CALPAIN-1 ACTIVITY IS RELATED TO DRIP LOSS IN COMMERCIAL PORK PRODUCTION

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Introduction
Unexplained variability in meat quality (tenderness, appearance and water-holding capacity) is a consistent concern for the meat industry in both domestic and export markets. Specific to research on pork, there is a great deal of existing knowledge on the effect of individual factors such as animal stress (Hemsworth *et al.*, 2002), nutrition (Warriss *et al.*, 1998), genotype (Lonergan *et al.*, 2001) and a number of post-mortem factors (Schäfer *et el.*, 2002). However, individual factors do not completely explain variability in quality due to substantial and complex interactions between a number of factors, such as genotype, management factors, nutrition and stress (D'Souza *et al.*, 1998a, Warris *et al.*, 1998). Figure 1 represents the experiments we have undertaken to assess the interactions of genotype, behaviour, handling, nutrition and post-mortem conditions in determining phenotypic measures of meat quality (colour, drip loss, tenderness) in commercial practice in Ontario and assessing underlying mechanisms by biochemistry and gene expression studies (both in muscle and brain tissue). This paper describes a subset of the data, specifically relating calpain activity in ham and loin muscles immediately post mortem to drip loss and shear force.

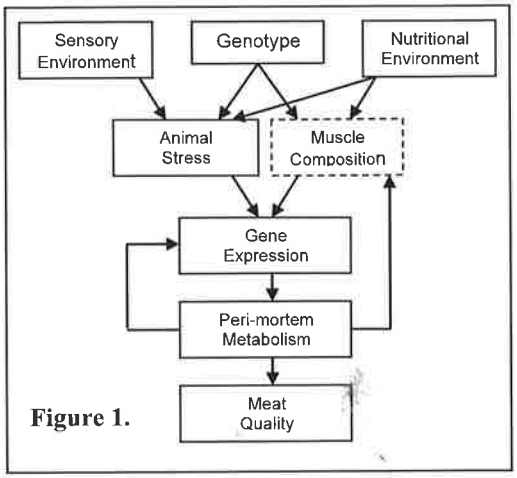


Figure 1.

Materials and Methods
12 barrows and 12 gilts from 24 commercial producers and 2 university farm-based herds (n=624) were subjected to on-farm behavioural tests and behaviour studies on shipping and reception at a common commercial slaughter plant. Producers were selected to represent a range of management and nutritional practices and inherently chosen to give a reasonable snapshot of variation seen in, commercial practice in Ontario. At the slaughter plant, blood samples were collected immediately at sticking for measurement of cortisol, glucose, lactate and CPK. The pH and temperature in *longissimus* and *semimembranosus* muscles were monitored at 1, 2, and 24 hours post mortem (pH_{1h}, pH_{2h}, pH_{24h}). Loin and ham samples were shipped to the University of Guelph Meat Laboratory for measures of backfat thickness, loin eye area, total lipid content, colour (La*b*), drip loss, cooking loss, processing yield and cooked meat Warner-Bratzler (WB)shear force measurements.
Subsamples of *longissimus* and *semimembranosus* muscles taken immediately post-mortem were snap-frozen in liquid nitrogen and subsequently stored at -80°C. Following analysis of the meat quality results, a sub-set of 65 loin and ham samples representatively spanning the range of drip loss and shear force values obtained were selected for measurement of net calpain-1 (micro-calpain) and calpain-2 (milli-calpain) activity by zymography, based upon the methods of Melody *et al.*, 2004. Four repeats of each activity determination were conducted per muscle sample. Internal controls of calpain-1 and -2 were used in each zymogram and the activity in each sample related to mg of pure enzyme standard. Statistical analysis of simple correlations and partial correlations (with one controlled variable) were performed using SPSS.

Results and Discussion
Variations seen in phenotypic measures on meat quality in commercial practice were consistent with the results of a previous US survey (Kauffman *et al.*, 1992). Colour (L –value) ranged from 35-62 and drip loss from 2.61% to 12.54% across both loin and ham samples. WB shear force values ranged from 1.07-5.43 Kg in ham samples and from 2.06-7.66 Kg in loin samples. Given the (intentionally) highly multivariate nature of the study, full multivariate analysis and

careful reconciliation of effects through gene expression differences are underway. However, it is instructive that significant effects can still be seen in simple correlations of two variables.

Figure 2 shows the correlation between the % drip loss and calpain-1 (micro-calpain) activity of all 65 ham and loin samples in the sub-set studied to date. There is a clear negative relationship; higher calpain-1 activity is correlated to lower drip loss. This negative correlation is highly significant ($0.01<p<0.02$). This result is consistent with the implications from previous studies (Kristensen and Purslow, 2001; Melody *et al.*, 2004) that degradation of cytoskeletal proteins by calpain reduces drip loss. Connections between myofibrils and the cell surface seem to be required in order to translate reduction in myofibrillar volume caused by the post-mortem fall in pH into reduction in cellular, and hence meat volume. Increased cytoskeletal degradation from higher levels of net activity of calpain-1 apparently reduces the ability to force water from the muscle cells out into extracellular spaces and drip channels.

Fig.2. Correlation between calpain 1 activity and % driploss of ham and loin

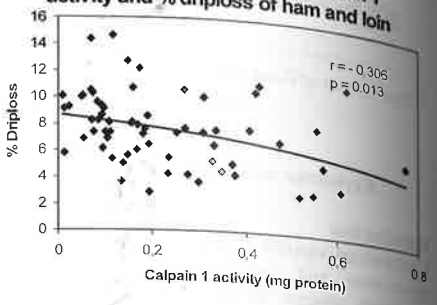
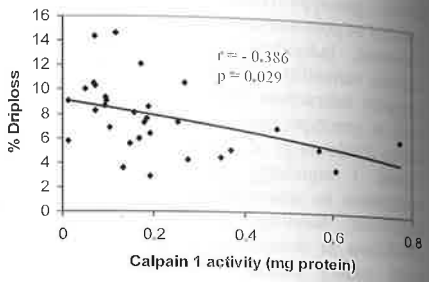


Figure 3 demonstrates that the negative correlation between drip loss and calpain-1 activity is significant (P=0.029) in semimembranosus samples (n=32). The correlation in loin samples only, although again showing a negative trend, is non-significant (P= 0.259).

Fig. 3. Correlation between calpain 1 activity and % driploss of ham only



Although calpain-2 (milli-calpain) was highly correlated with calpain-1 activity, no significant correlations with shear force or drip loss were seen in either ham or loin samples Simple correlations between WB shear force and calpain-1 activity were poor (P=0.756) and similarly, shear force was poorly correlated with drip loss (P=0.570).

WB shear force shows a significant (P=0.016) negative correlation with pH_{24h}. We therefore carried out partial correlation analysis, holding pH as the controlled variable, and examining the correlation between calpain-1 activity and WB shear force. No significant correlation was found (P=0.666).

Drip loss in both loin and ham samples showed a significant negative correlation (P=0.036) with pH_{1h} at and a highly significant (P=0.005) negative correlation with pH_{24h} (i.e. lower pH corresponded to higher drip loss). Because calpain activity is pH-dependent, pH is a co-factor in any simple correlation between drip loss and calpain activity. Partial correlation analysis of calpain -1 activity against drip loss with pH_{1h} or pH_{24h} as the variable held controlled shows significant negative correlations, with P=0.035 and P=0.013, respectively. Thus, calpain-1 activity is significantly related to drip loss even when pH variations are taken out of the picture.

Conclusions

The considerable interactions between genotype, management practices in commercial production, stress physiology, nutrition and post-mortem metabolism mean that there are highly multivariate influences on meat quality in commercial practice. Nevertheless, a clear and significant negative correlation exists between calpain-1 activity and drip loss, suggesting that this enzymic pathway is a common mechanism on which many of the influences may act.

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