final report

Project code: P.PSH.0228

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Date submitted: September 2006

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Process modelling

This is an MLA Donor Company funded project.

Meat & Livestock Australia and the MLA Donor Company acknowledge the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The concept of the Central Processing Management System (CPMS) that underpins the MQST programme depends on quantifying the interactions of processing variables so that meat quality outcomes can be predicted under a range of processing conditions. The core processing conditions that need to be managed are the rate of change of pH and temperature, and how these interact to affect quality will differ according to which muscle or species of meat animal is being considered. The meat quality attributes that need to be predicted by the model are tenderisation, colour and colour stability and water binding capacity.

Some important steps have already been taken to develop predictive models. MIRINZ produced a tenderness model which takes into account the temperature coefficient of ageing to calculate shear force under any conditions of chilling and storage temperature after rigor mortis has been attained. To account for the influence of pre-rigor processing conditions, which influence muscle shortening, a preliminary model framework for hot boned meat was developed for the Meat Research Corporation and further developed by Meat New Zealand. This model still needs further validation and refinement.

The Meat Quality model is continuing to develop a conceptual structure to account for the effects of processing on key meat quality attributes. The mathematical relationship that allows meat quality to be predicted needs first to be defined from physiological principles and controlled experiments; but, ultimately, the parameters used in the model need to be optimised against a substantial dataset.

The current research has incorporated and expanded new knowledge into the meat quality model in the following areas (see Diagram 1) :

- a ‘heat shortening component” by developing tenderness kinetics of heat shortened meat, and relating these data with a texture model.
- the meat colour stability component.
- the mechanistic muscle contraction model from the Biophysical Modelling programme into the meat quality model.

This research also evaluated the opportunities to upgrade the analysis of the Smart Simulation system. Accordingly, this objective was to develop a mathematical model of muscle contractions and evaluate the model’s ability to describe contractile behaviours in post mortem muscles.

The critical components of the model relating to contractile responses now includes the following:

1. Impulse generator: this specifies the electrical waveform characteristics and develops neural action potentials
2. Muscle membrane action potential
3. Calcium release and reuptake
4. ‘Active state’ – sensitivity of the contractile elements to calcium
5. Fatigue component, defined as a modification of the Active state
6. Force generation.

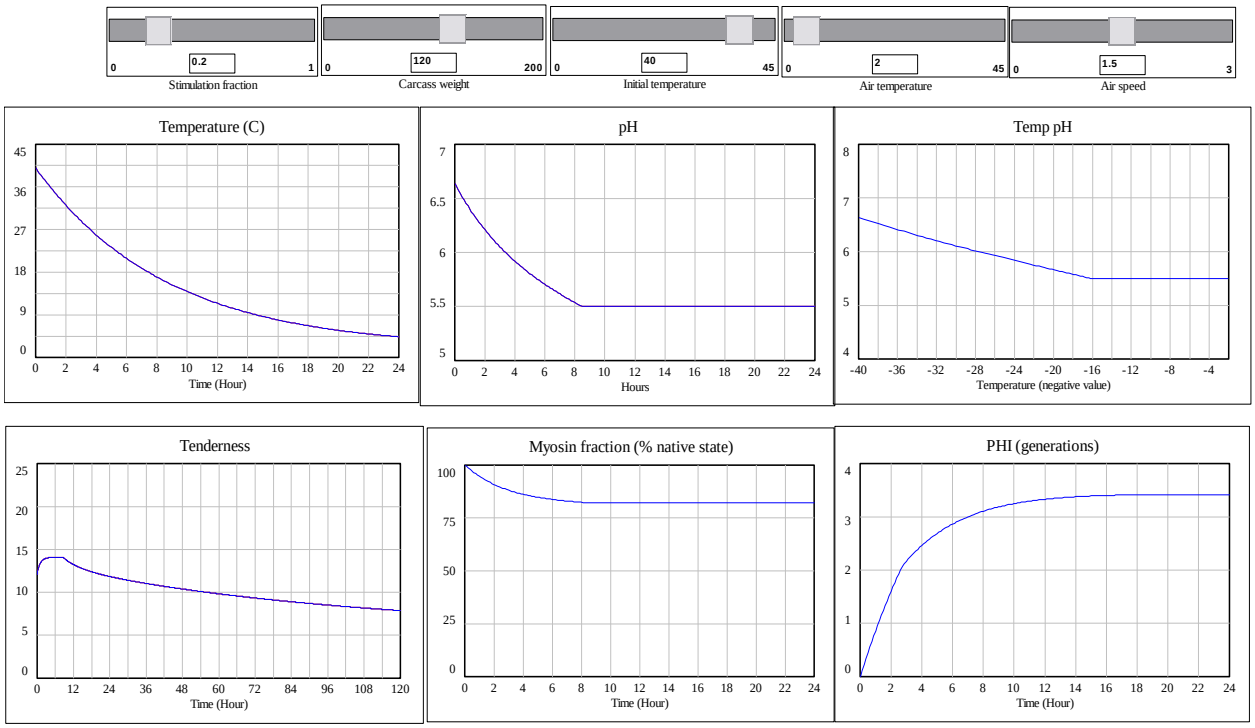


Diagram 1 – A screenshot of the Meat Quality Model showing input fields with modelled outcomes for a range of quality parameters.

This research evaluated two principal objectives: the first will be to continue the development of the pre-rigor model to accommodate the requirements of cold boning; the second developed the electrical stimulation component to meet the requirements of the Computer Process Management System (CPMS). Wherever possible, other projects within the MQST program contributed data to develop the models and were used to validate aspects of the model during development.

Substantive data and ongoing validation is required in order to allow the model to be used with any reliable, predictive capacity.

Contents

1 Background

The concept of the Central Processing Management System (CPMS) that underpins the MQST programme depends on quantifying the interactions of processing variables so that meat quality outcomes can be predicted under a range of processing conditions. The core processing conditions that need to be managed are the rate of change of pH and temperature, and how these interact to affect quality will differ according to which muscle or species of meat animal is being considered. The meat quality attributes that need to be predicted by the model are tenderisation, colour and colour stability and water binding capacity.

Two mathematical models have previously been developed at AgResearch: a procedure to calculate the rate of tenderisation under different chilling regimes for electrically stimulated lambs (tenderness model), and a more ambitious pre-rigor model for hot boned meat that calculates the effects of temperature on the rate of pH decline and consequent tenderisation and protein denaturation (Meat Quality Model; MQM). The parameters for the MQM were derived largely from the scientific literature or from experimental data. This model still needs further validation and refinement.

The pre-rigor model was developed for hot boning because hot boning simplifies many of the predictive requirements. Predicting variable temperature gradients within a carcass, or within large muscles in a carcass, could be ignored, as could the need to accommodate differences in the skeletal restraints that either limit the extent of shortening in some muscles, or actually cause stretching. These variables still need to be quantified to produce a pre-rigor model for cold boning.

A second component of the CPMS modelling requirements is to account for the effects of electrical inputs to a carcass. The electrical parameters of these inputs will vary depending on the processing objectives (stunning, immobilisation, rigidity), as will their timing relative to slaughter.

This project will undertake two principal objectives: the first will be to continue the development of the pre-rigor model to accommodate the requirements of cold boning; the second will develop the electrical stimulation component to meet the requirements of the CPMS. Wherever possible, other projects within the MQST programme will contribute the data to develop the models and will be used to validate aspects of the model as they are developed.

A critical requirement of the CPMS concept is the ability to predict the effects of processing conditions – in particular, rate of chilling and rate of pH decline - on meat quality attributes. An effective predictive model would allow processing conditions to be modified in response to market demands or, with on-line measurement of carcass traits, in response to the needs of each individual carcass. The main meat quality attributes that need to be predicted are tenderness, colour and colour stability, and water binding capacity.

A preliminary model to describe surface events responsible for meat colour has been developed in previous research. In the current research, this colour model will be uploaded in to the current model in order to understand the basis of meat colour and identify on farm and processing interventions that will allow better control of appearance and shelf life stability.

2 Project Objectives

The objectives of the research were :

- Incorporate a 'heat shortening component' into the meat quality model.
- Complete and validate the meat colour stability component of the model.
- Report on further validation of the meat quality model using data from on-going MQST experiments.
- Modify and incorporate the mechanistic muscle contraction model from the Biophysical Modelling programme into the meat quality model; evaluate the opportunities to upgrade the analysis of the Smart Simulation system.

3 Materials & Methods

3.1 Incorporate a 'heat shortening component' into the meat quality model.
A 'heat shortening component' will be developed and incorporated into the meat quality model by developing tenderness kinetics of heat shortened meat, and relating these data with a texture model.

3.2 Complete and validate the meat colour stability component of the model.

The validation of the colour model will require a suitable method to quantify metmyoglobin accumulation from the gradient images. Alternative software packages with the necessary features to achieve this are currently being evaluated. Once the procedure has been fully developed, validation will be completed. The aim is to ensure this is complete by the time the model is demonstrated to the NZ and Australian meat industry representatives.

3.3 Further validation of the meat quality model using data from on-going MQST experiments

As part of this on-going validation process, we have used data derived from the main experimental procedures used in the MQST during the current year. This experiment was to develop the calpain and substrate susceptibility assay and, for this purpose, the M. longissimus dorsi were hot boned and maintained at 15°C during the pre-rigor and subsequent ageing period. In addition to the shear force measurements required for the calpain assays, a number of additional measurements directed towards generating data for validating the model were undertaken. Also, where circumstances permitted, a second pre-rigor temperature treatment of 40°C, was added to the experiment to introduce an additional pre-rigor scenario.

The additional measurements included in the calpain experiments were:

1. rate of pH decline
2. water binding capacity (filter press method).
3. colour prediction

A total of 104 muscles were analysed at 15°C, of which 60 were also subjected to the 40°C pre-rigor temperature treatment.

3.4 Develop a calcium flux model to explain variations in contractile responses during the smart stimulation procedures; identify opportunities to upgrade the Smart Stimulation protocol.

The next stage was to determine how successfully the model could simulate real contraction data. To do this, the data collected in the CSIRO HSP experiment were used. Each carcass was dressed immediately following stunning by captive bolt and exsanguination, and without any previous electrical inputs. Each carcass was stimulated with 10 msec, 1A pulses; they initially received 3 isolated pulses at 1 second intervals, to obtain an individual twitch response, then a 15 second stimulation period of 15 Hz, which was then followed by 3 pulses each at 90 msec, 120 msec, 150 msec and 180 msec intervals. These increasingly extended pulse intervals allowed the calcium reuptake and crossbridge relaxation rates to be observed.

Very different contractile characteristics were recorded between carcasses, and this report will concentrate on two examples (carcass number 6 and 13) to evaluate the model performance. The objective was to define a set of parameters that could successfully model the twitch, tetanus and subsequent relaxation behaviours.

Followjng a literature search, the decision was made to base the model on the outline described by Riener & Quintern (Reiner R & Quintern J: A physiologically based model of muscle activation verified by electrical stimulation. Bioelectrochemistry and Bioenergetics 43 (1997) 257-264.) Some attractive attributes of this model are the ability to calculate fast and slow fibre activity independently, independent recruitment of fibres, based on physical fibre attributes, and a component for calculating fatigue. At this stage, two key simplifying assumptions are that the muscle is essentially isometric (to avoid the complication of varying force generation in response to sarcomere length and contraction velocity) and membrane effects resulting for stimulation at high frequencies (membrane fatigue, as described in earlier milestones). This last aspect can be included relatively easily and will be needed for stimulations rates much above 15 Hz, but the former would require a substantial increase in complexity and computational demand.

4 Results

4.1 Incorporate a ‘heat shortening component’ into the meat quality model.

4.1.1 Meat Quality Model – to date

The denaturation module of the Meat Quality Model (MQM) has, to date, concentrated on the denaturation of myosin, as measured by the kinetics of changes in myofibrillar density. The extent of denaturation is defined by temperature, pH and time (kinetics of the denaturation process), and these variables can be used to describe the measured changes in density. At rigor mortis, the myosin is stabilised by binding to actin, so denaturation (at least of the myosin) is assumed to cease at this point.

Myosin denaturation will have a significant impact on the water binding capacity of meat and on some key textural attributes that affect eating quality, and the modelling of these effects has been incorporated into the model. Myosin denaturation essentially ceases at rigor mortis and is also analogous to the pre-rigor muscle shortening events associated with post mortem temperature and is therefore sometimes known as ‘heat shortening’. The effects of cold shortening are already described in the model, but the events associated with so-called heat shortening are not.
Without doubt, muscle tissue will contract with exponentially increasing severity as the temperature increases. This behaviour is quite distinct form cold shortening, where the extent of contraction increases with decreasing temperatures. The distinction between the two shortening phenomena is when they occur: cold shortening depends on the presence of calcium and ATP,

and only occurs at near physiological pH values (the phenomenon ceases at pH 6 or below); whereas, heat shortening occurs as the muscle enters into rigor mortis, is calcium independent, and depends on conditions where ATP is nearly absent.

There is ample evidence that cold shortening causes meat to toughen (above the normal at-rigor values) in proportional to the extent of shortening. Although heat can cause muscle to shorten as much as cold, the at-rigor toughness is not increased relative to controls (where carcasses or cuts have been maintained at temperatures of less than 30 deg and greater than 12 deg), but is instead more tender. Where the effect of heat is most evident is in the ultimate tenderness (after ageing), where the meat fails to reach the same levels of tenderness compared with controls.

The implication of these results is that the sarcomeric shortening caused by elevated temperatures does not influence tenderness, but rather that these conditions influence the activity and survival of the calpain enzymes responsible for tenderness. It is recognised that the calpains will autolyse and therefore total activity is reduced. The simplest description of the events leading to increased ultimate toughness is based on the different temperature-dependent kinetics of proteolysis relative to autolysis: high temperatures accelerate the rate of proteolysis, but high temperatures also accelerate still more the rate of autolysis of the calpain enzymes. Hence, the total extent of proteolysis in high temperature meat is reduced by high temperature conditions.

4.1.2 Model of Calpain Autolysis

A temperature dependency of the rate of calpain autolysis has now been introduced into the model. Autolysis was modelled to begin at pH 6 and, for the sake of simplicity, made to be pH independent. Using the existing database on high temperature processing trials and subsequent meat tenderisation kinetics, a data fitting function in the modelling package, Vensim, was used to optimise the temperature dependency of the rate of calpain autolysis. The outcome of the model, expressed as tenderness values after 24 hours, 7 and 14 days of ageing across the cold- to heat-shortened spectrum, is shown in Figure 1 (a-c).

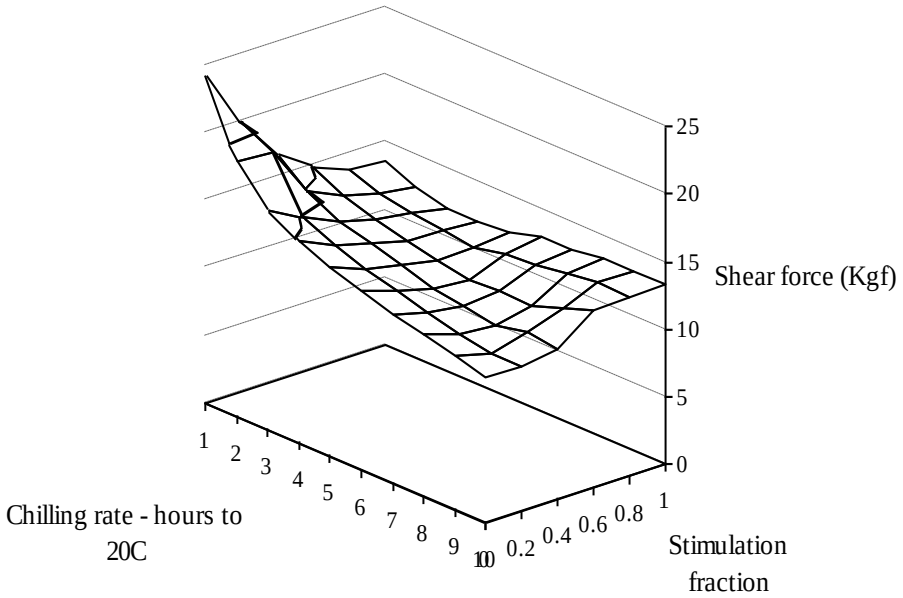


Figure 1a – Modelling of shear force of beef product across the chilling rate and stimulation continuum at 24 hour tenderness.

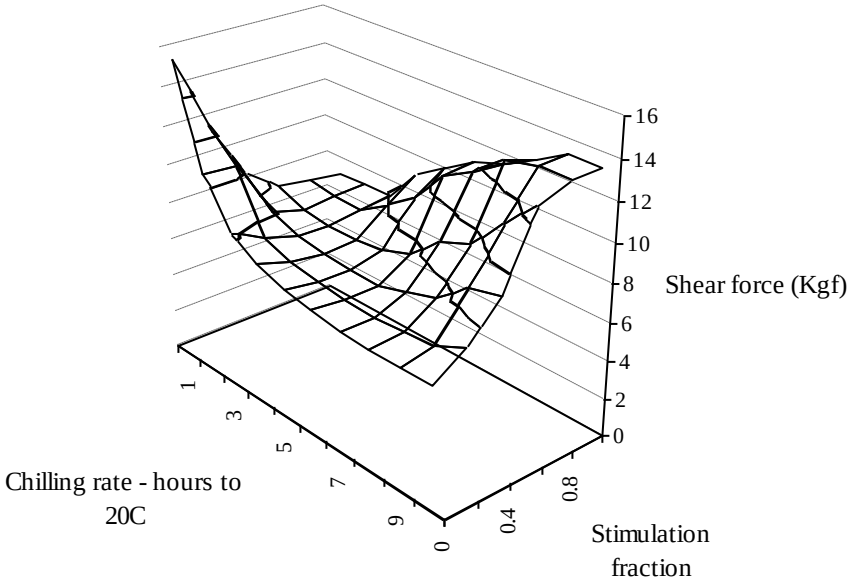


Figure 1b – Modelling of shear force of beef product across the chilling rate and stimulation continuum at 7 days ageing.

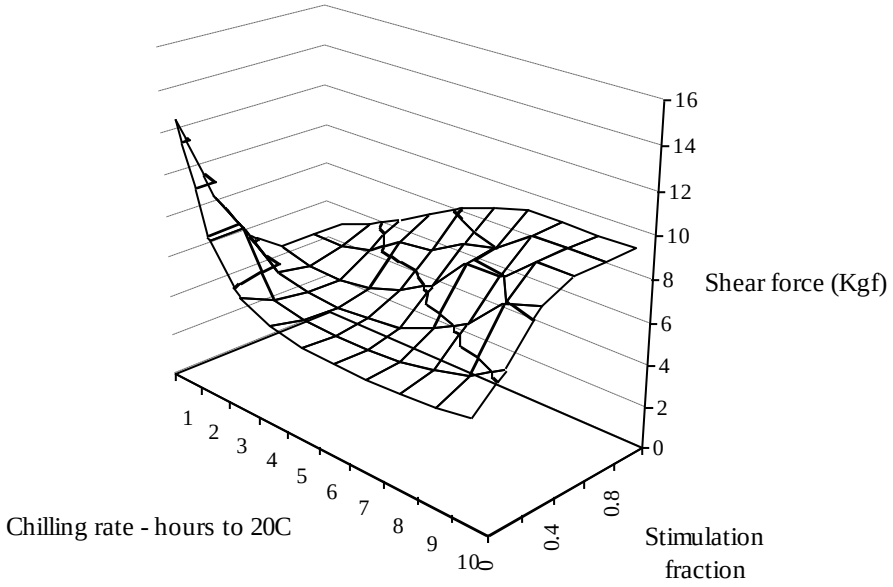


Figure 1c – Modelling of shear force of beef product across the chilling rate and stimulation continuum at 14 days ageing.

4.2 Complete and validate the meat colour stability component of the model.

A new component has been developed and added to the colour model. The key additions have been to include NADH as a substrate to the oxygen consumption rate (OCR) and reductase reactions, and introduce an NADH regenerating reaction. Fitting this model to experimental data is based on optimising the parameter for the equilibrium constant for the dehydrogenase reaction to identify the NADH concentration driving the OCR and MMB reductase reactions.

The validation of the colour model is underway although a suitable method to quantify metmyoglobin accumulation from the gradient images is required to complete this exercise (Diagram 2). Alternative software packages with the necessary features to achieve this are currently being evaluated. Once the procedure has been fully developed, validation will be completed.

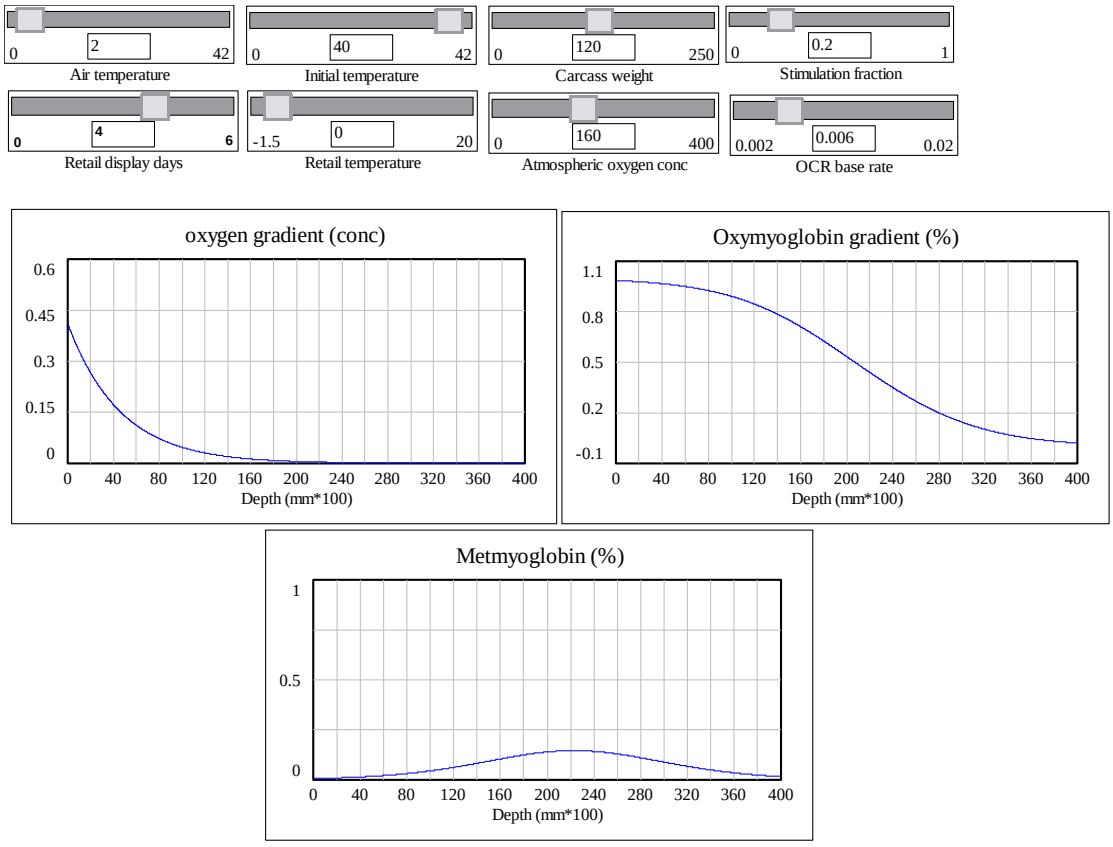


Diagram 2 – A screenshot of the Meat Quality Model showing input fields with modelled outcomes for a range of colour parameters.

3.3 Further validation of the meat quality model using data from on-going MQST experiments

3.3.1 Pre-rigor pH decline.

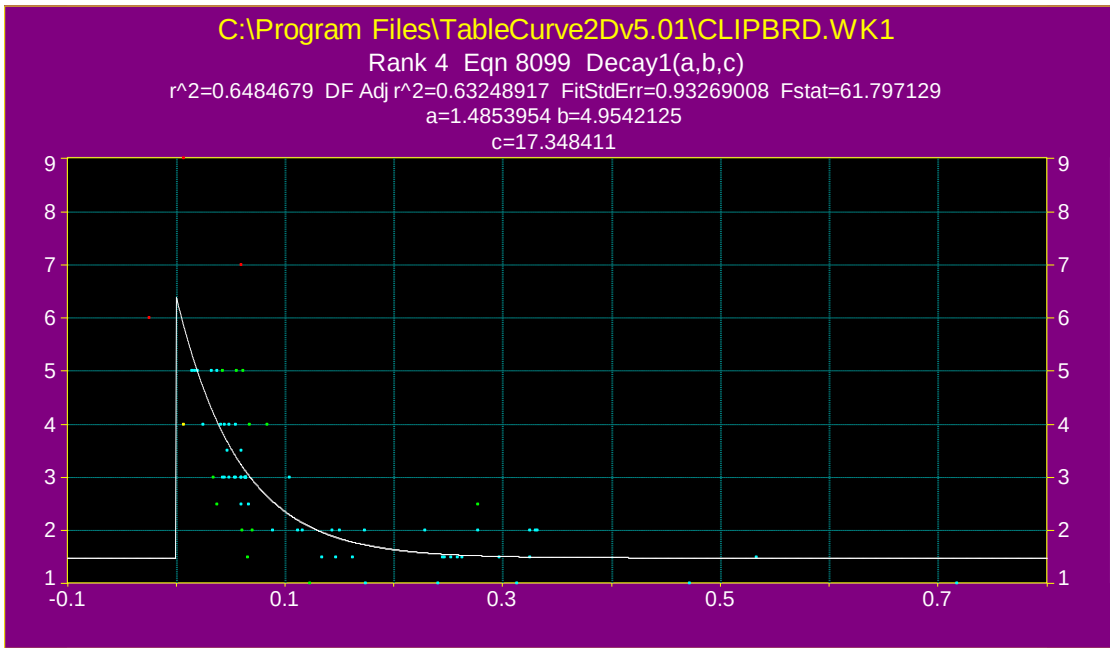
The average rate of pH decline at 15°C in these experiments was 0.167 pH units / hour, although the standard deviation was large, at 0.08. Increasing the temperature to 40°C increased the rate of pH decline to 0.436. These figures compare favourably with those currently used to calculate the temperature dependency of pH decline: (0.143 and 0.49).

3.3.2 Water binding capacity

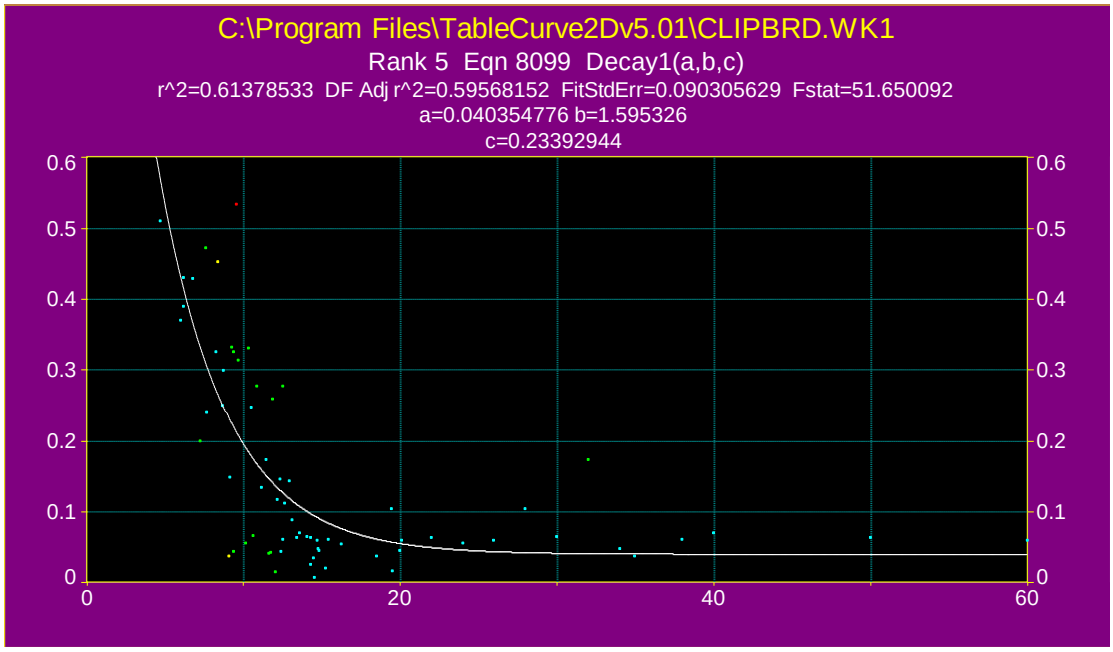
Water binding capacity (WBC) is typically measured either by the filter paper press method or by centrifugation. In both cases, the loosely bound water is extracted and measured. The model is based on the premise that water binding capacity is a function of denaturation of either the myofibrils (specifically myosin) or sarcomeric proteins. We have developed two novel assays to measure these directly: myofibrillar density (MD assay) measurements to determine lattice shrinkage caused by myosin denaturation; and the MTT assay, based on using the ability of the tissue to reduce tetrazolium salt (MTT assay), providing an index of reductive enzyme activity.

As expected, high pre-rigor temperatures depressed MTT reducing capacity (0.247 vs 0.051; P<0.001) and increased MD (1.59 vs 3.86; p<0.005). In contrast to previous experiments, the myofibrils from the high temperature treatment group were frequently dispersed through the density gradient, rather than forming a defied band at a single density point. The reasons for this are not clear at this stage, but we suspect that the lower pH of the samples at collection (in some cases as low as 6.2) means that the extent of denaturation of the myofibrils was more variable between samples. Some further work will be needed to clarify, and perhaps exploit, this observation but, until then, the density results need to be interpreted with caution.

The MTT and MF results were significantly correlated across all treatment samples and could be adequately described by an exponential fit.



The MD measurements did not provide an effective predictor of WBC in this experiment, in contrast to earlier experiments, probably because of the difficulties in identifying the true level of denaturation from the dispersed samples. The MTT results did, however, provide a useful prediction of WBC.

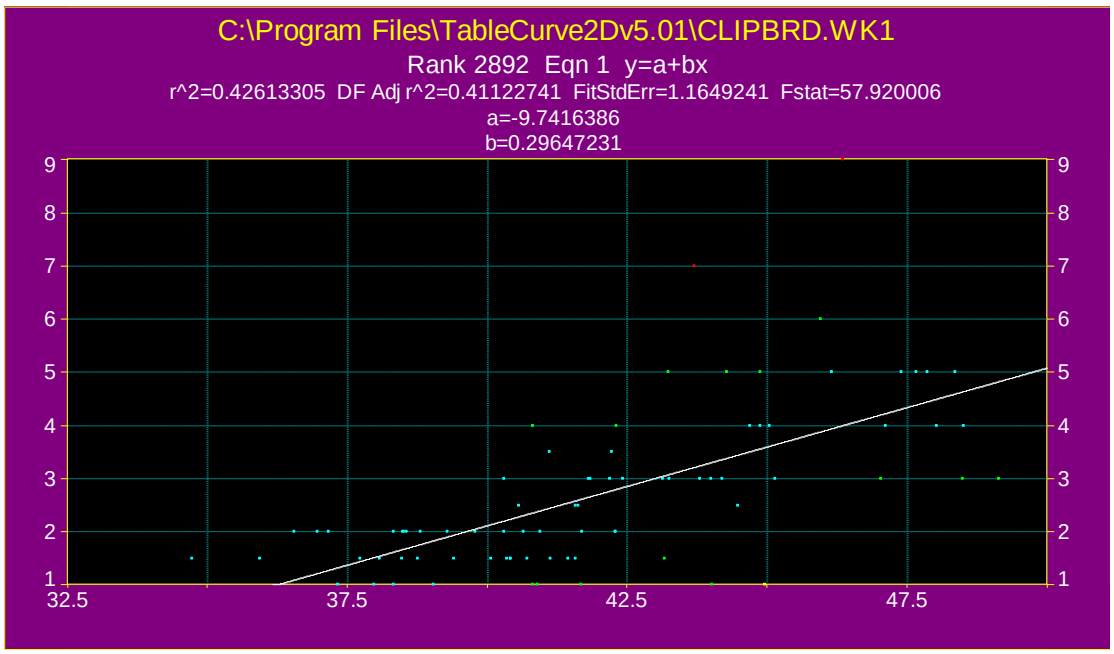


These results complement a more limited dataset produced during the development of the WBC module. Some further work that supports the role of MD and the potential benefit of combining both measures/predictions to predict WBC will be undertaken in the next set of milestones.

3.3.3 Predicting colour

Twenty four hour colour prediction, as it is affected by processing conditions, can have important commercial implications because of the use of colour as a grading attribute. Twenty four hour colour is a function primarily of oxygen consumption rate (OCR), which defines the depth of the oxymyoglobin gradient, and of the extent of myofibrillar shrinkage,which defines the amount of reflection. The MTT assay was specifically developed as a predictor of OCR, while the MD assay reflects the myofibrillar shrinkage.

As described earlier, the dispersed densities in the MD assay has limited its use in the interpretation of this dataset. However, notwithstanding this, a linear relationship between L* and MD was evident.



Similarly, the MTT assay was a reasonable predictor of bloomed meat colour (hue). The higher the MTT value, the more the colour transitions from the red (around a hue angle of 27) towards blue as a result of increasing OCR and increasing translucency (increasing MD)

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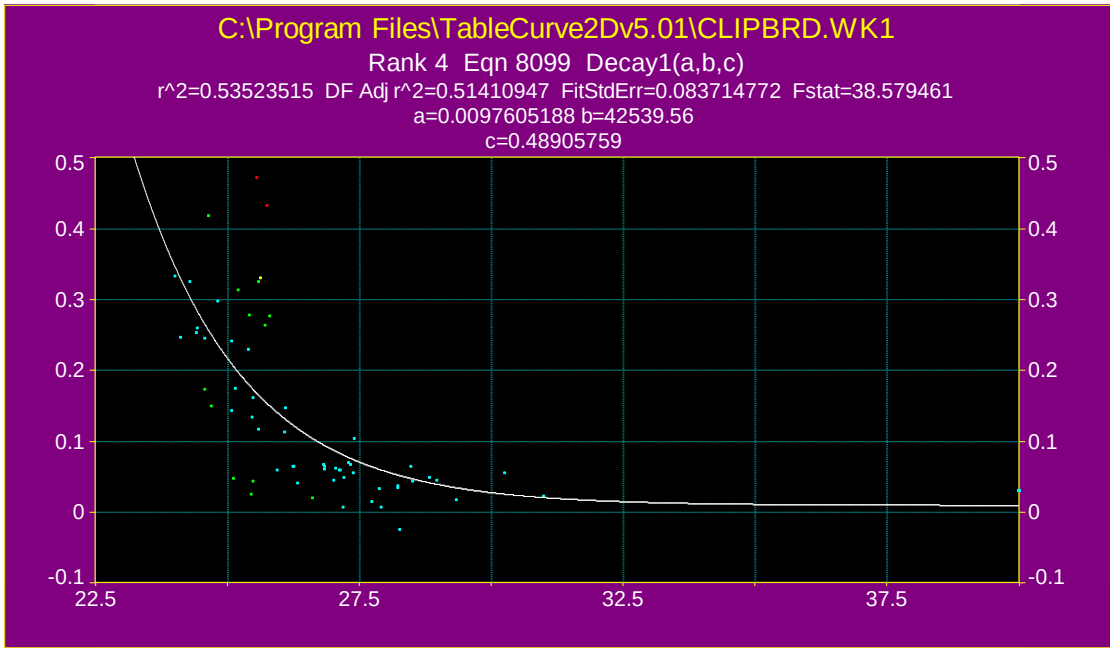


Ultimately, colour will need to be expressed as a combination of both the MD and MTT assays. At this stage, a multiple regressions shows little benefit in combining the two assays, but a better MD dataset could improve this prediction.

4.4 Develop a calcium flux model to explain variations in contractile responses during the smart stimulation procedures; identify opportunities to upgrade the Smart Stimulation protocol.

Earlier MQST milestones identified that the contractile characteristics of muscles can provide important information about the status of a muscle or carcass. This is used as part of the Smart Stimulation technology, where the whole carcass response is used to measure pH and to predict subsequent pH decline and tenderness development. Although a whole carcass response is the sum of the contraction of many muscles, the M. longissimus dorsi (LD) was found to be the dominant contributor.

A more muscle specific technique, based on measuring intramuscular pressure as an index of force of contraction, has also been used extensively to understand the interactions between muscle responses and stimulation parameters. As part oft his work, it was again identified that tenderness characteristics could be predicted from response characteristics. This was most notably recognised in a collaborative study with Drewe Ferguson, CSIRO, as part of a study to measure the heat shock protein response to cortisol infusion. Here, crude measures of the relaxation characteristics of the LD produced a correlation with initial tenderness of r^2 =0.65.

Although these results are highly encouraging, the muscle responses could only be considered a 'black box' in the absence of any mechanistic interpretation of the contractile behaviours. Relaxation and contraction characteristics are largely defined by the intracellular calcium control, as well as the sensitivity of the contractile elements to calcium, and a variety of mathematical models have been described in the physiology literature to simulate the function of muscles under different stimulation protocols. Such models would provide an ideal mechanism for interpreting the response characteristics measured using the intramuscular pressure procedures, and provide some indications of the underlying physiological events that may contribute to the tenderness predictions.

The most obvious differences between the responses of the two carcasses are seen in the rate of tetanised force development. The force equilibrated at its peak force in 6 sec in carcass 6 but about 1.5 sec in carcass 13. Also, carcass 6 showed a proportionally greater degree of relaxation during the test pulse sequence following the tetanus compared with carcass 13. In contrast, the behaviour of the twitch responses did not differ to any obvious extent between the carcasses. In part, this may be to the presence of more movement artefact associated with a single carcass contraction, which made the responses more difficult to isolate. In addition, the more extreme contractile conditions associated with a tetanised state may be needed to reveal underlying functional differences.

The results of a single set of parameters to fit the contractile behaviours of carcass 6 is shown in Figure 2.

Figures 3 & 4 show the tetanus and test pulse responses for carcass 13.

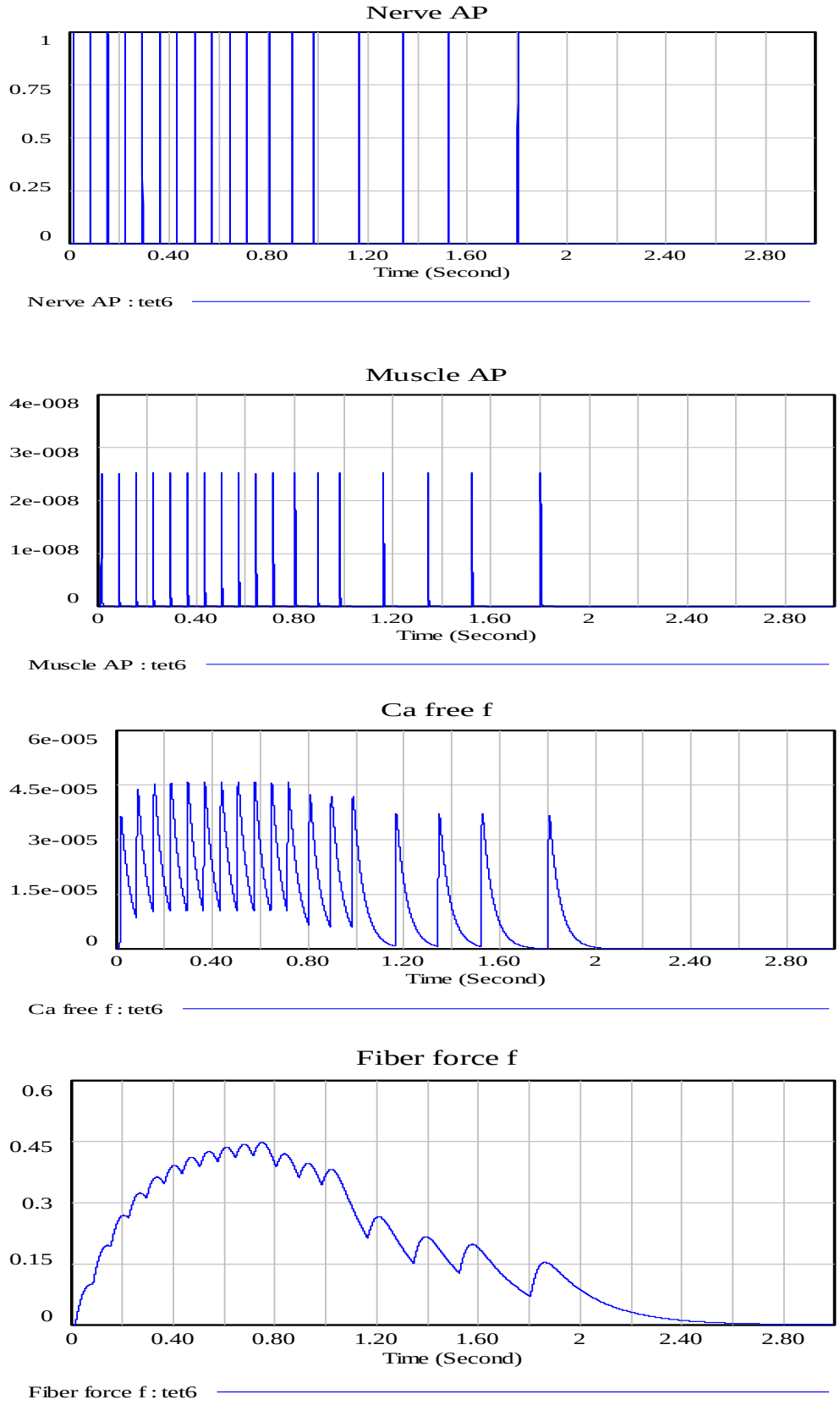
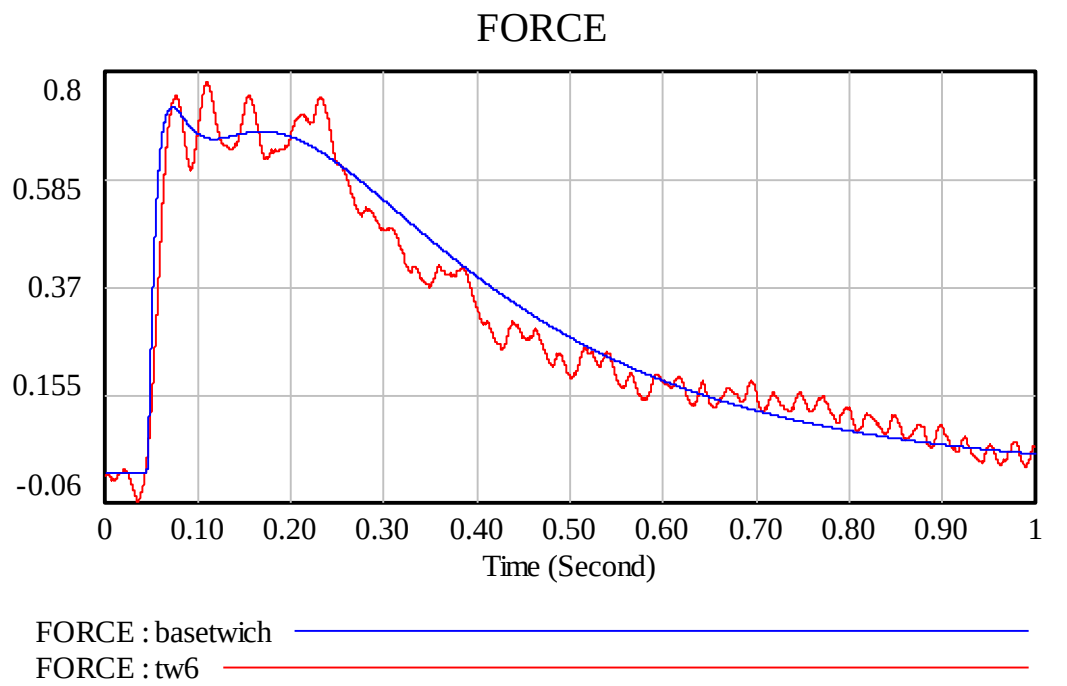


Figure 2: Main steps in the contraction model.

A: Twitch (measured data in red, predicted data in blue)



B: 15Hz tetanus followed by test pulses (measured data in blue, predicted data in red)

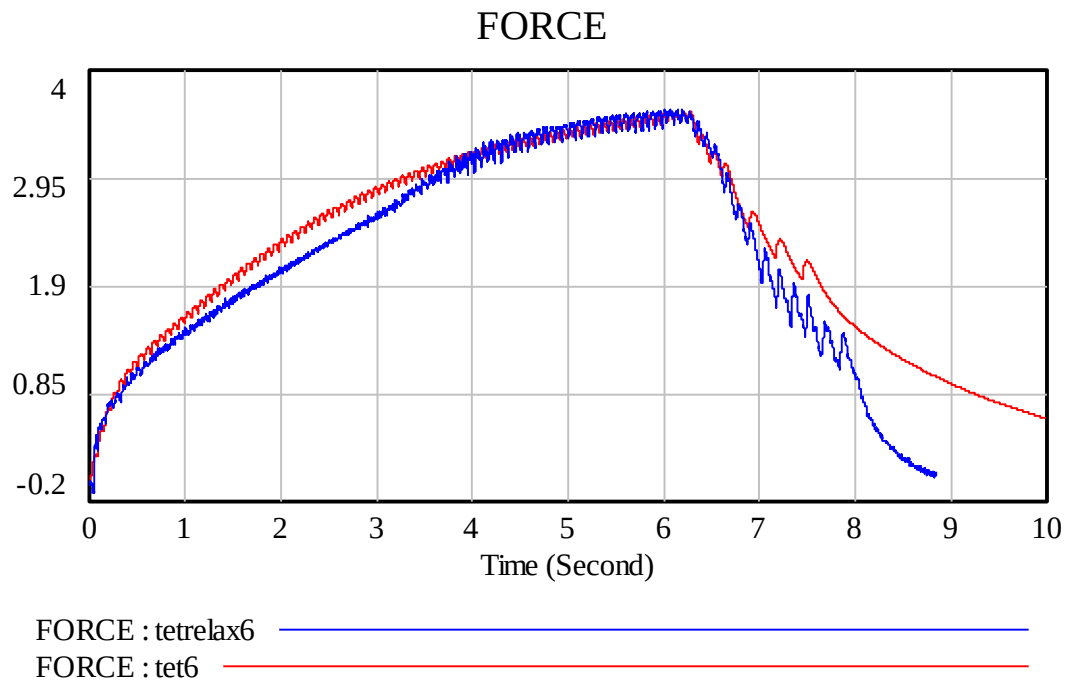


Figure 3A-B: Modelled force responses for a twitch, and the combined tetanus and test pulses for carcass 6.

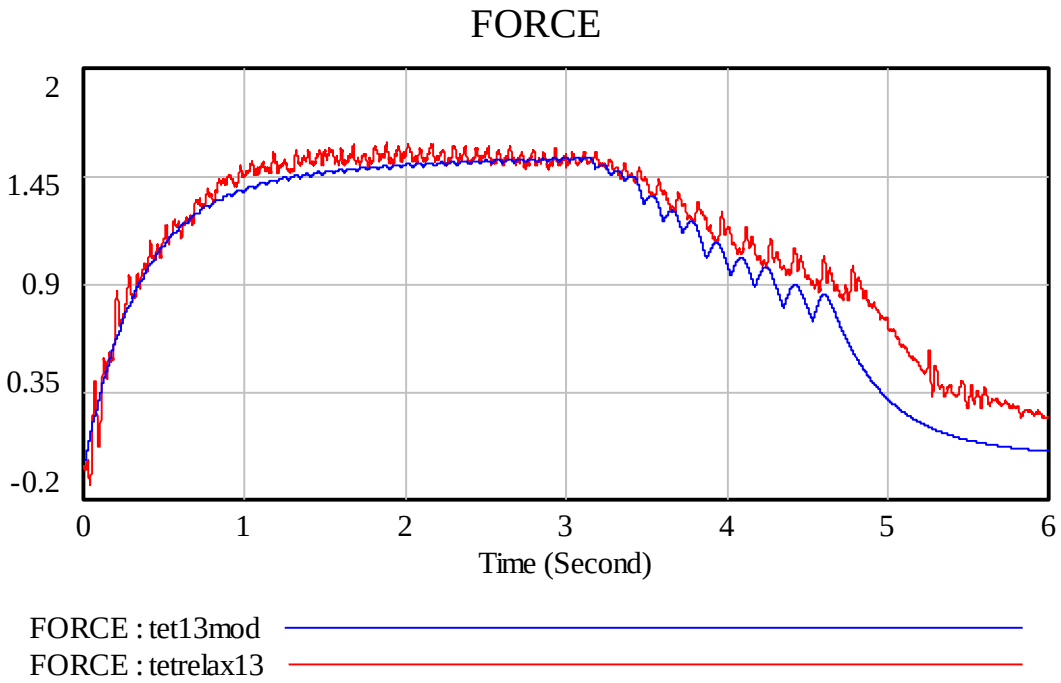
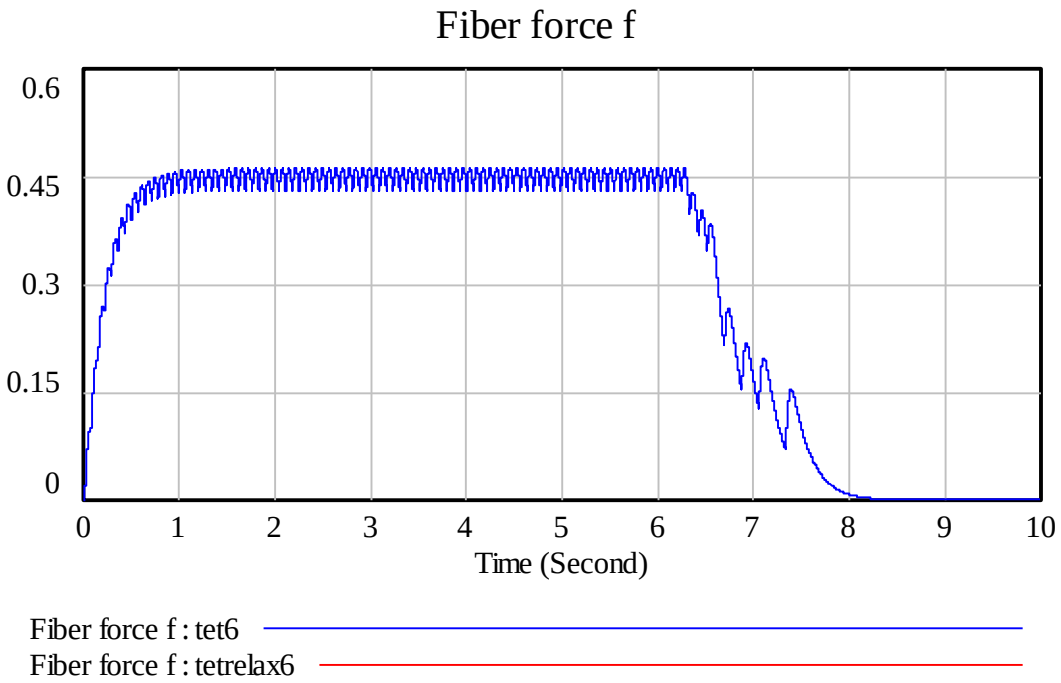


Figure 4: Tetanus and test pulse responses of carcass 13 (measured data in red, predicted data in blue).

In order to simplify the data fitting process, the period of the 15 Hz stimulation was shortened at the point where a force equilibrium became established (6 seconds for carcass 6 and 3 seconds for carcass 13) and the subsequent test pulses were concatenated to the end of the tetanus responses. Some difficulties in the model performance were experienced in simulating the sequence of test pulses, so the traces do not correspond exactly during this phase. Nevertheless, it is evident that an acceptable degree of correspondence was possible between the measured and simulated contractions, although the relaxation rate during the test pulses and following the end of the stimulation showed some divergence, slower in carcass six and faster in carcass 13.

Two separate set of response characteristics, nominally identified as fast twitch and slow twitch contractile behaviour, are needed to reproduce the full behaviour of the muscle, and the individual contractile characteristics of each fiber type are shown in Figures 5 and 6.

A: Carcass 6



B: Carcass 13

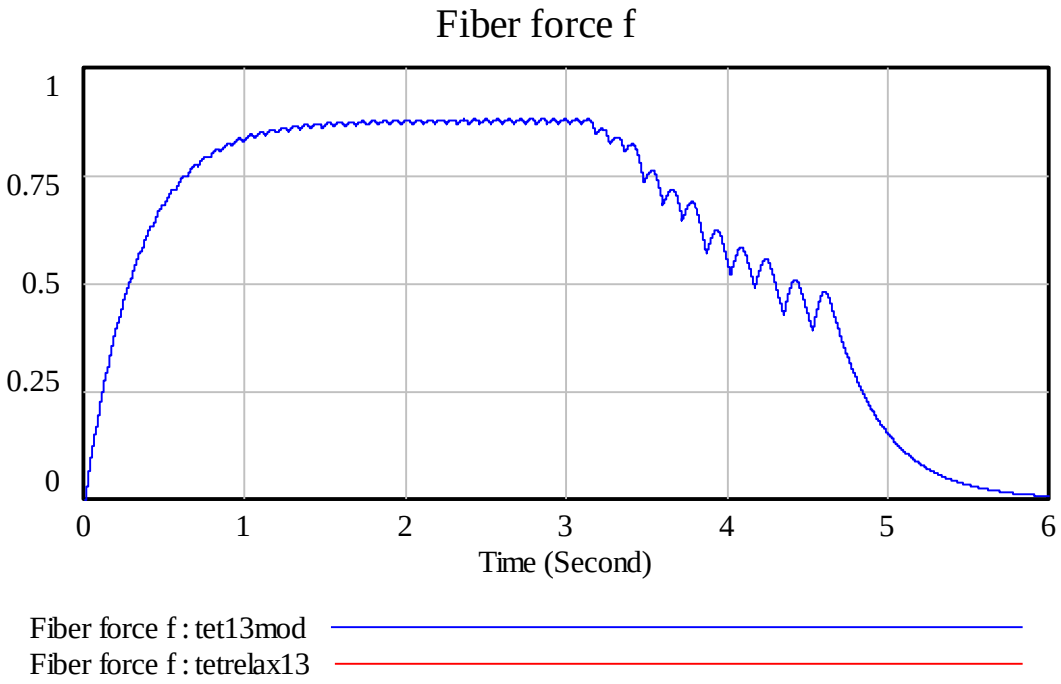
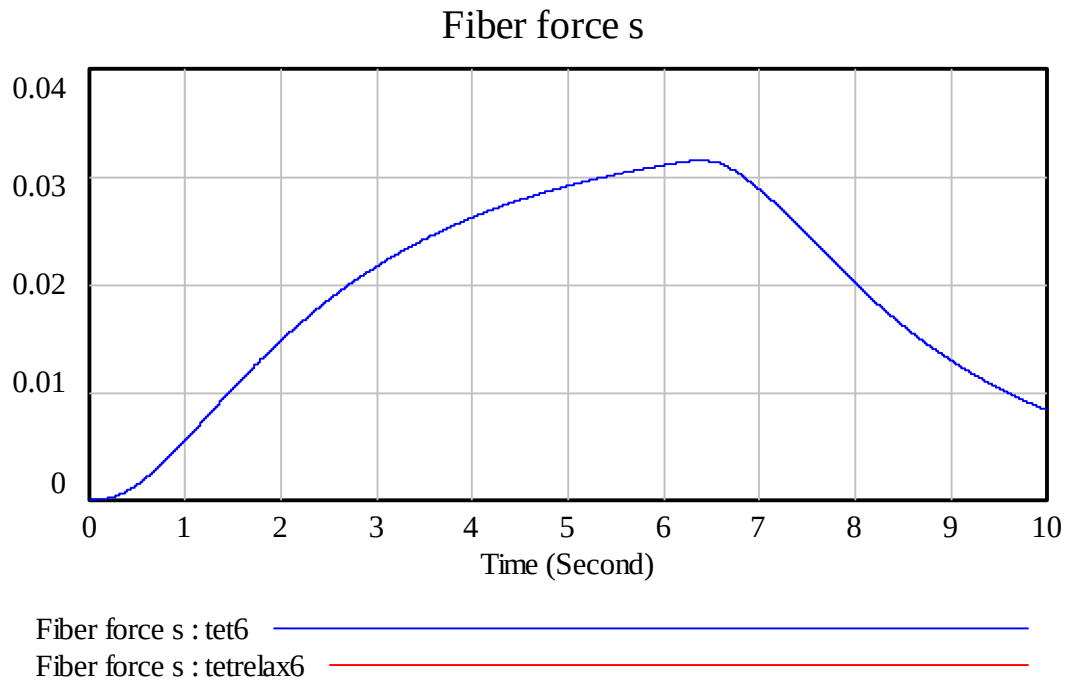


Figure 5A-B: Modelled fast twitch force generations for carcasses 6 and 13.

A: Carcass 6



B: Carcass 13

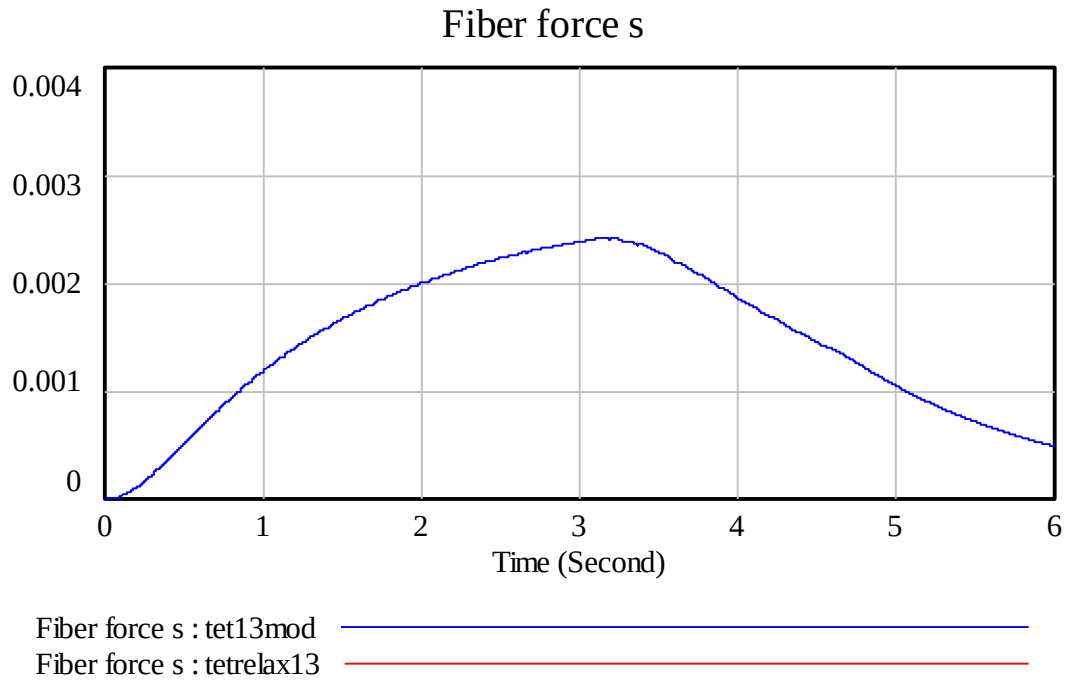


Figure 6A-B: Modelled slow twitch force generation for carcasses 6 and 13.

The contraction model would appear to show significant potential as a mechanism for understanding the contractile behaviour of post mortem muscles. However, the existing set of contraction data is not specifically tailored for this purpose, and permits more than a single solution in fitting parameters. For this report, 3 parameters describing the calcium flux behaviour and 2 for the fiber contraction behaviour were used, as well as a parameter describing the relative proportion of fast versus slow fibers. However, appropriate experimental conditions would help to resolve this difficulty, by making use of varying fibre type characteristics of different muscles, the different stimulation thresholds and rates of fatigue development. The objective would be to define a stimulation protocol that would provide the necessary controls to produce an effective fit of the key contraction parameters, and use the model to identify the unique structural and biochemical characteristics of a post mortem muscle.

5 Conclusion

A critical requirement of the CPMS concept is the ability to predict the effects of processing conditions – in particular, rate of chilling and rate of pH decline - on meat quality attributes. An effective predictive model would allow processing conditions to be modified in response to market demands or, with on-line measurement of carcass traits, in response to the needs of each individual carcass. The main meat quality attributes that need to be predicted are tenderness, colour and colour stability, and water binding capacity. These elements were developed in the current research.

Substantive data and ongoing validation is required in order to allow the model to be used with any reliable, predictive capacity.

6 Recommendations

The mechanisms by which processing defines meat quality are becoming well developed and can be measured though a range of new techniques that have been developed in this programme. As a spin-off , the transference of this understanding to industry via the optimisation of traditional processing technologies plus the introduction of the new generation of processing opportunities are underway using the Meat Quality Model as a platform.

However, despite processing refinements and controls, variable animal responses to processing conditions remain obvious and can be viewed as either a problem (variable product characteristics) or an opportunity. The opportunities are to identify the natural variation in the properties of New Zealand & Australian livestock as expressed under identical and controlled processing conditions.

The proposed objectives for the next phase of research are to use the principles and techniques to control the (otherwise dominant) processing conditions to define the natural variation in meat quality attributes. These objectives will be used to assess the ‘robustness’ of the techniques that have been developed, develop a database to continue the process of validation the MQM, and to identify production systems and marketing opportunities to exploit differing animal characteristics.

It is proposed the next phase of R&D will include using contrasting commercial processing conditions and identified producers, validate the MQM and use the model to define the extent of the ‘animal’ contribution to product attributes.