets customer demands. Based on genetic and management improvement, Canadian pork has been getting leaner while being produced more efficiently. The Canadian centre for swine improvement (CCSI, 2004) reports a decrease of 8.9 days to market, 131 fewer grams of feed per kg of weight gain, 1.7 mm less backfat, and 1.5 cm^2 more loin eye area in genetic progress of Canadian purebred breeding stock in the previous six years alone. These characteristics have all helped the production sector by improving lean growth efficiency, with loin eye area and backfat being the only traits considered in regards to product quality. Recently, more attention has been focused on improving traits specifically related to meat quality such as colour, water-holding capacity (drip loss), tenderness, and intramuscular fat (IMF) content. The meat packing industry is moving towards specialized grading grids that often include specific targets for carcass weight, backfat depth, lean yield, loin eye area, marbling (IMF) and colour. It is generally accepted that the average carcass is of acceptable quality but there is a wide variation in carcass quality and a significant number of carcasses not meeting acceptable quality standards.

Pork meat quality is affected by numerous factors including breed, genotype, feeding, pre-slaughter handling, stunning and slaughter practices, chilling, and storage conditions (Rosenvold & Andersen, 2003; Schäfer, Rosenvold, Purslow, Andersen, & Henckel, 2002). While many of these factors have been studied in isolation, interactions among these factors are poorly understood (Warriss et al., 1998). Pork quality is the result of a complex combination of factors, with interactions among the sensory environment, genotype and nutritional environment combining with peri-mortem metabolism to influence final meat quality.

1.1. Behavioural factors

Numerous studies have demonstrated the relationship between behavioural and physiological measures of stress in pigs at slaughter and subsequent effects on meat quality, particularly pale, soft and exudative (PSE) pork (Hemsworth et al., 2002; Warriss et al., 1998). It is widely recognized that the shipment and handling of pigs prior to slaughter causes significant stress to animals (Guise & Penny, 1989), and that individual responses to pre-slaughter stress vary considerably (Grandin, 1997). In terms of meat quality, stress-susceptibility has been mainly identified at the level of the muscle, specifically in mutations in the skeletal ryanodine receptor (sRyR) known to cause porcine stress syndrome (Fujii et al., 1991). However, differences in stress-susceptibility may also occur in areas of the brain responsible for emotional and neuroendocrine responses to stress. In rodent models, reductions in fear reactivity are characterized by lower corticotrophin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and higher numbers of glucocorticoid receptors in the hippocampus, which enhances negative feedback mechanisms of the hypothalamic–pituitary–adrenal (HPA) response to stress (Meaney, 2001). Boars heterozygous for the sRyR gene do not show differences in glucocortiocid receptor levels in the brain or stressor-induced HPA activation compared to wild type boars (Weaver, Dixon, & Schaefer, 2000a). However, variations in fear reactivity associated with differences in HPA function have been demonstrated in other lines of pigs (Weaver, Aherne, Meaney, Schaefer, & Dixon, 2000b). Therefore, variation in stress-susceptibility may be due to two separate mechanisms – at the muscle and at the brain. Behavioural stress responses are influenced by genetics, management and previous experience. Therefore, what is considered aversive by some animals may not produce a negative reaction in others. For example, Scott, Torrey, Stewart, and Weaver (2000) demonstrated that a genetic line of pigs selected for high lean growth showed increased anxiety in response to humans. Similar observations are reported by Grandin (1997), and associations between animal temperament and meat quality have also been observed in cattle (Petherick, Holroyd, Doogan, & Venus, 2002). Provision of environmental enrichment and/or positive interactions with humans on the farm can attenuate fearfulness and reduce stress at shipment and pre-slaughter (Beattie, O'Connell, & Moss, 2000; Geverink et al., 1998; Hill, McGlone, Fullwood, & Miller, 1998). In addition, it has been suggested that genetic and management factors can act synergistically to increase stress responses, thereby reducing meat quality (D'Souza, Dunshea, Leury, & Warner, 1998a). The relationships among individual differences in fear response, HPA activity and any consequent effects on meat quality have not been explored to date.

1.2. Nutritional factors

Modification of hog finishing diets can significantly impact water-holding capacity and eating quality of pork as noted in past reviews (Rosenvold & Andersen, 2003; Warriss et al., 1998). Researchers have investigated numerous dietary factors that influence meat quality, including protein quality and amino acid balance, protein to energy ratios, type of carbohydrates, fat quality, betaine, creatine, niacin, α-tochopherol, types of magnesium, vitamins E and C, glycolytic inhibitors and ractopamine among others (Apple, 2007; Apple, Maxwell, Stivarius, Rakes, & Johnson, 2002; Caine, Schaefer, Aalhus, & Dugan, 2000; D'Souza, Warner, Leury, & Dunshea, 1998b; Frederick, van Heugten, & See, 2004; Hamilton et al., 2002; Matthews, Southern, Bidner, & Persica, 2001; Peeters, Driessen, Steegmans, Henot, & Geers, 2004; Real et al., 2002; Stahl, Allee, & Berg, 2001). The impact of dietary interventions on pork meat quality has, in most cases, not been consistent across studies. This is largely due to interactions between various factors that influence pork meat quality – especially pig genotype and pre-slaughter stress – which have not been considered or are poorly understood (Warriss et al., 1998). However, a more complete understanding of the underlying mechanisms that influence pork meat quality could lead to the development of effective feeding and animal management strategies to enhance pork meat quality and reduce its variability.

Reducing muscle glycogen stores in pigs at slaughter can improve water-holding capacity and overall eating quality of pork meat, by preventing excess lactic acid production in muscle around the time of slaughter (Rosenvold et al., 2002). Muscle glycogen stores can be reduced by feeding diets that are low in glucogenic carbohydrates such as starch and sugars. The post-mortem generation of excess amounts of lactic acid from glycogen, induced by responses to external stressors and feeding adrenergic agonists (ractopamine), can increase the rate and extent of pH decline in muscle with subsequent negative effects on various aspects pork meat quality, including light meat color and reduced water-holding capacity (Rosenvold et al., 2002), especially in stress susceptible pigs. However, the impact of medium doses of ractopamine on pork meat quality has been small, somewhat inconsistent across studies, and is influenced by level and duration of feeding ractopamine, pig type and dietary protein level (e.g. Aalhus, Schaefer, Murral, & Jones, 1992; Armstrong, Ivers, Wagner, & Anderson, 2004; Uttaro et al., 1993). A recent study indicated that pigs fed

ractopamine had higher heart rates and altered behavioural responses to human presence and handling compared to controls (Marchant-Forde, Lay, Pajor, Richert, & Schinckel, 2003).

1.3. Genotype

Generally, approximately 30% of the variability in quality traits such as tenderness and water-holding capacity can be directly linked to genotype. The influence of the halothane and RN genes on water-holding capacity has been well recognized (Rosenvold & Andersen, 2003). However, even without the deleterious alleles of these genes, lower water-holding capacity has been found when selecting pigs for better feed conversion and improved lean growth efficiency (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001). These selected pigs were less tender than their control counterparts, which may be attributed to less marbling in the muscle and/or decreased proteolysis due to higher levels of calpastatin. Similarly, Huff-Lonergan et al. (2002) found poor water-holding capacity to be associated with decreased pork tenderness and flavour. The propensity for genetic lines to marble (IMF deposition) has been associated with improved water-holding capacity (Brewer et al., 2002), with consumers finding highly marbled pork chops (3.5% IMF) to be more desirable in tenderness, juiciness, and flavour than lean pork chops (1% IMF). A number of genes have been investigated for their role in meat quality such as *RYR1*(ryanodine receptor), *RN* (Rendement Napole) (Hamilton, Ellis, McKeith, Miller, & Parrett, 2000), *Pit1* (te Pas et al., 2001) fatty acid binding protein genes*(FABP3, FABP4* and *FABP5*; Ye, 2003),insulin-like growth factors *IGF*-1 and 2 (Casas-Carrillo, Prill-Adams, Price, Clutter, & Kirkpatrick, 1997) and leptin and leptin receptor *LEPTIN* and *LEPR* (Baratta et al., 2002). In addition to major gene effects, it is also widely recognized large interactions exist between genotype and environmental factors.

1.4. Post-mortem metabolism

Major meat quality parameters such as texture, colour and water-holding capacity are greatly affected by the metabolic processes that occur during the conversion of living muscle to post-mortem meat, and these in turn are greatly affected by the nutritional status, stress at time of slaughter and genetic susceptibility to stress. Variations in toughness of pork meat are primarily due to variations in post-mortem proteolysis, under the influence of the calcium-activated calpain enzymes and their inhibitor, calpastatin. Post-mortem pH and temperature affect enzyme activity but pre-mortem expression of calpains and calpastatin in muscle tissue is controlled by a large number of factors, some of which also involve calcium signalling pathways. One of these is stress; epinephrine (adrenaline) directly increases calpain expression in muscle cells (Ertbjerg, Lawson, & Purslow, 2000). Calpain expression may also be altered in some circumstances by nutritional manipulation (Kristensen et al., 2002, Kristensen, Therkildsen, Aaslyng, Oksbjerg, & Ertbjerg, 2004).

In terms of water-holding capacity, the role of post-mortem pH in the release of water from within the myofibrillar lattice of the muscle cells is well established (Offer et al., 1989), but the large variation in development of drip channels in meat is not understood. For drip losses to occur, water released within the cells has to be channelled to the surface and variations in drip channel formation have an obvious role in determining the actual amount of fluid loss (Offer et al., 1989; Schäfer et al., 2002). Calpain degradation of cytoskeletal proteins near the internal cell surface has previously been shown to affect how much water lost from the organelles within the cell can be transferred extracellularly. A recent publication has shown that degradation of transmembrane signalling proteins (integrins) by calpains is highly related to the time-course of drip channel development (Lawson, 2004).

1.5. Approach and objective of current study

Rather than take a traditional, multi-factorial experiment-based approach to investigate all possible interactions, we have elected to take a comparative approach using commercially-produced pigs in order to identify factors directly related to negative aspects of quality in an industry-applicable study population. Factors within the behavioural and nutritional environments that interact with genotype to contribute to animal stress and muscle composition are examined. Following through the production chain, differences in gene expression at the point of slaughter and peri-mortem metabolism are being measured and compared with meat quality measured after conventional carcass chilling. This will allow us to identify factors and pathways leading to poor or unacceptable meat quality, and design potential interventions to mitigate product quality issues.

This entire study is being conducted in two phases. Phase one, reported in this paper, has been carried out mainly in commercial facilities, and documents inputs from the production system through to post-mortem carcass handling and their relation to end-product quality parameters. This provides a realistic snapshot of the variations seen in the Ontario pork industry.

Phase two studies will examine specific behavioural and nutritional treatments designed to reduce stress and improve meat quality, and will be conducted under controlled conditions at University of Guelph Research Stations and at commercial farms.

2. Materials and methods

Nineteen commercial farms agreed to cooperate in this research trial. Each of the operations produced a cohort of 12 barrows and 12 gilts for the project. These hog producers represent a range in swine genetics to include animals with average to high lean growth potential, and from low to high marbling in the major breeds used in Ontario. Farm sizes range from 1,200 to over 10,000 hogs marketed per year. Feeding systems on these farms include liquid, wet–dry and dry only feeds. Breeding stock on these farms was sourced from six different genetics companies. One farm was sampled three times and one farm twice. In addition, 156 pigs from six genetic lines (24–32 pigs per genetic line) were reared at the University of Guelph's Ponsonby General Animal Facility to examine differences in behaviour, biochemical and meat quality traits when management is identical across genetic lines. Collection of data from on-farm behavioural assessment, carcass and meat quality evaluation have been carried out on 672 pigs (24 pigs from each of 28 cohorts).

At market weight (typical range 80–120 kg), all animals in a cohort were shipped to a commercial Ontario packing plant that uses carbon dioxide (CO_2) stunning in their harvesting operation. For both packing plant operational reasons and for data collection and sampling reasons, it was not possible that the cohorts from all producers were slaughtered on the same day, and so each cohort was slaughtered on a separate date. There is thus a confounding of two variables; producer and kill date.

2.1. Animal behaviour and farm management measures

Behavioural assessments were carried out both on-farm and during handling at the abattoir. At the farm, an open door test (van der Kooij et al., 2002) was used to assess the individual temperaments of study hogs. Animals were assessed as 'willing', 'intermediate' or 'reluctant' depending on their behaviour when allowed to voluntarily exit the home pen over a 3 min test. 'Willing' pigs ex-

ited the pen within 1 min, 'intermediate' pigs left within 3 min, and 'reluctant' pigs did not leave the pen. A management questionnaire was also completed on the farm, which consisted of 29 questions regarding feed, handling and housing practices.

The behaviour of individual pigs at the packing plant was quantified from video recordings of pig handling in the crowd pen and a single file chute that lead directly to the CO_2 stunner. Measures included the incidence of falls, backing, wedging and piling (Handling Incidents), as well as prodding and other contact by handlers (Human Intervention). Fighting during transport and lairage was indirectly measured by post-mortem lesion scoring on the head and shoulders (de Koning, 1985).

A study on the behavioural effects of different frequencies of interactions with humans was carried out as a separate trial. This, and the results of human interaction strategies to reduce variability on meat quality, will be reported separately.

2.2. Measurements at slaughter

At sticking, blood samples were collected for determination of cortisol (as an indicator of psychological stress) and glucose, lactate and creatine-phosphokinase (CPK), (as physiological indicators of stress). Small (10–15 g) subsamples of the Longissimus and semimembranosus muscles were removed as soon as practicable after slaughter and immediately frozen in liquid nitrogen. These were kept in liquid nitrogen and subsequently at −80 °C for calpain activity and gene expression analyses. Gene expression studies are ongoing and will be reported separately. The net activity of μ-calpain (Calpain-1) and *m*-calpain (calpain-2) were measured by casein substrate zymography (Therkildsen et al., 2004).

The pH and temperature in the Longissimus muscle (loin) and the semimembranosus muscle (ham) were monitored at 1, 2, 24 and 48 h post-mortem in the packing plant. Ambient plant temperature and humidity were also recorded. A section of the loin and the entire semimembranosus muscle from the left side of each carcass were then shipped to the University of Guelph Meat Laboratory for further analysis of meat quality

2.3. Meat quality measurements

Backfat and loin eye measurements along with objective measurements of lean colour (using the L^*, a^*, b^*system), pH, drip loss, and subjective measurements of firmness, colour, and marbling on Longissimus and semimembranosus muscles were measured. Muscle samples were also retained for total lipid analysis. Cooking losses of two boneless loin chops were measured in the preparation of the loin chops for determining Warner–Bratzler (WB) shear force. The semimembranosus muscle was injected with a conventional brine, smoked and then sub-sampled for WB shear force determination.

3. Statistical analysis

Table 1 summarises the variables investigated in this study, and categorises them into

1. "Principal input variables".
2. "Biochemical and gene expression measures".
3. "Post-mortem carcass measures".
4. " Major end-product quality measures".

This categorisation reflects the fact that variables in group (1) are largely under control by the producer and packer, group (4) are the outputs we wish to control, and groups(2) and (3) are measures to help understand the mechanisms and pathways leading from (1) to (4).

Even by simply identifying the many differences in gene expression (both up- and down-regulated) in muscle as just one variable, there are 56 variables in Table 1. Analysis of the huge and complex full data set generated will involve a number of approaches in order to fully understand the interactions between sensory environment, genotype and nutritional environment that contribute to variations in meat quality (specifically tenderness, colour and water-holding capacity). The purpose of our initial analysis is to quantify the magnitude of variability in a subset of group 4 vari-

Table 1
Variables studied

Principal Input variables	Biochemical and gene expression measures	Post-mortem carcass conditions	Major end-product quality measures
Primary animal variables:	*Sticking – muscle measures:*	*Loin pH*	*Colour*
Animal ID (ear tag, tattoo #)	Gene expression:	1 h Post-mortem	Ham L^*
Gender	Upregulated & downregulated proteins	2 h Post-mortem	Ham a^*
Farm	Calpain 1 activity	24 h Post-mortem Final (48 h)	Ham b^*
Kill date	Calpain 2 activity		Loin L^*
Genotype			Loin a^*
Nutrition type			Loin b^*
Behaviour on-farm:	*Sticking- blood measures:*	*Ham pH*	*Driploss*
Temperament (willing/interm./reluctant)	Lactate	1 h Post-mortem	% Ham
	Glucose	2 h Post-mortem	% Loin
	CPK	24 h Post-mortem Final (48 h)	
	Cortisol		
Crowd pen measures		*Loin temperature*	*Shear force*
Total time		1 h Post-mortem	WB Ham
Number in batch		2 h Post-mortem	WB Loin
Handling incidents		24 h Post-mortem Final (48 h)	
Human interaction			
Chute measures:		*Ham temperature*	%Cooking loss (ham)
Total time		1 h Post-mortem	%Cooking loss (loin)
Handling incidents		2 h Post-mortem	
Human interaction		24 h Post-mortem Final (48 h)	
Lesion score		Carcass weight	Loin firmness
		Carcass grade index	Loin wetness
			Loin marbling
			Loin Japanese colour
		Packing plant conditions:	
		Plant temperature	
		Plant humidity	

ables most relevant to industry concerns, and to assess which of the other variables (and especially group (1) "inputs") that are particularly related to these. For this purpose, the end-product quality variables selected as most relevant were:

- Water-holding capacity (% drip loss).
- Toughness (WB shear force).
- Colour (L^*-value)

Partial least squares (PLS) analysis was used (SPSS Inc. Chicago, USA) to fit a linear statistical model to the dataset for all variables where values exist for most or all of the 672 animals (i.e. excluding the few variables where subsets only were analysed). Input variables with low center-scaled b estimate (close to 0) and low Variance in prediction (Vip > 0.8)were rejected as poor candidates for inclusion in the model. This is a similar analysis to that used by Schäfer et al. (2002) to model parameters affecting drip in pork from stressed (exercised) and control pigs.

The linear model is in the form:

$$\begin{aligned}&\text{Variation in (quality parameter)}\\&= a\%\ (\text{input variable1}) + b\%\ (\text{input variable2})\\&\quad + c\%\ (\text{input variable3}) \ldots + p\%\ (\text{variable } n) \ldots\\&\quad + \text{residual variability}\end{aligned}$$

4. Results

Table 2 reports the average values, range and standard error values for carcass condition and end-product quality parameters. Colour (L^*-value) ranged from 27 to 64 and drip loss ranged from 2.216.4% in the loin, with a similar range in the ham. WB shear force values ranged from 1.67 to 5.43 kg in ham samples and from 2.08 to 8.21 kg in loin samples. Comparisons among meat quality parameters show strong associations that support standard definitions of PSE and DFD pork. For example, the final pH (pH at 48 h post-mortem) for loin was negatively correlated with loin colour and loin and ham drip loss, and positively correlated with initial (1 h) and final ham pH. Similarly, the final pH of the ham was negatively correlated with ham shear (tenderness), colour (L^*) and drip loss, and positively correlated with initial ham pH and initial and final loin pH.

Clear differences exist in WB shear force values between Longissimus and semimembranosus muscle samples, with the average shear force in the cooked loin samples of 4.6 kg being much higher than the average for the smoked ham samples at 3.0 kg. Apart form this difference due to post-mortem treatment, there are also differences in other parameters between muscles, although these are less obvious. For example, the mean drip loss for Loin samples is 7.95% and for Ham samples is 7.71%. Given the huge range (2.2–16.4%) of drip loss values observed, these means look similar. However, a paired t-test (i.e. comparing % driploss in ham to % drip loss in loin in each pig) shows that % drip loss in ham samples is significantly higher than for loin samples ($P < 0.01$). Looking at Pearson correlation coefficients between ham and loin drip loss with various pH measures, it is clear that for both muscles the highest absolute value for correlation coefficients is between drip loss and final pH. However a notable difference is the larger absolute values of correlation coefficients between ham drip loss and pH at all time points, compared to loin drip loss with loin pH levels.

Average values, min–max range and standard error values for blood analyses at slaughter and for behavioural measures are summarised in Table 3.

Table 2
Average values and ranges for meat quality measures across 28 cohorts of 24 animals from 19 farms and 6 University-based groups (total $n = 672$)

Variable	Mean	Std.Error	Minimum	Maximum
Loin 1 h pH	6.124	0.010	5.340	6.840
Loin 2 h pH	5.855	0.011	5.090	6.570
Loin 24 h pH	5.702	0.010	5.210	6.480
Loin final pH	5.590	0.005	5.200	6.290
Ham 1 h pH	6.086	0.009	5.430	6.770
Ham 2 h pH	5.857	0.010	5.150	6.540
Ham 24 h pH	5.748	0.012	5.210	6.670
Ham final pH	5.684	0.007	5.330	6.580
Loin 1 h temp °C	37.252	0.527	28.300	38.700
Loin 2 h temp °C	29.911	0.116	19.700	32.900
Loin 24 h temp °C	2.934	0.082	0.000	8.000
Ham 1 h temp °C	37.866	0.531	25.700	38.900
Ham 2 h temp °C	33.596	0.552	18.000	34.900
Ham 24 h temp °C	3.554	0.074	0.500	7.900
Carcass weight/kg	92.095	0.319	69.100	122.800
Lean yield/kg	60.624	0.242	3.000	91.900
Ham colour L^*	45.315	0.122	30.285	59.740
Ham colour a	9.646	0.057	4.910	15.260
Ham colour b	2.982	0.055	−1.370	9.590
Loin colour L^*	48.852	0.125	27.210	64.810
Loin colour a	7.505	0.043	4.710	13.190
Loin colour b	2.894	0.053	−0.600	10.870
Ham dripLoss %	7.950	0.087	2.260	16.430
Loin dripLoss %	7.713	0.076	2.930	14.890
Ham shearForce kg	2.995	0.024	1.670	5.430
Loin shearForce kg	4.597	0.042	2.080	8.210
% Cook loss ham	29.142	0.171	17.780	43.180
% Cook loss loin	21.650	0.141	10.020	31.610
Subjective carcass scores:				
LoinFirm	2.877	0.018	1.000	4.000
LoinWet	2.799	0.024	1.000	5.000
LoinMarble	2.326	0.030	1.000	5.000
Loin Jap colour	2.845	0.018	1.000	4.000
Loin NPPC colour	2.859	0.018	1.000	4.000

Table 3
Average and range of blood analysis and behaviour measures

Variable	Mean	Std. error	Minimum	Maximum
Blood				
Lactate (mmoll/L)	18.564	0.207	6.000	38.400
Glucose (mg/dL)	186.427	2.134	70.640	466.560
CPK (U/L)	4094.351	500.017	208.000	303000.000
Cortisol (ng/mL)	68.728	1.663	5.210	258.420
Behaviour				
Crowd				
Total time (s)	57.960	3.700	0.000	926.000
Batch size	14.240	0.333	0.000	38.000
Human Intactn.	3.165	0.133	0.000	20.000
Total Hnd. Inc.	2.043	0.078	0.000	9.000
Chute				
Total time (s)	32.170	3.017	2.000	1083.000
Human Intactn.	1.517	0.072	0.000	12.000
Total Hnd. Inc.	0.768	0.064	0.000	23.000

Batch size = number of pigs in the crown pen.
Crowd total handling incidents = sum of Jam, Back out, Piling, Escape and Fall frequencies.
Chute total handling incidents = sum of Back out, Piling and Flip over frequencies.

During on-farm testing, the individual temperaments of study hogs were assessed as 'willing', 'intermediate' or 'reluctant' with regard to their willingness to leave the pen using a standardized "open door" test. Comparison of pig temperament to behaviour at the packing plant shows that 'willing' pigs were more ($P < 0.05$) frequently involved in piling events in the crowd pen whereas 'reluctant' animals tended to receive more ($P < 0.10$)

human interventions (hits or prods) in the crowd pen. Measures of time spent in the crowd pen, number of pigs in the crowd pen, frequency of human interactions and handling incidents (e.g. balking, jamming, falling, piling) positively correlated ($P < 0.05$) with one-another. Frequency of piling in the chute was related ($P < 0.05$) to two measures of the stress response, blood lactate and glucose. Pigs that received more human interventions (hits or prods) in the crowd pen also had higher ($P < 0.05$) lactate and CPK. Initial findings on some aspects of these behavioural results have been summarised by Brown et al. (2007).

As expected, various measures of stress response corresponded with reductions in meat quality. Blood lactate levels were associated with pH and colour values in both the ham and loin, and significantly correlated ($P < 0.05$) with higher shear values in the ham. Glucose levels were correlated ($P < 0.05$) with initial pH and % drip loss in the ham, as well as colour and % drip loss levels in the loin, traits that are associated with PSE pork. Elevated CPK was correlated ($P < 0.05$) with % drip loss in both loin and ham, and colour values of the ham.

4.1. Post-mortem enzyme activity and meat quality

An initial analysis of variations in calpain activity in a subset of these animals ($n = 65$) has been presented previously (Purslow et al., 2006).This subset was selected to simply cover the high- to-low ranges in drip, colour and shear force in each cohort. A significant ($P = 0.013$) negative relationship between calpain-1 activity and drip loss was reported. Calpain-2 activities were not significantly correlated with drip loss. Correlations between shear force (cooked meat toughness) and calpain-1 activity were poor ($P = 0.756$) and similarly, shear force was poorly correlated with drip loss ($P = 0.570$). Removal of pH as a covariant revealed that a significant proportion of the correlation between drip loss and calpain activity was not simply due to variations in pH, pointing to other factors affecting this enzymic degradation pathway (Purslow et al., 2006). Further analysis of calpain activities in samples selected for gene expression studies is underway and will be reported separately.

5. Linear model using PLS analysis

The PLS model is designed to answer the initial question:

What are the most important 'input variables ' (in terms of Table 1) that account for the majority of variability seen in the three most obvious pork quality parameters (colour, water-holding, tenderness)?

This has allowed us to rank the importance of the 22 "input variables", six of the biochemical measures and 18 "carcass condition" variables in explaining the variability of the "end-product quality" measures. Table 4 shows the proportion (in the range 0–1) of the total variation in each of three meat quality parameters (Lightness in colour, or *L*-value; shear force or toughness; drip loss or water-holding capacity) that can be explained by each factor.

The analysis shows that variations between individual producers and the interaction of this with day at the packing plant (these two factors are confounded together in "Kill Date") are of overwhelming importance in determining variations in colour, tenderness and water-holding capacity of pork. This "Kill date" factor alone accounts for 16– 35% of variation in quality measures in both ham and loin. The fact that packing plant temperature also figures in the top six most important factors for drip from the loin muscle and tenderness (shear force) for both ham and loin suggests that there is a strong contribution from ambient conditions in the packing plant to variations in quality. As expected, pH values at 1 h post-mortem, 2 h and 48 h (final pH) are strong indicators of variations in all quality parameters measured.

Given the dominance of "Kill Date" as a factor, it is perhaps not surprising that no significant interactions between the 21 factors listed arose from the analysis. There was however the expected linkage between pH at various times post-mortem, and between temperature in the muscles at 1 and 2 h post-mortem. To a large extent these are not truly independent variables.

6. Discussion

This first phase of the study was designed to provide a snapshot of the variations in quality that exist in commercial practice in the

Table 4
Proportion of variation in drip, colour and shear values in both loin and ham muscles that is explained by each of the 21 variables listed in the first column (Factor)

Factor	Loin			Ham		
	Drip	Colour	Shear	Drip	Colour	Shear
Kill date	0.355	0.183	0.227	0.221	0.164	0.259
Gender (F)	0.002	0.051	0.011	0.001	0.03	0.005
Gender (M)	0.002	0.051	0.011	0.001	0.03	0.005
Temperament (willing)	0.007	0.001	0.018	0.002	0.01	0.007
Temperament (intermediate.)	0.031	0.003	0.083	0.001	0.018	0.037
Temperament (reluctant)	0.028	0.013	0.049	0.01	0.003	0.025
Carcass weight	0.041	0	0.01	0.045	0	0.029
Lean yield	0.018	0.019	0	0.004	0.062	0.057
Lactate	0.024	0.182	0.033	0.045	0.054	0.119
Glucose	0.038	0.138	0.041	0.061	0.084	0.037
CPK	0.009	0.026	0	0.007	0.032	0.008
Cortisol	0.016	0	0.017	0.001	0.001	0.021
Crowd HI	0.013	0	0.023	0.014	0.014	0.015
Chute HI	0.003	0.007	0.018	0.004	0.01	0.006
pH (1 h)	0.044	0.136	0.107	0.094	0.14	0.103
pH (2 h)	0.099	0.134	0.12	0.127	0.092	0.087
pH (final)	0.106	0.017	0.08	0.172	0.179	0.011
Meat temperature (1 h)	0.042	0.003	0	0.075	0.018	0.054
Meat temperature (2 h)	0.004	0.028	0	0.059	0.015	0.013
Plant temperature	0.107	0.001	0.109	0.046	0.005	0.101
Plant humidity	0.012	0.007	0.042	0.009	0.038	0

In the Factor column, HI = human interaction scores, plant temperature and plant humidity refer to ambient conditions in the packing plant on the day of slaughter. The factor "Kill Date" is confounded with individual producers as a factor, as the animals from each producer were sent to slaughter on a different date. In each column, the six largest contributors to the variation in that particular characteristic are shaded.

Ontario pork industry. Variations in drip loss (2–16%; average 7.7% in loin and 7.95% in ham) are considerable and a high water purge from carcasses represents loss of potential yield as well as quality. Variations of this nature are consistent with values reported elsewhere. Kauffman, Cassens, Scherer, and Meeker (1992) in a survey of the US industry for the National Pork Producers Council found that more than 50% of pork sampled from 14 packing plants had drip losses in excess of 6%. Meisinger (2003) stated that variability in quality results in losses of $8.08, or 8% of the liveweight value, per slaughtered pig in the US industry. von Rohr, Hofer, and Kunzi (1999) surveyed quality variations in Swiss pork. The large number of colour measures taken in this study ($n = 4055$) yielded an average L^* value of 55.6 (SD = 3.4). Drip measures on a much smaller sample size ($n = 64$) yielded an average drip loss of 5.4% (SD = 5.2%). In terms of economic value to the Swiss industry, von Rohr et al. (1999) defined carcasses with drip <5% and $L^* > 58.3$ as being of highest economic value. The minimum–maximum range of drip loss seen in our present study is comparable to the 2–12% range seen in Danish pigs in the experiments of Schäfer et al. (2002) comparing the water-holding capacity of pork from stressed and non-stressed animals. Støier, Aaslyng, Olsen, and Henckel (2001) reported lower average drip loss from Danish pigs in a commercial slaughterhouse. Conventionally handled pigs yielded loins with an average drip loss of 3.7%, and low stress handling prior to slaughter reduced this to 3.2%. Hansen, Claudi-Magnussen, Jensen, and Andersen (2006) in their Table 6 report the average % drip loss for conventionally produced and organic Danish pork to be in the range 6.05–6.53%.

Schäfer et al. (2002) reported a linear model based on PLS analysis to describe the influence of various factors on drip loss from stressed and non-stressed groups of pigs produced at a research site. In this fairly well-controlled experiment using one genotype and standardized feeding and handling regimes, the majority of variations in drip loss from Longissimus muscle could be accounted for by variations in post-mortem pH and muscle temperature alone. In our study, very considerable variations in the average colour, drip loss and toughness occurred between different cohorts produced at different farms and taken to slaughter on different days. The "Kill date" parameter explained 16–35% of variability in drip loss, colour and toughness from both muscles. This parameter compounds both between-producer effects and variations from day to day in the slaughterhouse. In the 1992 NPPC survey in the US (Kauffman et al., 1992) which looked at output from 14 slaughter plants, considerable variation was found between plants and between each day of the week at plants.

Lammens et al. (2007) surveyed pH development in pork loins and ham, and electrical conductivity in hams from pigs processed through five Belgian slaughterhouses. While their main finding was on the quality differences between animals produced under a quality assurance protocol and others, they did note differences between measurements at different slaughterhouses, which varied between loin versus ham muscle and time post-mortem of the measurements. In our current study we found that ambient temperature in the plant was a major factor in drip loss from the loin muscle and in toughness of both loin and ham muscles. When ranking the average drip, colour and toughness values of each cohort in our study, it was noted that the two cohorts repeated from one farm and the three cohorts repeated form another showed very different positions in the rank order. This also supports the notion that part of the variability due to "Kill date" is indeed due to day-to-day variations at the packing plant.

In terms of basic mechanisms, the rate and extent of both pH and temperature fall post-mortem is known to affect the degree of denaturation of the S1 region of myosin heads and to affect the myofilament lattice spacing post-mortem (Offer et al., 1989). Lattice spacing is also affected by sarcomere length, and the combination of these two effects means that drip loss from Longissimus varies with sarcomere length. Drip loss is high in shortened pork muscle (Bertram, Purslow, & Andersen, 2002; Honikel, Kim, Hamm, & Roncales, 1986) compared to rest-length muscle, and is decreases in muscle taken into rigor at extended sarcomere lengths (Bertram et al., 2002). The change in myofilament spacing pre- to post-rigor, as followed by X-ray diffraction studies, is greatest at short sarcomere lengths and progressively smaller at extended sarcomere lengths (Purslow et al., 2001). Although this change in myofibrillar spacing during rigor development is the major molecular mechanism of water expulsion from within myofibrils, there are a number of other structural mechanisms that determine the degree of drip loss from meat as a whole tissue, and so it is not surprising that at all sarcomere lengths the amount of fluid lost as drip is only about 20% of that predicted by the change in myofibrillar lattice spacing alone (Purslow et al., 2001). Of these other structural mechanisms, the physical integrity of cytoskeletal proteins, cell membrane permeability and extracellular space development all have the potential to be influenced by proteolytic enzymes.

Animal genotype, finishing diet, housing conditions, effects of production systems on animal behaviour and stress, immediate ante-mortem nutrition effects, transport and handling stress at the slaughter plant, stunning method and post-mortem carcass treatment are all known to be factors in pork quality variations. Given that there are undoubtedly also interactions between many of these factors, it is obvious to state that mechanisms and biochemical pathways controlling variations in toughness, colour and water-holding capacity are complex. This first phase of our study has provided us with a snapshot of the extent of the variations in commercial practice and some of the factors influencing this. In our continuing studies on gene expression in muscle from animals showing a wide range of quality parameters, we hope to identify some patterns of up- and down-regulated gene expression that may provide some explanation of the variability in pork quality. It goes without saying that the number of possible candidate genes and pathways is large. Scheffler and Gerrard (2007) review a number of these known pathways and pay particular attention to the overarching role of AMPK in controlling post-mortem glycolysis in anaerobic conditions. Milan et al. (2000) showed that the RN^- gene is a mutation in the PRKAG3 gene on chromosome 15, which encodes an isoform of a regulatory subunit of AMPK, and this mutation has a major effect on glycolytic potential, leading to a low ultimate pH. We look forward to the results from our ongoing gene expression studies to provide further evidence on the candidate pathways at the heart of variability in pork quality.

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