APPLYING A NOVEL METHOD FOR THE ANALYSIS OF BEEF ULTIMATE pH IN THE DETECTION OF QUANTITATIVE TRAIT LOCI

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ABSTRACT

Beef ultimate pH (pH_U) is an economically important trait related to meat quality. Values of pH_U higher than the normal 5.5 have a detrimental effect on tenderness, colour and keeping quality. The amount of lactic acid that is produced by the conversion of the glycogen stored in the muscle at time of slaughter (G_0) determines pH_U.

A novel biochemically-based approach for pH_U analysis was evaluated in the detection of quantitative trait loci (QTL) affecting this characteristic. The procedure proposed by Pleasants *et al.* (1999) transforms pH_U to the underlying glycogen generating a new variable, named predicted glycogen (PG_0). This model may overcome the limitations in pH_U investigations derived from its typical skewed distribution, characterised by a peaked primary mode at 5.5 and a long tail that comprise high pH_U values. In addition to PG_0, G_0, pH_U and the logarithmic transformation of pH_U (LpH_U) were analysed in: a simulated back-cross involving two inbred lines based on a model including a QTL and polygenic effects influencing G_0 and thus pH_U; and in experimental data from a reciprocal back-cross between Jersey and Limousin implemented by AgResearch.

The significance levels achieved by LpH_U did not differ from pH_U, indicating that there was no advantage of using this transformation. Evidence of QTL was clearer for PG_0 than pH_U in the simulation. A better performance of PG_0 compared to pH_U was observed when there were more elevated pH_U values. Results from the experimental data did not confirm the superiority of PG_0 in QTL detection. With the exception of one value of 6.2, pH_U data obtained in the experiment were close to 5.5.

It is concluded that PG_0 may improve the significance in QTL searching compared to pH_U when pH_U include high values that lead to the typical skewed distribution. The new procedure can also be exploited in other investigations utilising pH_U. Additional research work involving the characterisation of G_0 and pH_U is recommended to re-evaluate the parameters assumed in the implementation of this innovative approach.

Key words: beef, ultimate pH, muscle glycogen, quantitative trait loci

Dedicated to Mariano Navajas
and Afranio Velosa-Guzman,
two precious souls

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CHAPTER ONE

INTRODUCTION

The quality of product, in addition to the quantity produced per unit input, affects the economic efficiency of any meat-producing system. In an ideal marketplace, the value of beef presented in a similar way is determined by aspects of meat quality differences, which will impact returns from the production systems (Purchas *et al.*, 1989). Although "taste", price and healthfulness were identified as the three primary motivators related to meat purchase and consumption, if beef fails to meet quality expectations, price and healthfulness were irrelevant (Chambers and Bowers, 1993).

Ultimate pH is a meat characteristic that is not directly evaluated by consumers but has a strong influence on some of the most relevant quality attributes: colour and tenderness. Meat colour is the first criterion used by consumers to judge meat quality and acceptability. High pH values (>6.0) lead to dark cutting or dark, firm and dry meat which is rejected by consumers because of its unacceptable colour (Abril *et al.*, 2001). On the other hand, intermediate pH beef (5.7-6.2) has been associated with reduced tenderness, which has been rated by consumers as the most important aspect of eating quality (Tarrant, 1998). High pH values also reduce the shelf life due to altered bacterial growth (Gill and Newton, 1981).

Meat pH is often used as a means of monitoring meat quality and a pH below a threshold of pH 5.8 is usually demanded for chilled beef markets (Wright *et al.*, 1994). The percentage of carcasses that fail this specification in New Zealand has been estimated to be about 10 to 30% (Graafhuis and Devine, 1994; Smith *et al.*, 1996). These percentages indicate that too many carcasses have variable and sub-optimal meat quality parameters. An evaluation of the economic impact of this problem in New Zealand suggests that a lower incidence of high pH leads to financial benefits by reducing costs and increasing the potential price of the product (Wright *et al.*, 1994).

Several studies have been carried out to understand factors contributing to high pH beef. However, the distorted distribution of ultimate pH has hindered this task. The frequency distribution of ultimate pH in slaughtered animals typically presents a peak

around pH of 5.5 and a long upper tail with a variable proportion of values up to 7.0-7.2.

Recently a biochemically-based approach to analyse beef pH has been developed. The gradual fall in muscle pH following slaughter, from approximately neutral values (7.0) to around 5.5, results from the post-mortem accumulation of lactic acid in the muscle that is produced by the conversion of glycogen. If muscle glycogen levels at slaughter are low, the supply of substrate for glycolysis is limited resulting in a lower concentration of lactic acid and a higher ultimate pH. Pleasants *et al.* (1999) proposed a mathematical model that includes knowledge of the biochemical pathways from glycogen to lactic acid in a manner suitable for statistical analysis. In this study, the new approach is applied in the detection of quantitative trait loci (QTL) for ultimate pH.

It is now known that a proportion of the variation in some economically important traits can be attributed to one or few major genes, known as QTL. Knowledge of the existence and chromosomal location of QTL can be exploited through breeding programs utilizing strategies that include molecular information (MAS, marker-assisted selection). MAS is especially appealing in the genetic improvement of meat quality traits as it would allow the evaluation of live breeding animals of both sexes at a young age and this may increase the genetic response compared to traditional methods of selection.

Different methods have been developed to identify QTL using linked markers (Haley and Knott, 1992; Knott *et al.*, 1996). However, the standard techniques have not specifically incorporated the information about the biological processes involved in the expression of a specific trait. The objective of this study is to evaluate the effect of applying the new approach in QTL detection in two situations: a simulated back-cross involving two inbred lines; and in experimental data that was collected in a project carried out by AgResearch and the University of Adelaide, using a double-back-cross between Jersey and Limousin. The variables to be analysed include muscle glycogen in the live animals, actual ultimate pH, logarithmic transformation of pH and glycogen predicted from the pH information based on the new methodology.

CHAPTER TWO

REVIEW OF LITERATURE REGARDING BEEF ULTIMATE pH

2.1 INTRODUCTION

The present review is focussed on beef ultimate pH. It covers main points such as the influence of ultimate pH on important meat quality traits as well as describing the biological process that determines pH and its relationship with glycogen levels immediately preceding slaughter. A comprehensive description of the biochemically-based approach for the analysis of ultimate pH that was proposed by Pleasants *et al.* (1999) is also presented.

2.2 ULTIMATE pH IN BEEF

2.2.1 Association between pH and meat quality

After slaughter, beef pH falls from approximately 7.0-7.2 to typically 5.5. Good quality beef should have an ultimate pH of about 5.5, while higher values are associated with a negative effect on beef quality attributes such as colour, shelf life and tenderness (Tarrant, 1989).

Beef colour is one relevant criterion used by consumers to evaluate meat quality and acceptability (Conforth, 1994). Beef that does not present bright attractive colour is perceived by consumers as unwholesome or to be from old animals and therefore less tender. Figure 2.1 shows the relationship between ultimate pH above 5.5 and lightness in beef from bulls and steers. Higher pH values lead to darker beef because superficial layers of the meat are more translucent, and therefore more light is absorbed and less is reflected. Furthermore, less oxymyoglobin, which is the bright red form of myoglobin, is formed (Purchas and Aungsupakorn, 1993; Abril *et al.*, 2001).

Shelf life is a second attribute that is negatively influenced by high ultimate pH. An early spoilage is observed in meat with pH higher than 6.0. It was attributed by Gill

and Newton (1981) to a lower content of lactic acid and glucose compared to normal meat.

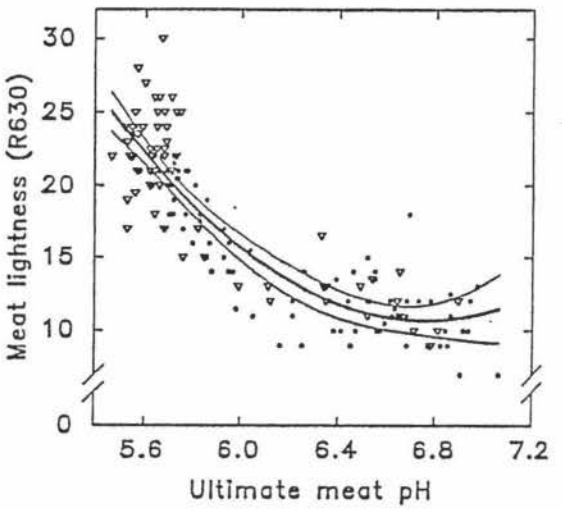


Figure 2.1: Changes in the lightness of meat, measured by the reflectance of light at 630nm, with increasing ultimate pH for samples of *longissimus* muscle from steers (open triangles) and bulls (solid dots).
Note: The cubic regression line with 99% confidence intervals is shown.
Source: Purchas and Aungsupakorn (1993)

Due to the poorer keeping quality and darker appearance of beef with high pH, a critical value of 5.8 or 6.0 is usually applied in the classification and grading systems. A maximum pH of 5.8 is one of the standards included in the Beef Quality Mark, introduced by Meat New Zealand (New Zealand Beef and Lamb Marketing Bureau Inc., 1997).

Meat tenderness is also related to post-mortem pH, although the relationship and its causes are less clear that in colour and keeping quality. Purchas and Aungsupakorn (1993) reported a curvilinear association between pH and tenderness (Figure 2.2). Although a greater tenderness is associated with pH values higher than 6.0, the dark colour makes the meat an unacceptable product to consumers. On the other hand, higher values of Warner-Bratzler force (lower tenderness) are found when meat presents intermediate levels of final pH (5.8 - 6.2).

Even though there is not a complete explanation to the lower tenderness at intermediate values of pH, it is partially explained by shorter sarcomere lengths in

beef (Purchas and Aungsupakorn, 1993) in addition to a reduced proteolytic activity at these pH values (Harper, 1999). However, Purchas and Yan (1997) found limited evidence to suggest that the greater toughness of intermediate-pH samples was due to a lower level of myofibrillar protein breakdown over a 20-day ageing period.

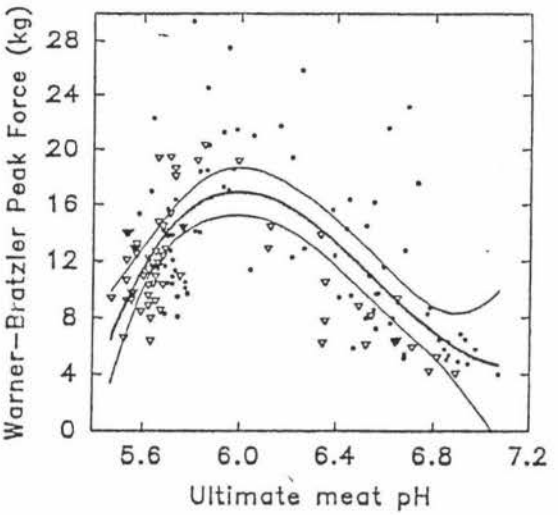


Figure 2.2: Cooked meat tenderness with increasing ultimate meat pH for samples of *longissimus* muscle from steers (open triangles) and bulls (solid dots).
Note: The cubic regression line with 99% confidence intervals is shown.
Source: Purchas and Aungsupakorn (1993)

2.2.2 Frequency distribution of ultimate pH

The ultimate pH distribution has been characterised in different beef producing countries. Figure 2.3 shows the frequency distributions of ultimate pH reported by Page *et al.* (2001) and Tarrant and Sherington (1980) based on information collected in commercial beef plants of USA and Ireland, respectively.

Although these results indicate that 70 to 80% of the slaughtered animals showed "normal" pH values between 5.4 and 5.6, there are 2.4% and 3.2% carcasses with pH above the critical point of 5.8. However, the average proportion of dark cutting in both cases is lower than that reported for other countries. The incidence of dark-cutting beef informed in different European countries during the 80's ranged, by categories, from 3.6 to 26%, 5.9 to 13%, and 2.4 to 3.7% for bulls, cows, and steers, respectively (Warris, 1990).

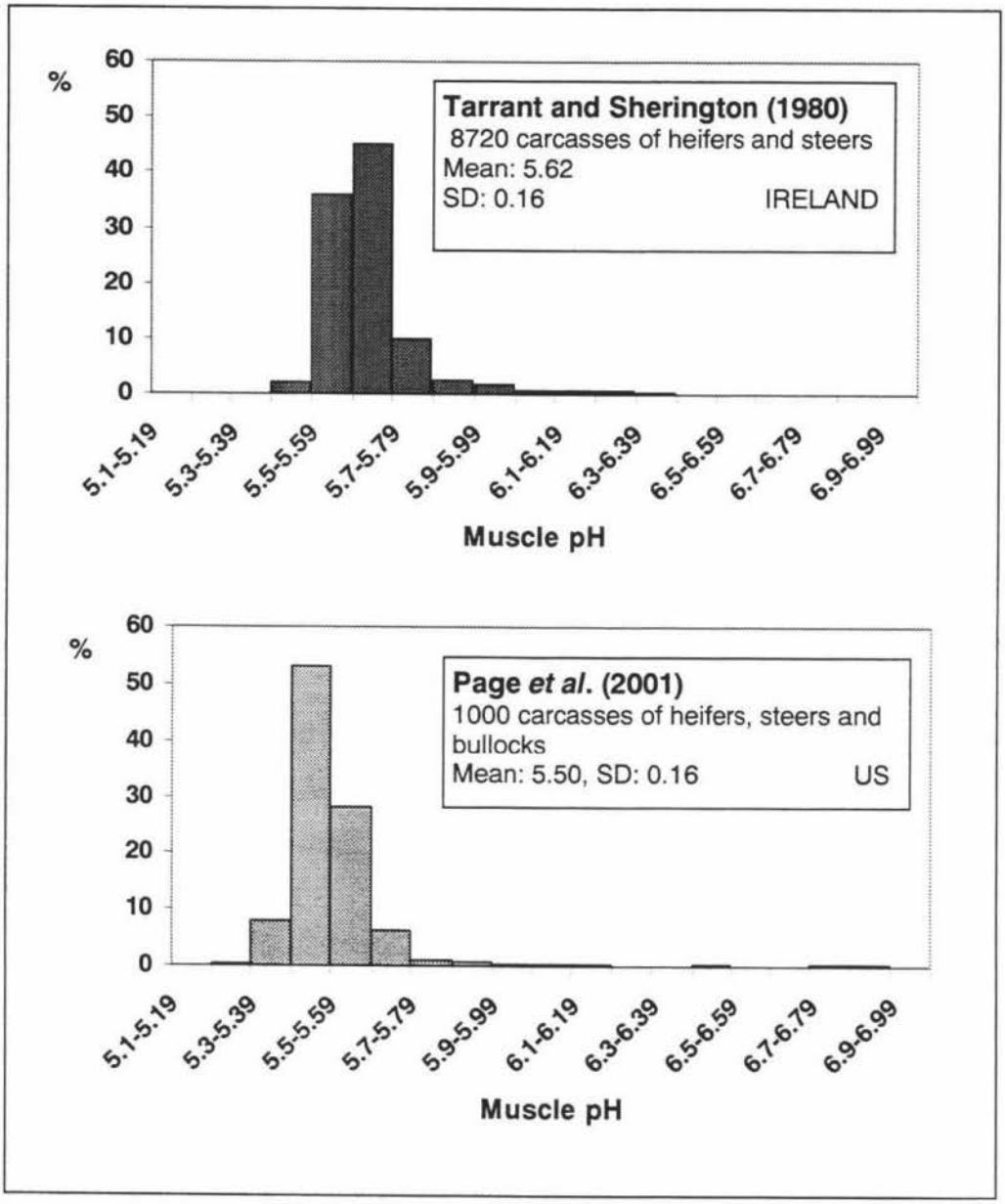


Figure 2.3: Frequency distribution of *longissimus* ultimate pH values in beef carcasses

Results from surveys carried out in New Zealand the last decade indicated an elevated incidence of high ultimate pH. Graafhuis and Devine (1994) found that 30% of approximately 3000 carcasses of prime steers presented pH values higher than 5.8 of pH while Smith *et al.* (1996) reported that 8% of 16905 carcasses exceeded the same value.

Despite the differences in the incidence in dark cutting in different countries, several authors reported similar non-normal distributions for ultimate pH in populations of commercially slaughtered animals (Tarrant and Sherington, 1980; Graafhuis and Devine, 1994; Smith *et al.*, 1996; Page *et al.*, 2001).

2.2.3 Biochemical determination of ultimate pH

Beef from non-stressed animals shows a decline in pH from approximately 7.0-7.2 to levels around 5.5 due to lactic acid accumulation, which is produced by glycolysis from the glycogen stored in the muscles. Figure 2.4 shows a schematic representation of the processes taking place about the time of death and those after slaughter that are involved in the conversion of muscle to meat. The decline of muscle pH is one of the processes involved in that conversion.

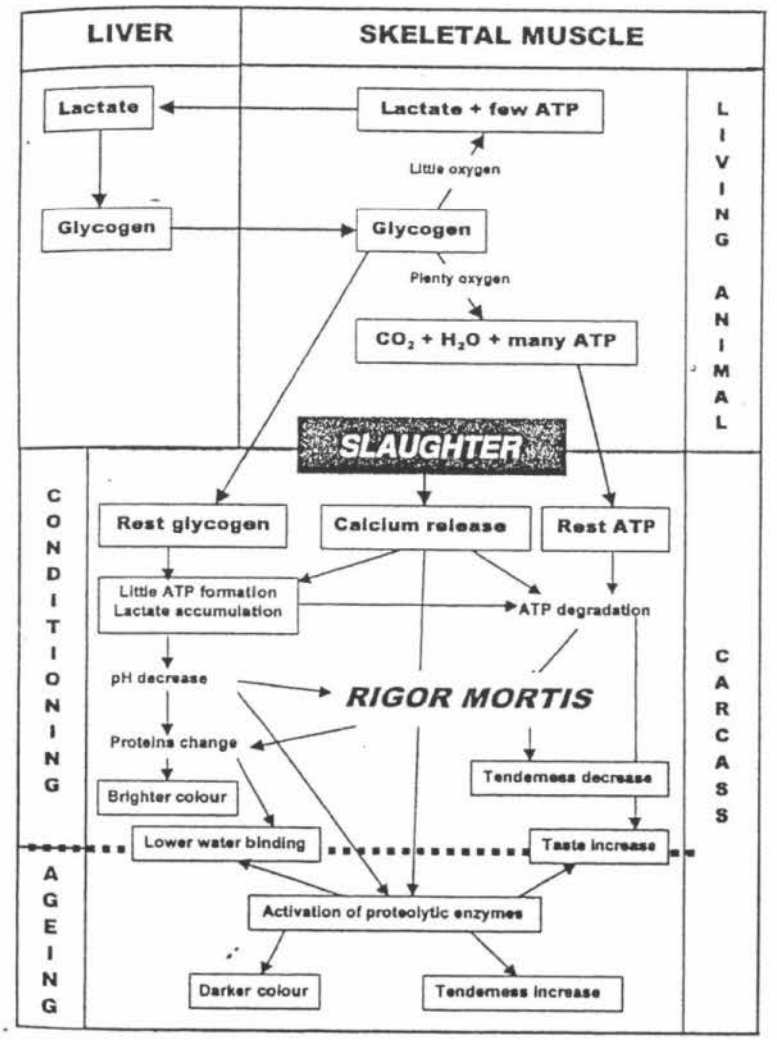


Figure 2.4: Schematic representation of peri-mortem metabolism in muscular tissue.
Source: Schreurs (2000)

Glycogen is a polymer of glucose that constitutes the muscle energy reserves. In order to utilise all potential energy of the glucose molecule, the metabolites of the glycogen degradation pathway have to go through three subsequent biochemical chains (Warris, 1990). These pathways, which are schematically represented in Figure 2.5, are glycolysis, citric acid cycle and oxidative phosphorylation. Glycolysis is the specific pathway by which monosaccharides such as glucose are converted into pyruvate. The pyruvate produces acetyl CoA that is the main compound in the common catabolic pathway that is made up of the citric acid cycle and oxidative phosphorylation.

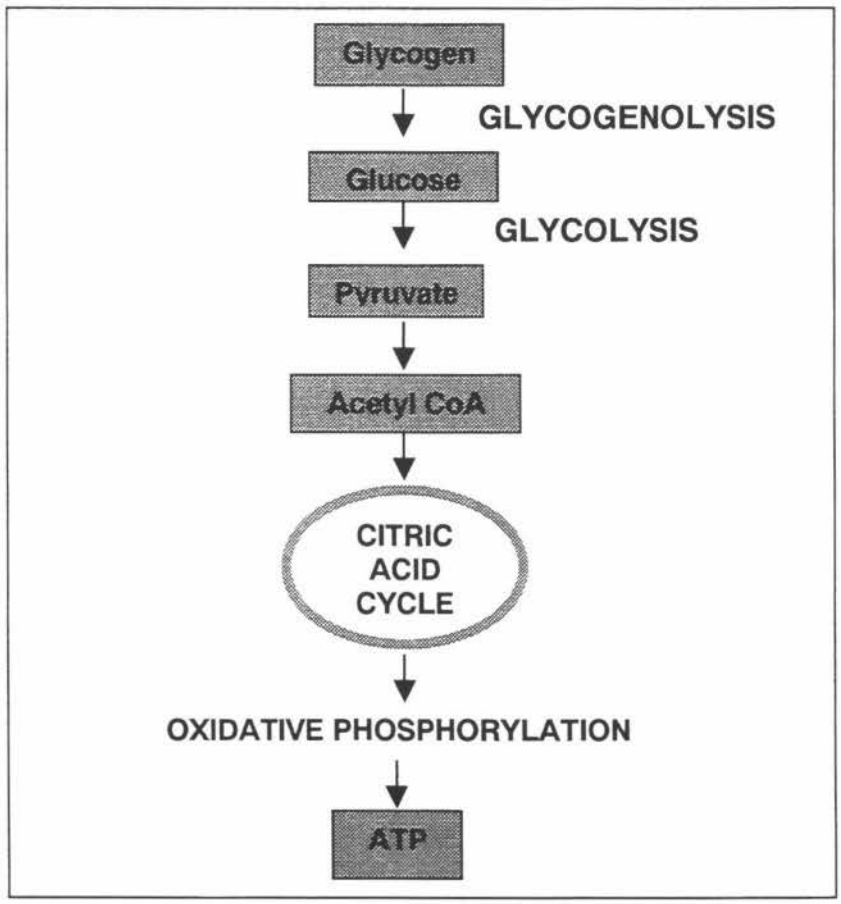
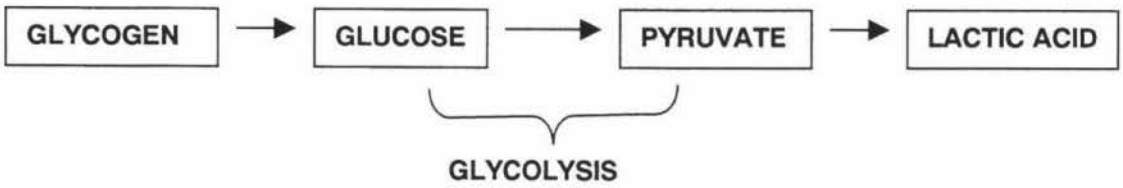


Figure 2.5: Schematic representation of catabolic pathways from glycogen

Because citric acid cycle and oxidative phosphorylation are aerobic processes, they are blocked when oxygen supply is not enough to maintain them (Schreurs, 2000). Thus, in anaerobic conditions the only way to generate some energy from glycogen stores is by glycolysis. After slaughter, the blood supply ceases and the supply of oxygen is interrupted. Energy is then provided by glycogen that generates few

adenosine triphosphate (ATP), as the main energy carrier, and large amounts of lactic acid by way of pyruvate:



Lactic acid production can also occur in live animals under anaerobic conditions, for instance during heavy exercise. However, great changes of pH in live animals are avoided due to the intracellular capacity of buffering considerable amounts of H+ and by the removal of lactate, which is converted into glycogen in the liver (glyconeogenesis) (Figure 2.4). Because the circulation is interrupted after slaughter, the accumulation of lactate is not completely counteracted and muscle pH will decrease (Schreurs, 2000).

The amount of lactic acid and the acidification of the muscle depend on the availability of glycogen. However, if glycogen is not limiting the production of lactic acid ceases when the enzyme systems will no longer function at low pH (Bendall, 1979). Although the reasons for this phenomenon are not clearly understood, two possible explanations have been suggested. The first one indicates that glycolysis ceases when adenosine monophosphate (AMP), required for ATP production, is not available. The second hypothesis points out that pH lower than 5.4 inactivates the glycolytic enzymes and then the process is stopped (Immonen and Puolanne, 2000).

The ATP produced by glycolysis is used by the muscles to maintain the relaxed state. However, the pH decline inhibits the re-synthesis of ATP and the concentration in the muscle decline. *Rigor mortis* occurs when the ATP concentration falls below a very low level of about 5 mmol/kg. The pH value of the muscle in *rigor mortis* is called ultimate pH (Lawrie, 1998).

2.2.3.1 RELATIONSHIP AMONG ULTIMATE pH, LACTIC ACID AND GLYCOGEN IN BEEF

The fall in post-mortem pH is a function of the amount of lactic acid produced by glycolysis in relation to the amount of lactic acid required to decrease muscle pH, which is called buffering capacity of the muscle.

The buffering capacity of muscles is their ability to resist the change of pH when acid or alkali is added and determines the molar concentration of lactic acid needed to lower the pH by one unit in one kg of muscle (Bendall, 1979). A mean buffering capacity of 51 mmol lactic acid/ pH x kg was reported by Puolanne and Kivikari (2000) for a pH range of 5.5-7.0. Bendall (1979) and Rao and Gault (1989) estimated buffering capacities of 60 and 49 mmol lactic acid/ pH x kg in *Longissimus dorsi*, respectively.

Figures 2.6a and b present the variation of ultimate muscle pH according to pre-slaughter muscle glycogen and final lactic acid concentrations, respectively. Data presented in Figure 2.6b shows that approximately 6.5 mg/g of lactate are required to reduce pH from 7.0 to 5.5. This amount is equivalent to a buffering capacity of about 48 mmol/ pH x kg.

Because two moles of lactic acid are produced per mol of glycogen by glycolysis, the amount of glycogen needed to decrease pH from 7.2 to 5.5 is about 45 mmol/kg expressed as glucose, with a range of 41 and 51 mmol/kg according to the different estimations of buffering capacities.

Low tissue glycogen levels limit the supply of substrates for glycolysis and hence slows the rate of pH decline due to a lower production of lactic acid, with a resultant higher ultimate pH. Even though reduced stores of glycogen lead to higher ultimate pH, there is a threshold level of glycogen in muscle, above which ultimate pH is independent of the glycogen concentration in the muscle. Consequently, the relationship between ultimate pH and glycogen concentrations at the moment of the slaughter is not linear. The non-linear association is explained by the fact that breakdown of glycogen by glycolysis ceases at approximately 5.5 of pH, independently of the amount of residual glycogen in the muscle (Warris *et al.*, 1984).

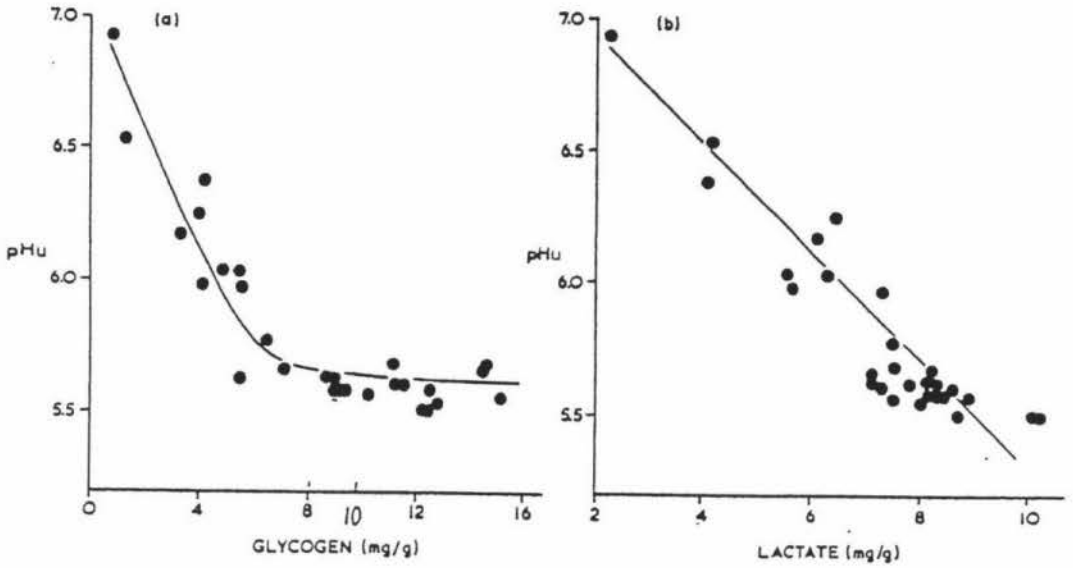


Figure 2.6: Relationships between (a) initial glycogen and (b) final lactate concentration and ultimate pH in *M. longissimus dorsi.*
Source: Warris *et al.* (1984)

Immonen and Puolanne (2000) reported a large variation in residual glycogen concentration, from 10 to 83 mmol/kg, at ultimate pH values between 5.4-5.6. This agrees with the concept that degradation of glycogen is inhibited at pH equivalent to approximately 5.5 and residual glycogen will exist at these values of pH. The concentrations of residual glycogen will vary according to the initial content at slaughter. However, Immonen and Puolanne (2000) also reported residual glycogen at higher pH values (pH≥6.0) that was not catabolised. The authors concluded that a minimum residual concentration may exist in which the denseness of the glycogen molecule limits further enzymatic degradation from glycogen to glucose.

A threshold of about 8-10 mg glycogen/g for the *longissimus* muscle of beef can be calculated from the results presented by Warris (1990) and Warris *et al.* (1984) (Figures 2.6a; b). The magnitude of the threshold is equivalent to 45 to 55 mmol glycogen/kg muscle, which agree with the values of glycogen required to lower the pH from 7.2 to 5.5 based on the buffering capacity estimations.

2.2.4 Pre-slaughter management and ultimate pH

High pH beef is caused by a lack of normal post-mortem acidification due to low muscle glycogen at slaughter. The depletion of muscle glycogen occurs in animals that have not been fed and this process is accelerated by excessive muscle activity and/or the action of stress (Tarrant, 1989).

In the live animal, muscle glycogen can be metabolised to release energy. A phosphorylase enzyme catalyses the conversion of glycogen into glucose (glycogenolysis). Under aerobic conditions, the pyruvate produced from glycogen by glycolysis enters the citrate cycle and the oxidative phosphorylation, and produces the required energy as ATP. The hormone adrenaline, secreted in response to external stressors, activates glycogenolysis and muscle glycogen is mobilised (Warris, 2000).

Muscle levels of glycogen at the time of slaughter depend on the content when the animals are removed from feed and the incidence of glycogen-depleting events prior to slaughter (Immonen *et al.*, 2000). From the farmgate to slaughter, cattle can be exposed to several stressors that include: fasting; dehydration; novel unfamiliar environments; transport; increased human contact; changes in the social structure (i.e by mixing animals from different origins); and climatic changes (Fergusson *et al.*, 2001).

The alternative of restoring depleted contents of glycogen has been studied. Although the repletion depends on the sex of the animals, mechanism of glycogen depletion and food offered in the recovery time, between 3 and 11 days are required to reach pre-stress concentration in muscle (Lacourt and Tarrant, 1981; McVeigh and Tarrant, 1982). Rates of glycogen re-synthesis are slower in ruminants that in monogastrics, probably explained by a lower availability of glucose in ruminants (Tarrant, 1989). Although Warris *et al.* (1984) pointed out that 2 days' rest with access to feed and water are needed to produce normal post-mortem acidification, it does not seem to be a suitable option to be applied in commercial conditions. Therefore, minimising the negative consequences of pre-slaughter stress is particularly relevant due to the long time taken for recovery of muscle glycogen reserves in cattle.

Animals may pass through several stages and activities during the pre-slaughter period in which the stress should be reduced. Nevertheless, the potential impact of improving handling in the different stages is related to their effect on meat pH.

Because of the impact of ultimate pH on beef quality, an important amount of research has been dedicated to investigate the effect of pre-slaughter conditions on glycogen and/or meat pH. The existing international knowledge associated with the problem of high pH was discussed in two important workshops in the 80's. Fergusson *et al.* (2001) recently presented a review on the critical pre-slaughter factors affecting ultimate pH, among other meat quality traits.

Factors such as sex and mixing unfamiliar animals in the pre-slaughter period have been identified as stressors that may predispose to dark-cutting. According to Gregory (1998), the most important cause of dark cutting is the sex of the animals. Bulls tend to present higher ultimate pH than steers, heifers and cows (Graafhuis and Devine, 1994; Warris, 1990;Tarrant, 1989). Strenuous efforts and emotional stress associated with the re-establishment of social hierarchy among unfamiliar animals would explain the higher predisposition to dark cutting under this condition especially in bulls (Augustini, 1981;Puolanne and Aalto, 1981;Grandin, 1989).

Mustering and yarding are on-farm management factors that may have a greater effect on the levels of muscle glycogen (Graafhuis and Devine, 1994). However, Lambert *et al.* (1998) and Lambert *et al.* (2000) concluded that neither the moderate exercise imposed during mustering nor yarding/fasting markedly affected muscle glycogen of well-fed steers. In addition, Lambert *et al.* (2000) pointed out that increased emphasis on animal handling during mustering and yarding is unlikely to lower the incidence of high pH beef in well-fed animals.

Transport and lairage have been claimed as pre-slaughter stages in which the stress may reduce muscle glycogen levels and determine high ultimate pH. Long-distance transportation tends to increase ultimate pH (Tarrant, 1989), whereas short or moderate travelling distances are unlikely to affect ultimate pH (Tarrant, 1989; Warris, 1990) or affect muscle glycogen concentration for well-fed steers (Lambert *et al.*, 2000). However, Fergusson *et al.* (2001) pointed out that there are caveats to these statements because of influence of the condition of the cattle, the nutritional history and the holding time in lairage. The definition of the optimal lairage time is a critical issue that has not been completely clarified. Although the lairage phase represents an opportunity for animals to recover from the physical and emotional stress associated with handling, marketing and transport, it may be an additional cause of stress because the abattoir is an unfamiliar environment. The slow repletion rates estimated in cattle determine that animals with low muscle glycogen concentrations would

require more than two days to reach levels that would minimise the risk of dark cutting. Wythes *et al.* (1980) indicated that resting animals after a long journey (1400 km) for two days rather than one reduced the ultimate pH values in bullocks. On the other hand, short resting periods before slaughter have been recommended after short transport periods (Puolanne, 1989). Purchas (1992) reported that 28h-period at the abattoir resulted in higher ultimate pH compared to 4 hours, whereas Peachey (1999) did not find a significant effect in ultimate pH between the same holding times. Fergusson *et al.* (2001) pointed out that it is difficult to draw general conclusions as the effect of lairage will vary depending on the pre-slaughter glycogen concentrations, animal sex and transport duration/distance.

The extent of glycogen depletion in the pre-slaughter period is likely to be due to the effect of a series of glycogen depleting events rather than any one such event (Devine and Chrystall, 1989). The influence of multiple factors acting simultaneously may explain the difficulties in obtaining clear conclusions about the influence of the different effects. Efforts to identify causes of variation in the occurrence of high ultimate pH in commercially slaughtered animals have not successfully identified the determining factors for high ultimate pH and/or their relative incidence (Graafhuis and Devine, 1994; Navajas *et al.*, 1996; Smith *et al.*, 1996; Purchas and Keohane, 1997).

The study of ultimate pH and the determining causes presents some difficulties, besides the multiple factors that may affect. Some trials with the objective of estimating the effect of pre-slaughter stress on ultimate pH could not find any significant associations due to the absence of high pH values (Peachey, 1999; Webby *et al.*, 1999). This was attributed by the authors to quiet handling of the animals. Moreover, Fergusson *et al.* (2001) pointed out that the response to the stress can be moderated through the previous experience of the animals. Cattle that are routinely exposed to human contact, such as experimental animals, may experience less stress under the normal stressors than individuals without this previous experience. On the other hand, when the stress acts reducing the muscle glycogen contents, high pH values are registered. High values of ultimate pH lead to a skewed distribution that may also affect the statistical analysis. With the exception of Tarrant and Sherington (1980), none of the reviewed articles discussed this problem.

2.3 A DYNAMICAL MODEL DESCRIBING THE RELATIONSHIP BETWEEN MUSCLE GLYCOGEN AND ULTIMATE pH

Studies of ultimate pH in beef are complicated by its skewed frequency distribution, which cannot be converted to a normal probability density by logarithmic transformations. A mathematical model that describes the conversion of glycogen to lactic acid in a manner suitable for statistical analysis has been proposed by Pleasants *et al.* (1999). This approach is detailed next.

Pleasants *et al.* (1999) derived the probability density of ultimate pH in slaughtered animals from a pair of coupled differential equations that express the change of muscle glycogen (G) and pH (pH) over time:

$$k_1 \frac{dG}{dt} = (5.5 - pH)\, y(G) \qquad [2.1a]$$

$$k_2 \frac{dpH}{dt} = (5.5 - pH)(7.2 - pH)\, y(G) \qquad [2.1b]$$

where k_1 and k_2 are constants related to the rates of these equations and $y(G)$ is a function of glycogen that describes how the loss of glycogen varies throughout the acidification of muscles by the transformation of muscle glycogen to lactic acid.

After slaughter, glycogen in the muscle is converted by glycolysis to lactic acid that leads to the decrease of meat pH. Expression [2.1a] captures the finding that the rate of change of muscle glycogen depends on the availability of muscle glycogen and the muscle pH. Glycogen is the substrate for glycolysis and its concentration will vary according to the initial levels and the amount that is catabolised. If the muscle glycogen levels at slaughter are low, the supply of substrate for glycolysis is limited resulting in a lower concentration of lactic acid and a higher ultimate pH. Higher levels of glycogen determine greater amounts of lactic acid and consequently lower values of pH, until pH reaches values about 5.5. The breakdown of muscle glycogen ceases when pH is approximately 5.5, even if residual glycogen remains undegraded, probably through pH inactivation of the glycolytic enzymes.

The rate of change of pH is affected by the concentration of muscle glycogen and by the buffering capacity of the muscle (expression [2.1b]). Muscle pH values immediately after death are close to neutrality (7.0-7.2). Low availability of muscle

glycogen will lead to small changes from the initial neutral pH. On the other hand, greater glycogen concentrations imply a larger acidification and lower pH values. The rate of change of muscle pH is affected by the buffering capacity, which indicates the amount of lactic acid that is required to decrease muscle pH. Muscle pH falls slowly at values close to neutrality, due to buffering capacity, and at pH levels near to 5.5 when the glycogen breakdown is inhibited and accumulation of lactic acid stopped.

Dividing [2.1b] by [2.1a] the relation between pH and G is then expressed as:

$$\frac{k_2}{k_1}\frac{dpH}{dG} = 7.2 - pH$$

Define $\frac{1}{k} = \frac{k_2}{k_1}$, with $k = \frac{k_1}{k_2}$, the previous equation is then equal to:

$$\frac{1}{k}\frac{dpH}{dG} = 7.2 - pH \qquad [2.2]$$

The former differential equation has the following solution:

$$pH = 7.2 - \frac{1}{k}C.e^{-kG} \qquad [2.3]$$

where:

pH and G are muscle pH and muscle glycogen, respectively, and C is a constant related to the content of glycogen at the time of slaughter.

The solution of the differential equation [2.2] can be confirmed by differentiating [2.3] by G:

$$\frac{dpH}{dG} = \frac{7.2 - 1/k\,C.e^{-kG}}{dG}$$

$$= +C.e^{-kG}$$

According to [2.3] $$pH = 7.2 - \frac{1}{k}C.e^{-kG}$$

Then $$\frac{1}{k}C.e^{-kG} = 7.2 - pH$$

And $$C.e^{-kG} = k(7.2 - pH)$$

Substituting $C.e^{-kG}$ we obtain:

$$\frac{dpH}{dG} = k(7.2 - pH)$$

and re-arranging the previous equation, we get the original differential equation [2.2]

$$\frac{1}{k}\frac{dpH}{dG} = (7.2 - pH)$$

Calling $G^* = kG$ (i.e re-scaling glycogen) and $C^* = \frac{1}{k}C$ (some constant), equation [2.3] is re-written as:

$$pH = 7.2 - C^*.e^{-G^*} \qquad [2.4]$$

Considering that the constant C^* is related to the muscle glycogen at slaughter (t=0), named Go, and by including the condition $pH = 7.2 - \delta$, where δ is a constant recognising that muscle pH must be lower than 7.2 at t=0, C^* is defined as:

$$C^* = \delta e^{G_0}$$

Substituting C^*, equation [2.4] can be re-reformulated as:

$$pH = 7.2 - \delta.e^{Go-G^*} \qquad [2.5]$$

Defining $\varepsilon = \delta / e^{G^*}$, ultimate pH ($pH_U$) can be expressed as a function of muscle glycogen at slaughter by:

$$pH_U = 7.2 - \varepsilon.e^{Go} \qquad [2.6]$$

Transforming [2.6] and manipulating the result, it is possible to transform the actual value of ultimate pH to the original glycogen level (G_O):

$$\varepsilon.e^{Go} = 7.2 - pH_U$$

$$e^{Go} = \frac{7.2 - pH_U}{\varepsilon}$$

$$\ln\left(e^{Go}\right) = \ln\left(\frac{7.2 - pH_u}{\varepsilon}\right)$$

$$G_O = \ln\left(\frac{7.2 - pH_U}{\varepsilon}\right) \qquad [2.7]$$

As muscle pH decreases after slaughter, the conversion of glycogen to lactic acid is reduced until a pH of 5.5 is achieved. At this level of pH no further glycogen can be catabolised because the glycolytical pathway is inhibited. Therefore, an ultimate pH of 5.5 is determined by muscle glycogen levels that are equal or higher than the minimum amount of muscle glycogen required to achieve a pH around 5.5.

Suppose G_O is exactly the amount of glycogen to obtain an ultimate pH of 5.5, with the residual glycogen G equal to zero, and substitute in [2.5]:

$$5.5 = 7.2 - \varepsilon.e^{Go}$$

Then

$$\varepsilon.e^{Go} = 7.2 - 5.5 = 1.7$$

$$e^{Go} = \frac{1.7}{\varepsilon}$$

$$\ln\left(e^{Go}\right) = \ln\left(\frac{1.7}{\varepsilon}\right)$$

$$G_0 = \ln\left(\frac{1.7}{\varepsilon}\right) = CG$$

This quantity of glycogen, named critical glycogen (CG), is the minimum amount that leads to pH=5.5. Note actual pH varies around 5.5 due to measurement error, which will be dealt with later. Knowledge of the biochemical process indicates that since pH reaches 5.5, the glycolytic pathway is stopped. All levels of G_O equal or higher than CG will lead to ultimate pH of 5.5:

$$Go \geq \ln\left(\frac{7.2-5.5}{\varepsilon}\right) = CG \qquad [2.8]$$

If a muscle contains an amount of muscle glycogen higher than *CG*, an amount equivalent to *CG* will be converted to lactic acid. The difference between the initial value and *CG* is residual glycogen that is not catabolised because glycolysis has been inhibited.

The critical glycogen *CG*, as well as the fact that those levels of glycogen equal or higher that *CG* will achieve an ultimate pH around 5.5 are indicated in Figure 2.7. The proportion of muscles with pH = 5.5 is then determined by the proportion of muscle glycogen values equal or higher than *CG*, that is indicated in Figure 2.7 as α. In statistical terms this is expressed as:

$$P[pH_u = 5.5] = \int_{CG}^{\infty} f(G_0)dG_0 = \alpha \qquad [2.9]$$

The probability density of ultimate pH can be described in terms of the glycogen probability density as:

$$P[pH_u] = \begin{cases} \int_{CG}^{\infty} f(G_0)dG_0 & for \ \ pH_U = 5.5 \\ \beta\varepsilon^{G_0} f(G_0) & for \ \ pH_U > 5.5 \end{cases} \qquad [2.10]$$

where the first line refers to the proportion of pH_U = 5.5, which are explained by proportion of muscle glycogen equal or greater than *CG* (expression [2.9]). The second line represents the remaining probability density of pH_U that is explained by the proportion of muscle glycogen distribution below *CG*. The parameter β is chosen to make $P[pH_u]$ a probability density.

A measurement error of pH is included in the definition of the observed probability density of ultimate pH. The measurement error (me) comprises the instrumental error associated to the measurement of pH and other random factors. It is assumed to be a random normal variable with mean zero and variance σ^2_{me}:

$$me \sim N(0, \sigma^2_{me}) \qquad [2.11]$$

The observed probability density for ultimate pH is defined by the convolution of the measurement error density [2.11] and the density of the actual ultimate muscle pH [2.10] (Pleasants *et al.*, 1999):

$$P\left[pH_U\right] = N\left(5.5, \sigma^2_{me}\right) \int_{CG}^{\infty} f\left(G_0\right) dG_0 + \beta\varepsilon e^{G_0} f\left(G_0\right) * N\left(0, \sigma^2_{me}\right)\Big|_{G_0=0}^{CG} \qquad [2.12]$$

where * is the convolution operator and the second term applies for G_0 values from 0 to CG.

Substituting in expression [2.12] $\int_{CG}^{\infty} f\left(G_0\right) dG_0$ by α and β by $(1-\alpha)$:

$$P\left[pH_U\right] = \alpha N\left(5.5, \sigma^2_{me}\right) + (1-\alpha)\varepsilon e^{G_0} f\left(G_0\right) * N\left(0, \sigma^2_{me}\right)\Big|_{G_0=0}^{CG} \qquad [2.13]$$

The probability density of ultimate pH comprises two terms. The first term corresponds to an α proportion of the observed distribution that has mean and variance equal to 5.5 and σ^2_{me}. The remaining $(1-\alpha)$ of ultimate pH is determined by the $(1-\alpha)$ proportion of muscle glycogen that corresponds to those values between 0 and critical glycogen.

Using these results, Pleasants *et al.* (1999) showed that the probability function of ultimate pH (pH_U) can be expressed as a mixed probability density in terms of the frequency of G_0:

$$P\left[pH_U\right] = \underbrace{\alpha N\left(\ln\left(1.7/\varepsilon\right), \sigma^2_{me}\right)}_{\text{I}} + \underbrace{(1-\alpha) N\left(\mu_{G_L}, \sigma^2_{G_L}\right)}_{\text{II}} \qquad [2.14]$$

where: μ_{G_L} and $\sigma^2_{G_L}$ are the mean and variance, respectively, of the glycogen levels below the critical glycogen.

The association between muscle glycogen and ultimate pH, according to this approach, is illustrated in Figure 2.7. The peak in the ultimate pH distribution corresponds to those animals with muscle glycogen equal to or greater than the

critical glycogen level (CG) required to achieve an ultimate pH of 5.5 (I in [2.14] and Figure 2.7). Thus, those animals with muscle glycogen equal or higher than the critical glycogen will have an ultimate pH around 5.5. Consequently, the variability of glycogen concentration is not expressed in terms of ultimate pH, with the variation around the average explained by the measurement error (me).

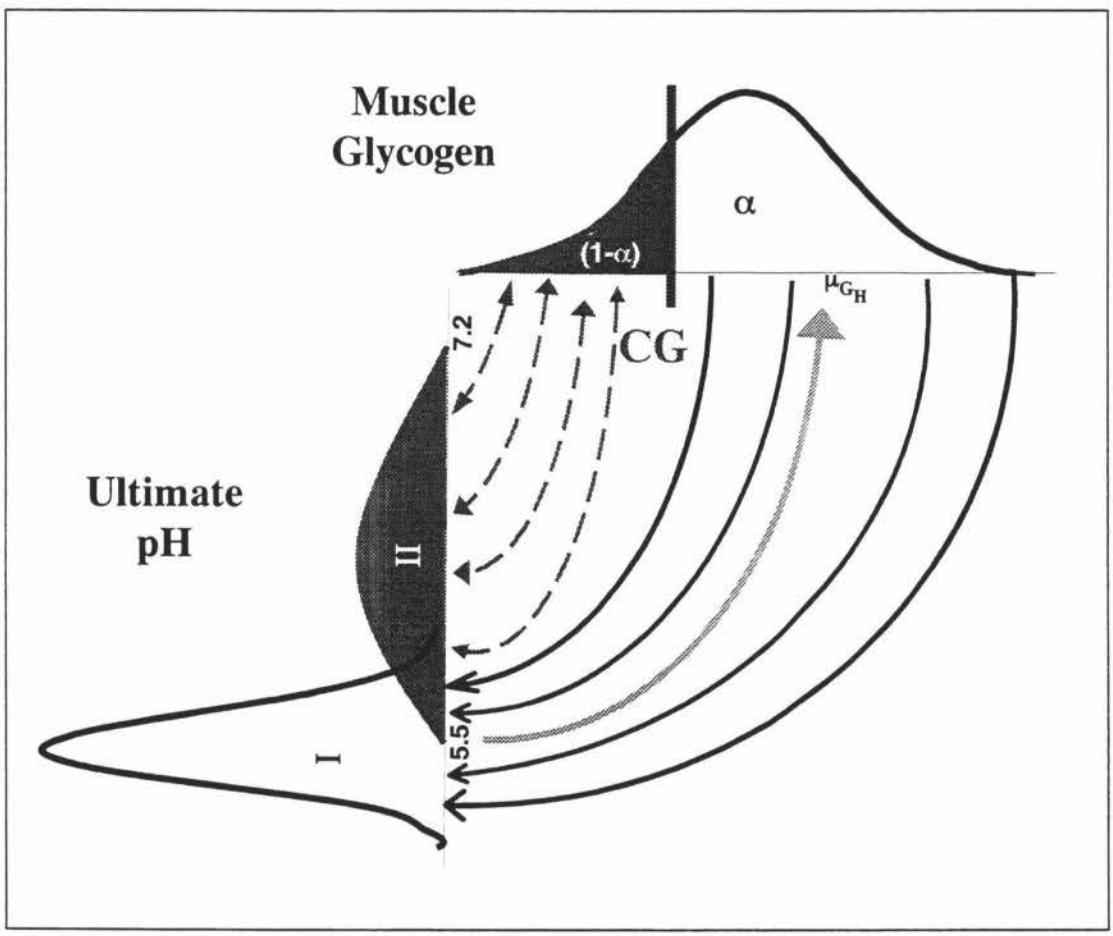


Figure 2.7: Illustration of the relationship between muscle glycogen and ultimate pH. Adapted from Pleasants *et al.* (1999)

On the other hand, the tail in the frequency distribution is explained by glycogen levels lower than critical glycogen. These glycogen concentrations generate ultimate pH higher than 5.5 and lower than 7.2 depending on the amount of muscle glycogen at slaughter, with pH described directly by equation [2.6]. The total variance in the second normal distribution in the mixture (II in [2.14] and Figure 2.7) includes the

effect of the measurement error (σ^2_{me}). Nevertheless, the variance in the tail also reflects the differences in glycogen at slaughter, transformed from ultimate pH based on [2.7] and named as G_L.

The mathematical model proposed by Pleasants *et al.* (1999) allows the transformation of ultimate pH data to the underlying glycogen content, according to information of glycogen that is contained in ultimate pH distribution. Using expression [2.7] it is possible to estimate the original glycogen levels from the ultimate pH data for those observations that belong to the tail of the pH distribution.

The biochemical inhibition of glycolysis at pH of 5.5 makes it impossible to restore the variation in muscle glycogen that is associated with the data in the peak around 5.5. Although the individual differences in muscle glycogen for these animals have been lost in this part of the distribution, it is possible to map them to the most probable value that corresponds to the mean of glycogen content at slaughter. Pleasants *et al.* (1999) defined the mean as the critical glycogen value (CG) (see equation [2.14]). However, the most probable value of glycogen is the mean of glycogen for the white area of muscle glycogen distribution (α in Figure 2.7). The mean of muscle glycogen values higher than CG (μ_{G_H}) can be estimated as the mean of a truncated normal distribution where CG is the truncation point.

As a transformation of ultimate pH data, predicted glycogen reflects the information content of pH. By this method, the information around the peak of ultimate pH is discounted in favour of the data contained in the tail of the distribution, which is more informative in terms of the variability of glycogen (Pleasants *et al.*, 1999). Assuming the procedure adequately captures the biochemical nature of ultimate pH, this transformation should give more power to the investigations related to beef ultimate pH.

CHAPTER THREE

DETECTION OF SIMULATED QTL FOR BEEF ULTIMATE pH APPLYING A BIOCHEMICALLY-BASED APPROACH

3.1 INTRODUCTION

The approach to identify quantitative trait loci (QTL) in most traits has been based on the combined analysis of molecular and phenotypic information by searching for significant associations. Different methodologies are available to find the location of a QTL on a chromosome and to estimate the additive and/or dominant effects. However, the knowledge of biological processes underlying the expression of a specific trait has not been expressly included in the standard techniques.

The ultimate pH in beef is an economically relevant characteristic, where values higher than 5.5 negatively affect meat attributes such as tenderness and colour (Purchas and Aungsupakorn, 1993; Smith *et al.*, 1996). The frequency distribution of muscle ultimate pH is non-normal, presenting a peaked primary mode at 5.5 and a longer upper tail with a suggestion of a secondary mode at high values. This skewed distribution has made the studies of ultimate pH more difficult by affecting the inference that can be made. Although logarithmic transformation of a positive skewed variable gives a normal distribution (Lynch and Walsh, 1998), Pleasants *et al.* (1999) pointed out that it failed to convert a normal probability density in ultimate pH.

Beef ultimate pH is determined by post-mortem accumulation of lactic acid in the muscle. This is produced by the conversion of glycogen that existed in the muscle at the time of slaughter. A mathematical model that describes the conversion of glycogen to lactic acid in a manner suitable for statistical analysis has been proposed by Pleasants *et al.* (1999). The objective of this study is to evaluate the effect of applying the novel approach in the detection of QTL for ultimate pH in a simulated back-cross design involving two inbred lines.

3.2 MATERIALS AND METHODS

The influence of applying the new approach to QTL detection was analysed by simulation, which was programmed in A Programmers Language (APL, 1999). The simulation included four distinct parts:

1. Simulation of continuous variation for muscle glycogen in a back-cross design between two inbred lines.
2. Derivation of ultimate pH from glycogen values
3. Transformation of pH to the underlying glycogen levels based on the biochemically-based approach.
4. Interval mapping by regression analysis.

Two sizes of an additive QTL were simulated.

3.2.1 Simulation of muscle glycogen data in a back-cross design

3.2.1.1 PARENTAL INBRED LINES

Phenotypic data of muscle glycogen at slaughter were simulated for two completely inbred lines, L1 and L2. Based on a phenotypic standard deviation σ_{Go} and assuming a heritability of 30% ignoring the QTL, the additive polygenic value for each animal was sampled from a distribution N (0, σ_a), with σ_a the additive polygenic standard deviation equal to $\sqrt{h^2\sigma_{Go}^2}$. Arbitrary values of 30 and 15 units were assumed in the simulation for the phenotypic mean and standard deviation, respectively, of muscle glycogen.

The genetic effect for glycogen included the additive polygenic value and that due to the QTL alleles. Five loci were simulated for all the animals in the base populations with a different allele fixed in each inbred line. These loci represent four markers and the QTL. The real distances among markers and the position of the QTL are illustrated in Figure 3.1.

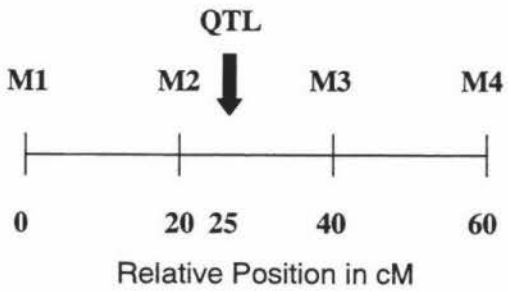
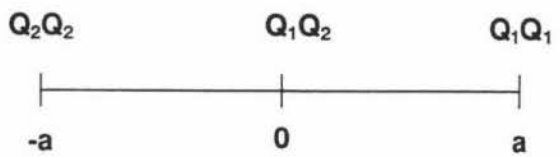


Figure 3.1: Relative positions (in cM) of four simulated genetic markers (M) and the QTL

The genotypic effects of the homozygous QTL genotypes were defined as –a and a for Q_2Q_2 and Q_1Q_1, respectively, with a being the additive genetic deviation from the genetic mean of the homozygotes. The dominance genetic deviation (d) was defined as zero, and thus the genetic effect of the heterozygote Q_1Q_2 is equal to the genetic mean of the homozygotes:



Two scenarios, which differ in the size of the QTL effect, were simulated. The size of the QTL was defined as a. For the first scenario, the additive genetic deviation of the QTL was one polygenic additive standard deviation (σ_a), whereas in the second scenario it was equivalent to 0.5 σ_a. Recall σ_a reflects polygenic effects other than the QTL.

Residual components from a distribution N $(0, \sigma_e)$, where σ_e is the residual standard deviation, were sampled and added to the genetic components in order to obtain the phenotypic glycogen at slaughter. The residual standard deviation is equal to:

$$\sigma_e = \sqrt{(1-h^2)\sigma_{Go}^2}$$

3.2.1.1 FIRST-CROSS AND BACK-CROSS

100 males and 100 females were randomly chosen from each inbred line. Sires and dams originating in different lines were randomly mated to produce the first-cross (F1)

individuals. Two F1 males were randomly selected and each one was mated to 200 inbred females that were randomly chosen from L1.

The additive polygenic value was calculated in the F1 and back-cross generation as:

$$½g_s + ½ g_d + \phi$$

where:

g_s and g_d are the polygenic genetic values of sires and dams, respectively, and

ϕ = Mendelian sampling with $\phi \sim N (0, \sigma_{MS})$ and where the Mendelian sampling standard deviation (σ_{MS}) is equivalent to $\sqrt{½\sigma_a^2}$

The haplotypes of markers and QTL alleles that were transmitted to the next generation were simulated using recombination rates based on Haldane 's mapping function:

$$r = \frac{1 - e^{-2d}}{2}$$

where

r = recombination rate

d= map distance in cM.

Haldane's function assumes that there is no interference, so crossovers occur randomly and independently in the chromosome. Therefore, the presence of crossover in one region of the chromosome does not affect the frequency of crossovers in adjacent regions (Lynch and Walsh, 1998).

3.2.2 Prediction of ultimate pH and back-transformation to glycogen

3.2.2.1 ULTIMATE pH

Ultimate pH data was simulated in the back-cross animals from the phenotypic records of muscle glycogen. The calculations were based on the mathematical model developed by Pleasants *et al.* (1999) that incorporates the biochemical pathways involved in the determination of post-mortem muscle pH. Ultimate pH is a consequence of acidification of muscle from neutral values about 7.2 at slaughter. The acidification is determined by accumulation of lactic acid, which is produced by the breakdown of glycogen through the glycolytic pathway.

The achieved ultimate pH will depend on the content of glycogen in the muscle at the time of slaughter. Low levels of glycogen will determine high ultimate pH and lower pH will be achieved from greater concentrations of glycogen, until the muscle has a pH of 5.5. Glycolysis ceases at this value of pH and the accumulation of lactic acid is stopped, independently of the amount of glycogen that has not been catabolised. A critical glycogen level has been defined as the amount of muscle glycogen required to achieve an ultimate pH of 5.5. Quantities of muscle glycogen lower than critical glycogen will be associated with ultimate pH between 7.2 and 5.5, depending on the specific amount of glycogen in the muscle at the time of slaughter. On the other hand, glycogen contents equal or higher than critical glycogen will determine pH values of 5.5 independently of the specific value of muscle glycogen.

Consequently, ultimate pH (pH_U) was computed as a function of the muscle glycogen content at slaughter for those animals with glycogen levels lower than the critical glycogen. It was calculated using the following equation:

$$pH_U = 7.2 - e^{0.05(G_o - 10)} \qquad [3.1]$$

where G_O is the muscle glycogen content at slaughter. The parameters were chosen so ultimate pH looked real, where $e^{0.05(-10)}$ is ε in equation [2.6].

The critical glycogen level, which represents the amount of muscle glycogen required to achieve an ultimate pH of 5.5, was calculated based on equation [2.8] as:

$$CG = \frac{\ln(1.7)}{0.05} + 10 = 20.65$$

Because glycolysis is inhibited at pH=5.5, an ultimate pH of 5.5 was assigned for those animals presenting muscle glycogen equal or higher than the critical glycogen. In such animals, residual glycogen would exist in the meat at ultimate pH.

A measurement error, which includes instrument error associated with the measurement of muscle pH and other random factors, was included. It was assumed to be a normally distributed random variable with mean zero and variance σ^2_{me}, with σ^2_{me} being the variance of the measurement error (me). Although an accuracy in terms of standard deviation of ± 0.05 is often called by the meat industry, Jansen (2001) reported that potential sources of error associated with unrecognised effect of

temperature might increase this value to 0.1. The variance of the measurement error was therefore assumed equal to 0.01. Components from this distribution were sampled and added to the previously calculated values of pH.

3.2.2.2 PREDICTED MUSCLE GLYCOGEN

Pleasants *et al.* (1999) showed that the probability function of ultimate pH can be expressed as a mixture of two normal distributions in terms of the frequency of muscle glycogen.

Muscle glycogen values were predicted from the ultimate pH records based on this assumption. The parameters of both sub-populations (means and variances) and the mixture proportion were estimated by maximum likelihood using the iterative algorithm proposed by Hosmer (1973).

3.2.2.2.1 ESTIMATION OF THE PARAMETERS OF A MIXTURE OF TWO NORMAL DISTRIBUTIONS

The actual pH density function (f_{pH_U}) is a mixed distribution that is defined as:

$$f_{pH_U} = p_1 f_{pH1} + p_2 f_{pH2} \qquad [3.2]$$

where:

f_{pH1} and f_{pH2} are the density functions of two normal random variables with means μ_1 and μ_2, and variances σ_1^2 and σ_2^2, respectively.

p_1 and $p_2 = 1 - p_1$ are the proportions of each variable in the mixture.

The components of the mixed distribution and the parameters are illustrated in Figure 3.2.

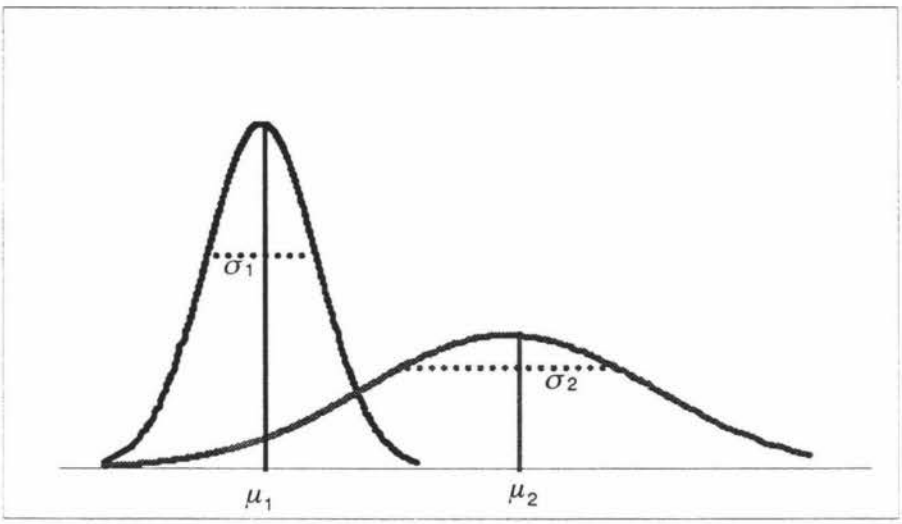


Figure 3.2: Diagram of the two normal distributions included in the mixture. Means (μ_1 and μ_2) and standard deviations (σ_1 and σ_2) are indicated

Considering that p_2 can be defined as $1-p_1$, the vector of unknown parameters (θ) is:

$$\theta = \left(p_1, \mu_1, \sigma_1^2, \mu_2, \sigma_2^2\right)$$

The ln-likelihood function is:

$$L(\theta) = \sum_{j=1}^{n} \ln\left(f_{pH_U}(x_j)\right)$$

where:

x_j represents the independent observations of ultimate pH, with j = 1, 2, ...n, and

n is the total number of observations in the mixed distribution.

Define the weights w_{1j} and w_{2j} as :

$$w_{1j} = \left[p_1 f_{pH1}(x_j)\right] / \left[f_{pH_U}(x_j)\right]$$

$$w_{2j} = \left[p_2 f_{pH2}(x_j)\right] / \left[f_{pH_U}(x_j)\right]$$

where $f_{pH1}(x_j)$, $f_{pH2}(x_j)$ and $f_{pH_U}(x_j)$ are the density functions for pH_1, pH_2 and pH_U, respectively.

Iterative estimates for the five parameters in θ are given by:

$$\hat{p}_1 = \frac{1}{n}\sum_{j=1}^{n}\hat{w}_{1j}$$

$$\hat{\mu}_1 = \frac{\sum_{j=1}^{n}\hat{w}_{1j}\cdot x_j}{\sum_{j=1}^{n}\hat{w}_{1j}} \qquad \hat{\mu}_2 = \frac{\sum_{j=1}^{n}\hat{w}_{2j}\cdot x_j}{\sum_{j=1}^{n}\hat{w}_{2j}}$$

$$\hat{\sigma}_1^2 = \frac{\sum_{j=1}^{n}\hat{w}_{1j}\cdot\left(x_j-\hat{\mu}_1\right)^2}{\sum_{j=1}^{n}\hat{w}_{1j}} \qquad \hat{\sigma}_2^2 = \frac{\sum_{j=1}^{n}\hat{w}_{2j}\cdot\left(x_j-\hat{\mu}_2\right)^2}{\sum_{j=1}^{n}\hat{w}_{2j}}$$

where the estimated weights ($\hat{w}_{1j}$ and $\hat{w}_{2j}$) in iteration t, uses the respective means ($\hat{\mu}_1$ and $\hat{\mu}_2$) and variances ($\hat{\sigma}_1^2$ and $\hat{\sigma}_2^2$) estimated in the previous iteration (t-1).

Starting values for the parameters were 0.5, 5.5, 6.0, 0.009 and 0.09 for p_1, μ_1, μ_2, σ_1^2 and σ_2^2, respectively. Convergence was assessed from the squared change in estimates from one round to the next. Iteration was stopped when this convergence criterion reached 1×10^{-3}.

The iterative algorithm proposed by Hosmer (1973) may present singularities in the likelihood function $L(\theta)$ and therefore it is not guaranteed to converge to a solution. All iterative approaches require a starting value and a poor option can result in the iteration converging to a solution that is a local maximum but not the true maximum-likelihood solution (Lynch and Walsh, 1998). The biological basis of the problem being analysed indicates reasonable initial values for at least some of the parameters. In the particular case of ultimate pH, 5.5 was considered a reasonable value for the mean of the distribution in the peak of ultimate pH because of the inhibition of glycogen breakdown at ultimate pH about this value. Similarly, the mean for the distribution in the tail of ultimate pH should be close to 6.0.

3.2.2.2.2 CALCULATION OF PREDICTED GLYCOGEN

For each animal the predicted glycogen values ($\hat{G}o$) at slaughter were calculated as:

$$\hat{G}o = w_1.\hat{G}_H + w_2.\hat{G}_L \qquad [3.3]$$

where:

w_1 and w_2 are the weights for each record according to the probability of that observed pH value belonging to pH_1 or to pH_2 distribution, respectively. The weights are functions of the estimated means, variances and mixture frequency of the two normal distributions.

$\hat{G}_H$ and $\hat{G}_L$ are the expected values of glycogen that correspond for the sub-populations pH_1 and pH_2 included in the mixed distribution pH_U.

$\hat{G}_L$ corresponds to those muscle glycogen levels that are below the critical glycogen value (CG) (Figure 3.3) and were estimated based on the actual ultimate pH according to the following formula derived from the equation [2.7] proposed by Pleasants *et al.* (1999):

$$\hat{G}_L = \frac{\ln(7.2 - pH_U)}{0.05} + 10 \qquad [3.4]$$

These points represent circumstances where ultimate pH was elevated, as all glycogen had been catabolised without creating sufficient lactic acid to reduce pH to 5.5

$\hat{G}_H$ is related to the glycogen distribution that is above critical glycogen and the expected value is equal to the mean of that proportion of the distribution (Figure 3.3):

$$\hat{G}_H = \hat{\mu}_H = \hat{\mu}_{Go} + \frac{\hat{\sigma}_{Go}.h_{CG}}{p_{CG}} \qquad [3.5]$$

where

$\hat{\mu}_H$ is the mean of a truncated normal distribution with lower limit equal to CG

$\hat{\mu}_{Go}$ is the mean of muscle glycogen in the live animals

$\hat{\sigma}_{Go}$ is the standard deviation of muscle glycogen

h_{CG} is the height of the standard normal curve at the critical glycogen (CG)

p_{CG} is the total area to the right of CG, which was previously estimated as $\hat{p}_1$.

The transformation from ultimate pH to the underlying muscle glycogen works so that a change in pH is mapped to a change in glycogen. The back-transformation depends on the information about glycogen that is in the pH distribution. Because of the inactivation of glycolysis at pH of 5.5, it is not possible to restore the variability of the underlying glycogen for those pH values about 5.5, which corresponds to the glycogen distribution that is above critical glycogen. However, it is feasible to relate the observations in the peak of pH=5.5 to a representative glycogen level. Although Pleasants *et al.* (1999) proposed the critical glycogen as the value to be assigned, in this study ultimate pH of 5.5 were traced back to the mean of the glycogen distribution that is above critical glycogen. The most probable value of muscle glycogen for pH levels around 5.5 is the mean of that part of glycogen distribution. This mean ($\hat{\mu}_H$) was calculated as the mean of a truncated normal distribution, by [3.5], in which the critical glycogen value is the truncation point (Figure 3.3).

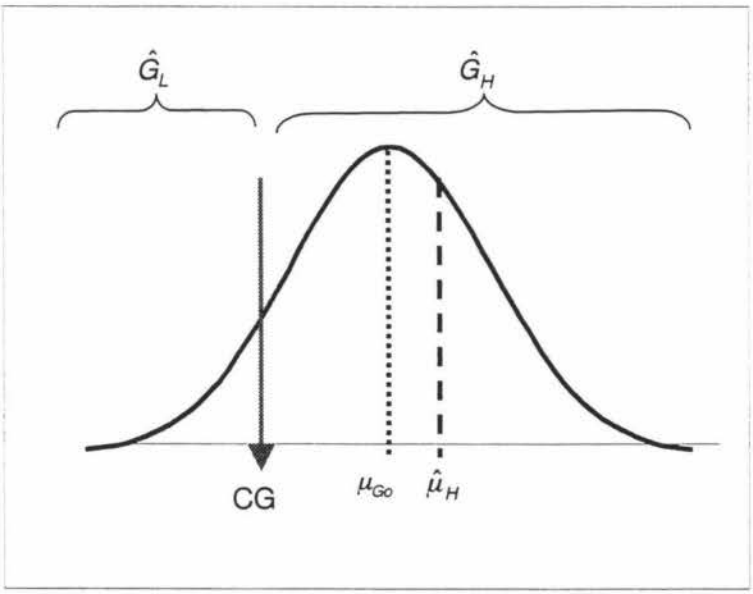


Figure 3.3: Diagram of glycogen distribution indicating the estimated glycogen values and critical glycogen (CG)

Assuming σ_{Go} equal to 15, the "unknown" μ_{Go} was estimated (denoted $\hat{\mu}_{Go}$) by Newton's method (Press *et al.*, 1986) to solve:

$$f\left(\int_{CG}^{\infty}(2\pi)^{-1/2}\exp\left[-\frac{(CG-\mu_{Go})^2}{2\sigma_{Go}^2}\right]-p_{CG}\right)=0$$

The normal probability integrals were computed using the algorithm proposed by Dutt (Ducrocq, 1984).

Based on that estimation of the mean and the assumed value of σ_{Go}, h_{CG} was calculated as:

$$h_{CG} = (2\pi)^{-1/2} \exp\left[-\frac{(z)^2}{2}\right]$$

where z is equal to

$$z = \frac{CG - \hat{\mu}_{Go}}{\sigma_{Go}}$$

Due to the inhibition of glycogen breakdown at pH of 5.5, variation around the peak of ultimate pH (f_{pH1}; Figure 3.2) is explained by measurement error of ultimate pH. Information on the variability of muscle glycogen has been lost for those animals with ultimate pH values around 5.5. On the other hand, the variance in the tail of pH distribution (f_{pH2}; Figure 3.2) is explained by the measurement error plus the variability in muscle glycogen below the critical glycogen, reflecting the differences among animals in muscle glycogen concentration. Consequently, the transformation of ultimate pH to glycogen exhibits different variances depending from which sub-population in ultimate pH they were calculated.

The variance for predicted glycogen in the individuals with values below the critical glycogen (σ_L^2) was equivalent to the measurement error expressed in terms of glycogen units.

Assuming a value for the unknown variance of muscle glycogen (σ_{Go}^2), the variance for predicted glycogen greater than the CG (σ_H^2) was computed as the variance of a truncated normal distribution. It was calculated as the proportion of the variance above the critical glycogen value (CG) (Figure 3.3) using the following equation:

$$\sigma_H^2 = \left[1 - \frac{h_{CG}}{p_{CG}}\left(\frac{h_{CG}}{p_{CG}} - z\right)\right]\sigma_{Go}^2$$

where h_{CG}, p_{CG} and z were previously defined.

The different variances in predicted glycogen indicate different reliability in the information, which was taken into consideration in the interval mapping. Most of the information comes from those predicted glycogen values that comes from elevated ultimate pH, which are more informative in terms of the differences in muscle glycogen at slaughter.

3.2.3 Interval Mapping

Estimates of QTL significance were obtained by regression analysis at each putative QTL position in 1-cM steps, along the mapped chromosome. The regression was performed on the conditional probability of having a given number of L1 QTL alleles, assuming a QTL at the putative position and given the genotypes for each pair of flanking markers.

3.2.3.1 CONDITIONAL PROBABILITIES OF QTL GENOTYPE

A pair of linked markers M and N (Figure 3.4) is assumed to straddle a QTL locus denoted Q. Distances between these loci (d) are measured in centiMorgans. Given a map function, these distances can be converted to recombination fractions (r.) to calculate the likely fraction of gametes with each combination of marker alleles, as in Figure 3.5.

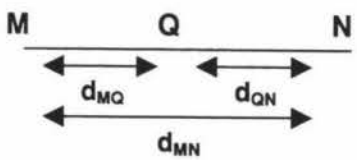


Figure 3.4: Map of section of chromosome between two markers, M and N, and a QTL, Q. d_{MN}, d_{MQ} and d_{QN} refer to the distances (in cM) between M and N, and between the QTL and each genetic marker, respectively

Figure 3.5 illustrates a back-cross design including one QTL (Q) and the two flanking markers M and N. Parental lines L1 and L2 differ in markers and QTL genotypes. It is assumed that the parental lines L1 and L2 present marker genotypes $M_1M_1N_1N_1$ and $M_2M_2N_2N_2$ and carry alleles Q_1 and Q_2 for the QTL, respectively.

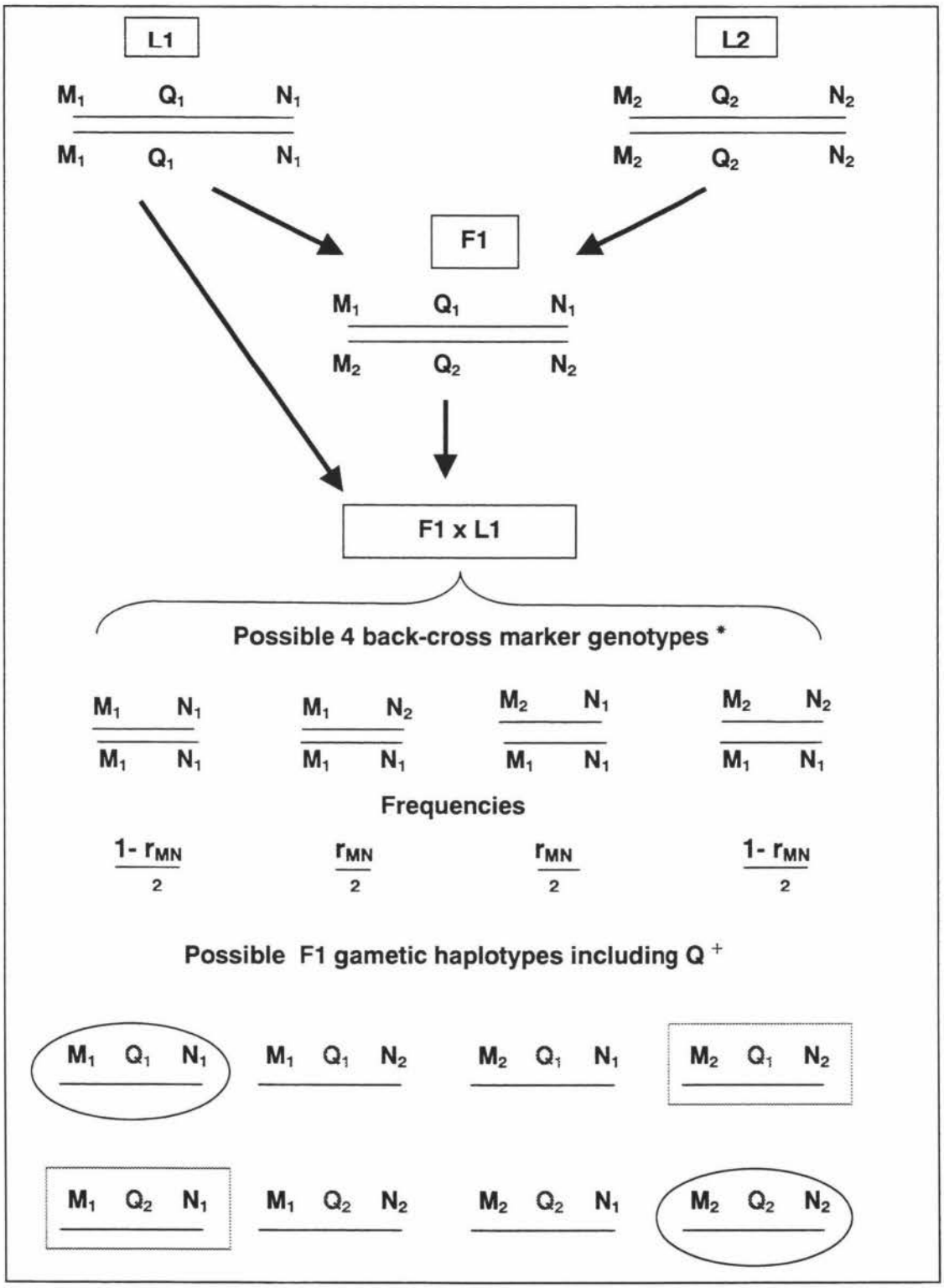
L1 and L2 are mated to produce an F1. In the F1 generation, all individuals have the same heterozygous genotype. The back-cross is generated by mating the F1 to one of the parental lines. In this study, it was mated to L1 and thus the genetic background of the back-cross individual is ¾ L1¼L2.

M_1N_1 is the only possible marker genotype for the gametes that are produced from L1. Thus, the possible marker genotypes in the back-cross are given by the four likely genotypes in the gametes for the F1 parents. These gametes include two recombinants (M_1M_2 and M_2M_1) and two non-recombinants (M_1M_1 and M_2M_2). The frequencies of recombinant and non-recombinant F1 gametes determine the frequencies of genotypes in the back-cross progeny. They are functions of the recombination rate between M and N (r_{MN})(Figure 3.5).

Each marker genotype may carry an L1- or L2-allele for the QTL. There are eight possible gametic haplotypes when the QTL is incorporated. Note that in this case only the chromosome from the F1 parent is shown (Figure 3.5). The gametic haplotypes include:

- non-recombinants produced without recombination between M and Q or Q and N (within a circle in Figure 3.5);
- single-recombinants produced when a recombination event takes place between M and Q or N and Q, and

double-recombinants originated when there is recombination between M and Q and Q and N (within a square in Figure 3.5).



* The symbols above the double line represent the paternal haplotype, whereas the symbols below indicate the maternal haplotype

+ The maternal haplotype is not shown, as these are all identical in the backcross to maternal L1

Figure 3.5: Illustration of a back-cross design including one QTL (Q) and the flanking markers M and N

The frequencies of the gametic haplotypes shown in Figure 3.5 are presented in Table 3.1a. The frequencies depend on the recombination parameters where r indicates the probability of a recombination event and (1-r) is the probability of no-recombination. For example, for M_1M_1 the probability of carrying Q_1 is explained by the probability of non-recombination among the QTL and the genetic markers: $(1-r_{MQ})(1-r_{QN})/2$. On the other hand, the haplotype $M_1Q_2M_1$ is possible if recombination events occur between M and Q and Q and N: $r_{MQ}.r_{QN}/2$. The probability for each QTL allele conditional on the marker haplotypes will depend on the probability of having inherited one particular QTL allele and the frequency of the marker haplotypes, which are indicated in Figure 3.5. The conditional probabilities for both QTL alleles are shown in Table 3.1b.

Table 3.1a: Probability of QTL alleles (Q_1 and Q_2) for the possible marker haplotypes

GAMETIC HAPLOTYPE	PROBABILITY OF:	
	Q_1	Q_2
M_1N_1	$(1-r_{MQ})(1-r_{QN})/2$	$r_{MQ}.r_{QN}/2$
M_1N_2	$(1-r_{MQ})r_{QN}/2$	$r_{MQ}(1-r_{QN})/2$
M_2N_1	$r_{MQ}(1-r_{QN})/2$	$(1-r_{MQ})r_{QN}/2$
M_2N_2	$r_{MQ}.r_{QN}/2$	$(1-r_{MQ})(1-r_{QN})/2$

Table 3.1b: Probability the QTL alleles (Q_1 and Q_2) conditional to the flanking markers haplotype

F1 GAMETE HAPLOTYPE	CONDITIONAL PROBABILITY OF:	
	Q_1	Q_2
M_1N_1	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$	$r_{MQ}.r_{QN}/(1-r_{MN})$
M_1N_2	$(1-r_{MQ})r_{QN}/r_{MN}$	$r_{MQ}(1-r_{QN})/r_{MN}$
M_2N_1	$r_{MQ}(1-r_{QN})/r_{MN}$	$(1-r_{MQ})r_{QN}/r_{MN}$
M_2N_2	$r_{MQ}.r_{QN}/(1-r_{MN})$	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$

3.2.3.2 REGRESSION ANALYSIS

The identification of QTL by linkage to marker loci is based on the identification of significant associations between the observed marker haplotypes and their means. A significant difference of means among the marker classes suggests the presence of a QTL linked to the markers (Falconer and Mackay, 1996).

The expected means of marker haplotypes are function of the proportion of each QTL genotype and the effect of the QTL. The means of the different marker haplotypes are presented in Table 3.2 where:

- the means are calculated as the sum of the products of Q_1 or Q_2 frequencies times the mean of the animals carrying Q_1 or Q_2 and then divided by the frequency of the marker haplotype group;
- the frequencies of the QTL alleles and marker haplotypes are given by the respective probabilities (Tables 3.1a and 3.1b and Figure 3.5); and
- the means of the groups of animals carrying Q_1 or Q_2, obtained from the F1 parent, are defined as:

$$\mu_{Q1} = \mu + \alpha$$

$$\mu_{Q2} = \mu$$

where μ is the mean of offspring carrying Q_2 and α is the difference in performance of an offspring with the Q_1 rather than the Q_2 allele, inherited from the F1 parent.

Table 3.2: Expected means of the different marker haplotypes

MARKER HAPLOTYPE	EXPECTED MEANS		
M_1N_1	$\frac{\frac{1}{2}(1-r_{MQ})(1-r_{QN})(\mu+\alpha)+\frac{1}{2}(r_{MQ}.r_{QN})\mu}{\frac{1}{2}(1-r_{MN})}$	=	$\mu+\frac{(1-r_{MQ})(1-r_{QN})}{(1-r_{MN})}\alpha$
M_1N_2	$\frac{\frac{1}{2}(1-r_{MQ})r_{QN}(\mu+\alpha)+\frac{1}{2}r_{MQ}.(1-r_{QN})\mu}{\frac{1}{2}r_{MN}}$	=	$\mu+\frac{(1-r_{MQ})r_{QN}}{r_{MN}}\alpha$
M_2N_1	$\frac{\frac{1}{2}r_{MQ}(1-r_{QN})(\mu+\alpha)+\frac{1}{2}(1-r_{MQ})r_{QN}\mu}{\frac{1}{2}r_{MN}}$	=	$\mu+\frac{r_{MQ}(1-r_{QN})}{r_{MN}}\alpha$
M_2N_2	$\frac{\frac{1}{2}(r_{MQ}.r_{QN})(\mu+\alpha)+\frac{1}{2}(1-r_{MQ})(1-r_{QN})\mu}{\frac{1}{2}(1-r_{MN})}$	=	$\mu+\frac{r_{MQ}r_{QN}}{(1-r_{MN})}\alpha$

The second column in Table 3.2 shows that the means of marker classes are explained by the means of the Q-alleles weighted by their frequencies. The equations in the third column are obtained after operating with the expression in the previous column, where the coefficients for α are the probability of having inherited Q_1 from the F1 parent, conditional on the marker haplotype (see Table 3.1b).

Consider that there is a QTL between the two markers M and N with r_{MN}=0.17. The QTL is closer to marker M that to N. The recombination rates between QTL and markers are: r_{MQ}=0.05 and r_{QN}=0.13. The proportions of marker haplotypes are 0.418 for M_1N_1 and M_2N_2, and 0.082 for M_1N_2 and M_2N_1. The proportions of recombinant markers are lesser than the non-recombinants because the markers are linked. Markers in linkage equilibrium (r_{MN}=0.5) have recombinants and non-recombinants in equal proportions (0.25).

Marker haplotype M_1N_1 has the higher probability of carrying Q_1 (0.415). Because the frequency of double recombination is low, the probability of having Q_1 for M_2N_2 is close to zero. The values for M_1N_2 and M_2N_1 are 0.06 and 0.02, respectively.

The conditional probabilities, according to equations in Table 3.1b, are 0.99, 0.75, 0.25, and zero for M_1N_1, M_1N_2, M_2N_1 and M_2N_2, respectively. These values indicate that 99% of the M_1N_1 individuals inherited the allele Q_1, whereas any M_2N_2 has this allele. This implies that the means of these marker classes are explained by μ_{Q1} and μ_{Q2}, respectively. On the other hand, the conditional probabilities for M_1N_2 and M_2N_1 are intermediate. Although 75% of the M_1N_2 animals carry Q_1, the absolute quantity of animals is low because the frequency of this class is only 8%. In this case, the mean of each marker haplotype is defined as a mixture of animals carrying Q_1 or Q_2. For instance, the mean of M1N2 can be expressed as $0.75\,\mu_{Q1} + 0.25\,\mu_{Q2}$.

The expected means of marker haplotypes in this example are μ, $\mu + 0.25\alpha$, $\mu + 0.75\alpha$ and $\mu + \alpha$, for M_2N_2, M_2N_1, M_1N_2, and M_1N_1, respectively. The expected means regressed on these probabilities are illustrated in Figure 3.6.

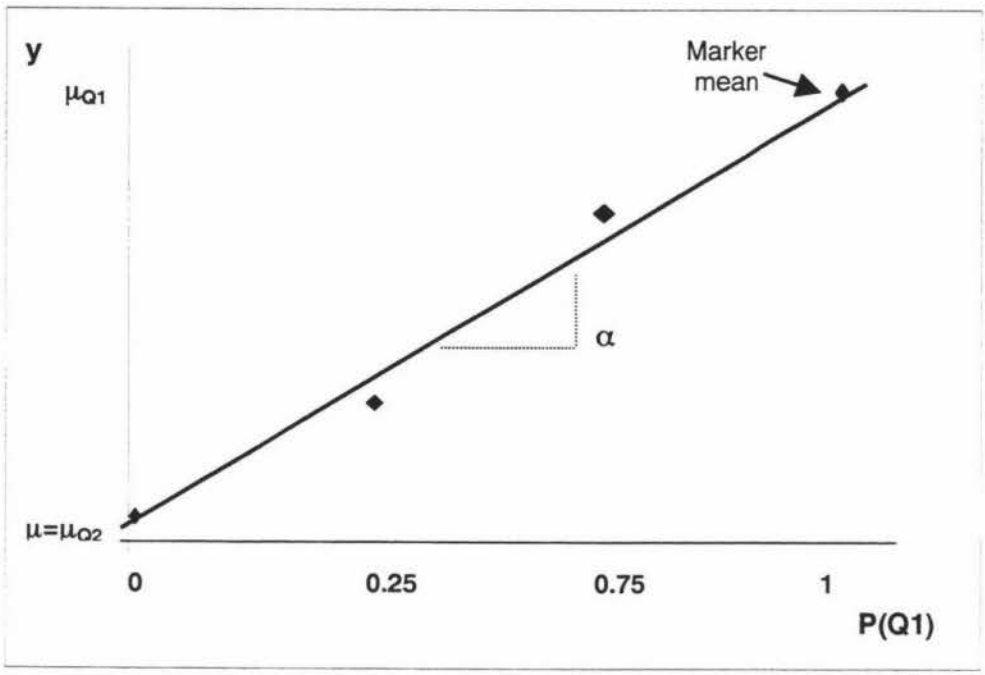


Figure 3.6: Regression of phenotypic values (y) on the probability of Q_1-allele of a QTL flanked by markers M and N

The probability of an individual having a particular QTL allele depends on the location of the QTL and the allele values at flanking markers. By moving a putative QTL along the chromosome, the most likely position of the QTL corresponds to the position where the best fit of the regression line is obtained.

The following single-QTL regression model was fitted at 1-cM intervals to muscle glycogen at slaughter, ultimate pH, the logarithmic transformation of pH and predicted glycogen in separate univariate analysis:

$$y_i = \mu + \alpha x_i + e_i$$

where:

y_i is the i^{th} phenotypic observation;

μ and α are the intercept and regression coefficient that were previously defined

x_i is the probability of having inherited a paternal (or F1) Q_1 for the i^{th} observation, conditional on the observed marker genotypes;

e_i is the random residual for i^{th} phenotypic observation.

The fitness of the regression was evaluated in this study by a *t*-like statistic defined as:

$$t\text{-statistic} = \frac{R(\alpha|\mu)}{MSE}$$

where $R(\alpha|\mu)$ is the sum square of the regression corrected by the mean and *MSE* the mean square error.

The regression analysis was performed for all the traits by the method of ordinary least squares (OLS), assuming that the residual errors are homoscedastic ($\sigma^2(e_i) = \sigma_e^2$) and uncorrelated ($\sigma(e_i, e_j) = 0$; $i \neq j$). However, predicted glycogen was also analysed by weighted least squares (WLS) to take into account the different variances of the observations.

The normal equations can be written in matrix form as:

$$\mathbf{X'V^{-1}Xb = X'V^{-1}y}$$

where **X** is the design matrix, **b** is the vector of parameters to be estimated, **y** is the vector of observations and **V** is the variance-covariance matrix of the observations. Under the assumption of homoscedasis and independence, **V** is equal to an identity matrix **I** and the normal equations simplify:

$$\mathbf{X'Xb = X'y}$$

When the observations are independent but have different variances such as for predicted glycogen, **V** is a diagonal matrix so that:

$$\mathbf{V} = \begin{bmatrix} \sigma_H^2 & & & 0 \\ & \sigma_H^2 & & \\ & & \cdots & \\ 0 & & & \sigma_L^2 \end{bmatrix}$$

where σ_H^2 and σ_L^2 are the variances for the predicted glycogen values above and below the critical glycogen (CG), respectively.

3.2.3.3 OVERALL SIGNIFICANCE LEVEL

Nominal significance was established by permutation analysis. Because marker classes contain two QTL genotypes, the phenotypic distribution within marker classes is a mixture of two normal distributions (Falconer and Mackay, 1996). Therefore,

critical values for test statistics assuming a normal distribution of phenotypes may not be appropriate. Permutation analysis derives an actual distribution for the test statistic and has the property of no dependence on the distribution of the data.

For a given QTL position, the phenotypes are randomly assigned to marker genotypes and the test statistic computed in each shuffle. This process is repeated several hundreds of times, generating an empirical distribution for the test under the null hypothesis of no association between phenotype and genotype (Churchill and Doerge, 1994). Based on the distributions of the test statistics, significant levels of 1%, 5% and 10% were calculated for a two-sided hypothesis test. The permutation tests were undertaken for the four simulated traits analysed by OLS (5000 shuffles). Predicted glycogen was also analysed by WLS (1000 shuffles).

3.3 RESULTS AND DISCUSSION

3.3.1 Simulated traits

Ultimate pH was computed from simulated muscle glycogen according to the knowledge of the biochemical process captured by the approach proposed by Pleasants *et al.* (1999). In the first step, it was calculated directly from muscle glycogen for those values below the critical glycogen, assuming that all glycogen is metabolised. Glycogen contents equal or greater than critical glycogen were initially mapped to a value of 5.5, because at this value of pH the conversion of glycogen to lactic acid is inhibited. Actual ultimate pH was obtained after including the measurement error. Thus, in pH range from 5.3 to 5.5 most glycogen levels are higher than critical glycogen (Figure 3.7). However, some glycogen levels close from below to critical glycogen are associated with these values of ultimate pH due to the random measurement error.

The understanding of the biochemical process indicates that muscle glycogen lower than critical glycogen leads to an ultimate pH higher than 5.5. Figure 3.7 shows that simulated pH values higher than 5.5 are mostly determined by lower muscle glycogen. Nevertheless, pH of 5.6 and 5.7 are given by glycogen levels above critical glycogen, which is explained by the effect of measurement error on the actual ultimate pH. On the other hand, ultimate pH values equal or higher than 5.8 are exclusively linked to muscle glycogen below critical glycogen.

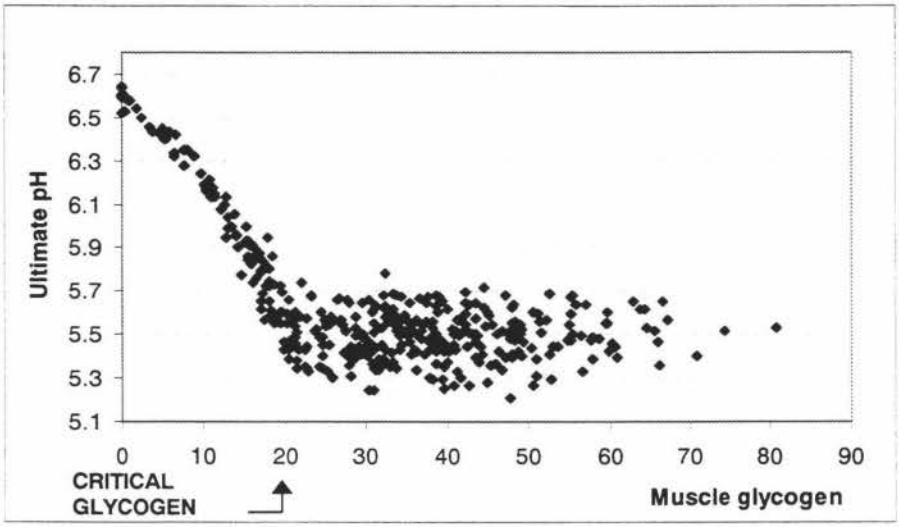


Figure 3.7: Association between muscle glycogen and beef ultimate pH based on simulated data

Ultimate pH values of 5.3 to 5.5 were traced back to predicted glycogen levels of approximately 35, which was the value estimated as the average of the portion of muscle glycogen distribution above critical glycogen ($\hat{G}_H$)(Figure 3.8). As ultimate pH increases the probability of belonging to the distribution (w_1) that corresponds to the peak of ultimate pH (f_{pH1}) decrease. Therefore, predicted glycogen has a lower influence of $\hat{G}_H$ and declines. Higher values of ultimate pH also indicate a greater probability (w_2) of belonging to the tail of pH distribution (f_{pH2}) with a more important effect of $\hat{G}_L$ in predicted glycogen calculation. $\hat{G}_L$ is the direct back-transformation of ultimate pH to glycogen assuming that the tail in pH distribution is determined by the glycogen levels lower than critical glycogen. Ultimate pH and predicted glycogen reproduce the expected linear association between pH and muscle glycogen for pH values higher than 5.7.

Predicted glycogen depends on the estimated values of the weights as well as the values for $\hat{G}_L$ and $\hat{G}_H$. Because weights w_1 and w_2 are functions of the estimated means, variances and mixture frequency of the two normal distributions, they rely on the solutions of the mixture of two normal distributions. The parameter estimates were 0.66, 5.51, 6.17, 0.01 and 0.11 in scenario 1 (QTL size =σ_a), and 0.69, 5.50, 6.03, 0.011 and 0.12 in scenario 2 (QTL size = 0.5σ_a), for p_1, μ_1, μ_2, σ_1^2 and σ_2^2, respectively.

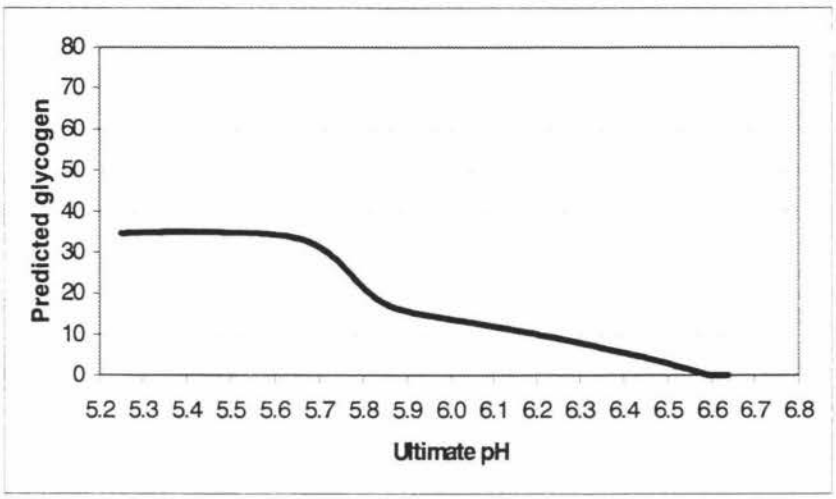


Figure 3.8: Relationship between ultimate pH and predicted glycogen in the simulated back-cross

$\hat{G}_L$ and $\hat{G}_H$ were calculated based on an glycogen distribution characterised by hypothetical values of mean and variance. Predicted glycogen had the expected values according to the assumed parameters for the underlying muscle glycogen. However, these values cannot be interpreted in biological terms because the assumed parameters do not show equivalence with the scarce published information about the parameters of glycogen distribution. Different authors reported that muscle glycogen content typically ranges from 60 to 130 mmol/kg in healthy well-fed and rested animals (Warris *et al.*, 1984; Tarrant, 1989; Lambert *et al.*, 1998; Immonen *et al.*, 2000), although levels of 200 mmol/kg have been reported in grain-fed cattle (Fergusson *et al.*, 2001). Furthermore, published results indicated that 45 to 55 mmol/kg muscle are required to lower pH from 7.2 to 5.5 (Warris *et al.*, 1984; Warris, 1990; Inmonen *et al.*, 2000). Despite the information available, knowledge is lacking in terms of average and variability of levels of muscle glycogen at slaughter.

Using real values of mean and variance for muscle glycogen implies re-calculating the parameter in equations that relates ultimate pH and muscle glycogen, as well as critical glycogen. If the captured information has correspondence with the actual magnitudes of muscle glycogen, it will facilitate the interpretation of results from a biological point of view.

3.3.1.1 DESCRIPTION OF SIMULATED MUSCLE GLYCOGEN, ULTIMATE pH AND PREDICTED GLYCOGEN

The amount and distribution of muscle glycogen in the foundation generations are very similar for both sizes of QTL (scenarios 1 and 2), when Lines 1 and 2 are considered together. Table A1.1 (Appendix 1) presents the estimated phenotypic means and standard deviations of muscle glycogen in the simulated parental lines, F1 and back-cross generations in both scenarios. The possible genotypes for the QTL are specified for all the generations and the back-cross animals are split into the two possible genotypes (Q_1Q_1 and Q_2Q_1). The difference in the average levels between Line 1 and Line 2 in scenario 1 (38 vs 23) is approximately twice the difference in scenario 2 (37 vs 27), reflecting the different QTL size simulated in each case. In the same way, the differences between the means of sub-populations Q_1Q_1 and Q_2Q_1 reflect the different QTL effects. Consequently, scenario 1 presents a greater difference between sub-populations in the percentage of animals below the critical glycogen (CG) than scenario 2 (Table A1.1).

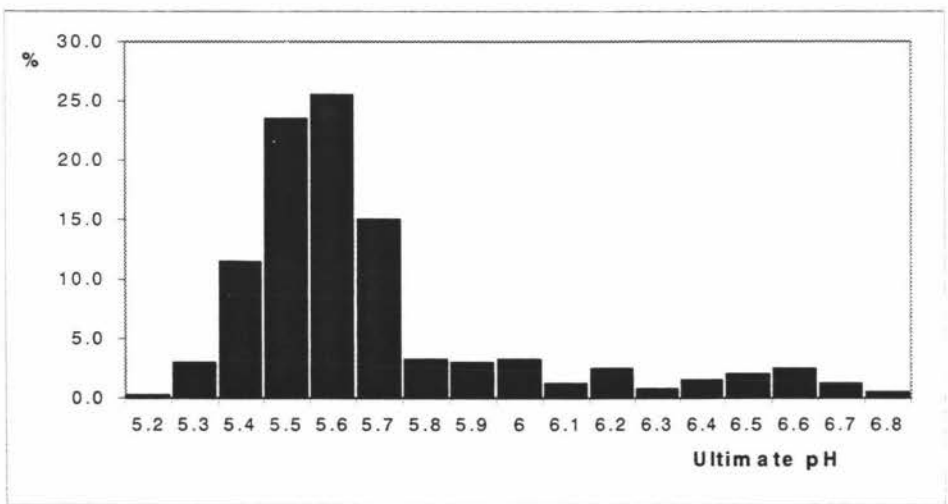


Figure 3.9: Frequency distribution of ultimate pH in the simulated back-cross (scenario 1: QTL size = σ_a)

Ultimate pH in the back-cross exhibited the expected skewed frequency distribution. Figure 3.9 shows the distribution for scenario 1 whereas the data that corresponds to scenario 2 is presented in Table A1.2. Ultimate pH data in the back-cross was similar in both scenarios because of the similar levels of glycogen. The percentages of records above 5.8 were 18.0% and 17.5% in scenarios 1 and 2, respectively. The

incidence of high pH is in the range of results reported in New Zealand by Graafhuis and Devine (1994) and Smith *et al.* (1996) as being obtained in commercial meat plants (30 and 8%, respectively). The logarithmic transformation of ultimate pH presented a skewed distribution that is similar to the frequency distribution of ultimate pH.

The highest values of predicted glycogen were derived from the peak of the pH distribution located around 5.5. Conceptually, they correspond to the proportion of the underlying glycogen distribution above the critical glycogen. On the other hand, the frequency classes below approximately 21 units of predicted glycogen (critical glycogen CG) correspond to those animals that presented the highest values of ultimate pH. The percentages of observations in the pH levels that correspond to the tail of the distribution are very similar to the percentage of predicted glycogen values below CG (Table A1.2). Although the means of predicted glycogen and the original muscle glycogen are in general very similar, the differences are smaller when the proportion of information below the critical glycogen increases. A similar trend is observed when comparing the standard deviation of both traits.

3.3.2 Interval mapping

3.3.2.1 PERMUTATIONS TEST

Table 3.3 presents the average of critical values for all positions and traits analysed by OLS. In addition, this table shows the average critical value for predicted glycogen analysed by WLS and the tabulated *t*-values for three significance levels (1%, 5% and 10%). Comparisonwise values obtained in several positions along the chromosome for each trait and both scenarios are presented in Tables A1.3, A1.4 and A1.5.

In agreement with Churchill and Doerge (1994) and Spelman *et al.* (1996), the comparisonwise critical values, for analysis by OLS, were relatively constant in both scenarios along the chromosome and similar to the corresponding tabulated *t*-values in all traits. All the analysed traits had very similar comparisonwise values, which was also reported by Spelman *et al.* (1996), who analysed five genetically correlated traits for marker-QTL effects: milk, fat and protein yield and fat and protein percentage. The similarity among the critical values for the correlated traits indicates that the same value is suitable in determining which QTL results are significant for the correlated

characteristics. The tabulated *t*-values are representative of the comparisonwise critical values for each significance level (α) in the analysed traits along the mapped chromosome.

Table 3.3: Comparisonwise threshold levels: average of all positions along the chromosome for interval mapping performed by ordinary least square (OLS) and weighted least square (WLS)

SIGNIFICANCE LEVELS (α)	OLS[1]	WLS[2]		TABULATED *t*-VALUES [3]
		Scenario 1	Scenario 2	
1%	2.58	3.25	3.27	2.58
5%	1.96	2.49	2.51	1.96
10%	1.65	2.10	2.12	1.65

[1] Representative value for all analysed traits
[2] For predicted glycogen
[3] Assuming normality

Predicted glycogen when analysed by WLS presents higher comparisonwise values than in OLS (Table 3.3) due to different approximated distributions of the *t*-statistic (Figure 3.10). The comparison between scenarios shows that the averages of the significant levels are slightly greater in scenario 2 compared to scenario 1.

WLS take into consideration the different reliability of the predicted glycogen data based on the different variances. Predicted glycogen that was mapped from the tail of pH distribution reflects the different glycogen contents among animals. On the other hand, those values traced from the peak of pH distribution present a low variance because in those individuals the variability of muscle glycogen disappeared. By including the variances for both groups, WLS maximises the effect of more informative data that corresponds to predicted glycogen calculated from the high pH values. This may produce an effect similar to reducing the number of observations with the consequently increasing in the magnitude of critical values.

The critical values for the WLS analysis show a higher variability among the positions along the chromosome in both scenarios that may be explained by the lower number of shuffles used (1000 in WLS vs 5000 in OLS). This variation increases when the significance levels decrease (Table A1.5). Larger numbers of shuffles provide more

reliable estimations of the critical values specially for more extreme values such as α=1% (Churchill and Doerge, 1994).

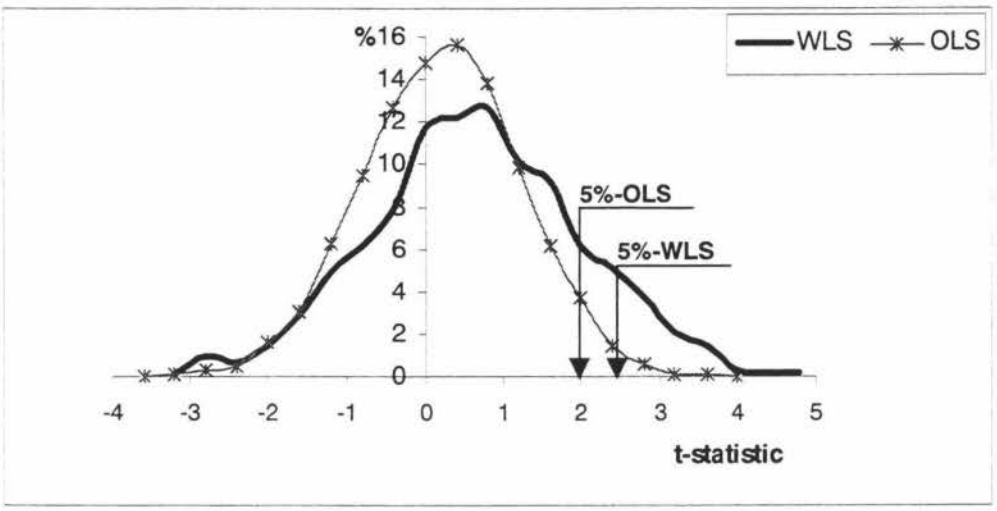


Figure 3.10: Approximate distribution of test-statistics for predicted glycogen analysed by weighted least square (WLS) or ordinary least square (OLS). Critical values for α=5% are indicated. (WLS: 1000 shuffles; OLS: 5000 shuffles)

3.3.2.2 QTL DETECTION

The absolute values of the *t*-test along the mapped chromosome for the four analysed traits in scenario 1 are illustrated in Figure 3.11. The *t*-test curves for all traits exceed the significant thresholds and reveal the presence of a QTL. The maximum *t*-value in each curve corresponds to the most likely position of the QTL. The results obtained for muscle glycogen and predicted glycogen (WLS) indicate that the QTL is located in position 27cM of the mapped chromosome, very close to the actual position (25 cM). Based on the test statistics for ultimate pH, logarithmic pH and predicted glycogen, the QTL is situated at 29 cM.

Although all the correlated traits suggest the presence of a QTL, ultimate pH and logarithmic transformation of pH presented the lowest peak in the test statistic. Both curves were very similar indicating that the logarithmic transformation did not modify the *t*-test compared to the ultimate pH.

On the other hand, predicted glycogen showed clearer evidence of a QTL by having a higher profile compared to the former traits. Figure 3.11 shows an effect of the method

used in the regression analysis. When the predicted glycogen was analysed by WLS, the back-transformation of ultimate pH to glycogen had a *t*-curve even higher and closer to muscle glycogen. Although the critical values are also higher for WLS, it indicates the presence of the simulated QTL.

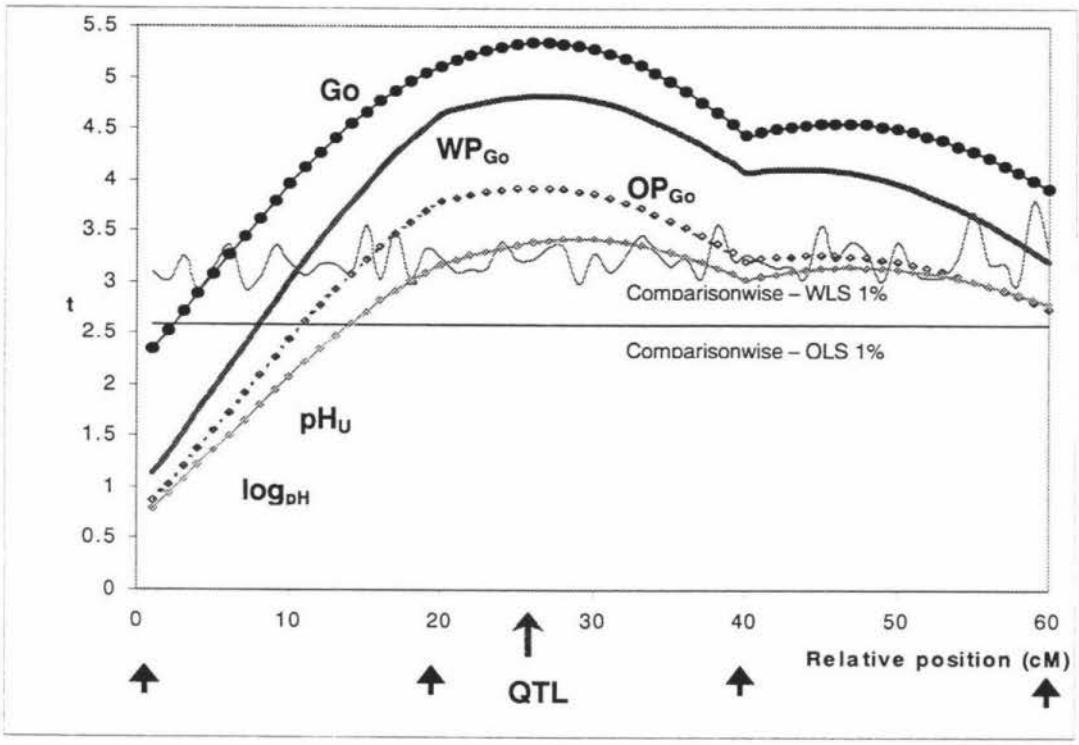


Figure 3.11: Curves of *t*-test absolute value along the chromosome for muscle glycogen (Go), ultimate pH (pH_U), logarithmic transformation of pH_U (log_{pH}) and predicted glycogen using OLS and WLS (OP_{Go} ,WP_{Go}) in scenario 1. (Arrows indicate positions of markers)

The values of the *t*-test for all the analysed points and all the traits are lower in scenario 2 than in scenario 1 (Figure 3.12). The smaller size of the QTL determines lesser differences between the sub-populations in scenario 2, which explains the dissimilarity in the test statistic curves.

Muscle pH and the logarithmic transformation showed very similar *t*-test curve also in scenario 2 (Figure 3.12). However, in this case neither of these traits exceeded any of the calculated critical values (Table 3.3). Although pH is determined by the glycogen levels, this characteristic and the logarithmic transformation failed to identify the QTL affecting glycogen. This fact can be explained by the smaller difference in the average

of both sub-populations compared to scenario 1 in addition to the their skewed distributions.

The test statistics of predicted glycogen (OLS) and muscle glycogen in scenario 2 are very close (Figure 3.12). In this case, the profile of predicted glycogen (OLS) is even more similar to muscle glycogen than in scenario 1. The higher percentage of observations in the tail of pH distribution determines that predicted glycogen in scenario 2 is comparatively more informative about the differences in the underlying glycogen that in scenario 1. The values in the tail of ultimate pH reflect the relationship between pH and glycogen and thus these observations reproduce the variability in muscle glycogen. This also explains the greater distance between predicted glycogen and ultimate pH profiles comparing scenarios 2 and 1.

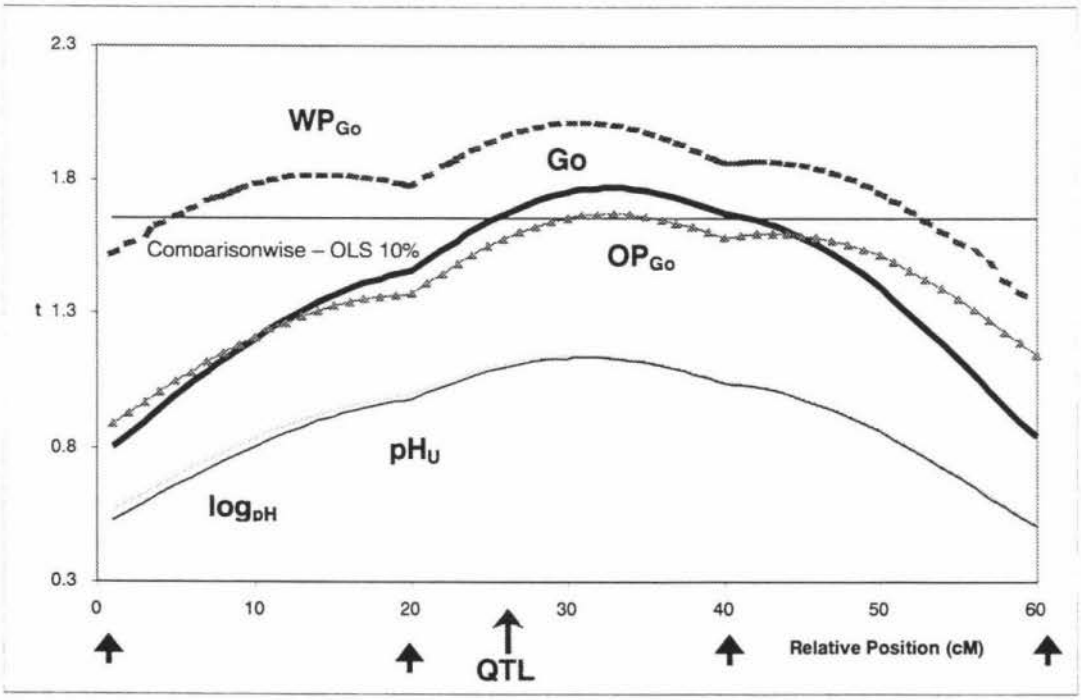


Figure 3.12: Curves of *t*-test absolute value along the chromosome for muscle glycogen (Go), ultimate pH (pH_U), logarithmic transformation of pH_U (log_{pH}) and predicted glycogen using OLS and WLS (OP_{Go} ,WP_{Go}) in scenario 2. (Arrows indicate positions of markers)

Although predicted glycogen and muscle glycogen significantly indicate the presence of a QTL at 33 cM (α =10%), the highest values of both characteristics scarcely exceed the comparisonwise levels. On the other hand, predicted glycogen (WLS

presented a higher profile than the former traits. However, the greater comparisonwise levels for WLS determine that in this case the highest value (31 cM) was slightly lower than the critical value (2.01 versus 2.04).

3.4 CONCLUSIONS

Ultimate pH is an economically important trait due to its effect on meat quality that affect the export markets that can be achieved and in some countries has a direct effect on the price of the products. Although ultimate pH is the economically important trait, this approach is orientated to the analysis of the underlying glycogen that determines ultimate pH. In this sense, this study was orientated to the detection of a QTL affecting muscle glycogen and consequently the ultimate pH.

The power in the detection of the QTL affecting muscle glycogen and therefore ultimate pH depends on the trait analysed. The logarithmic transformation of ultimate pH did not increase the power compared to ultimate pH, since they both presented the lowest test statistics independently of the size of the simulated QTL. Furthermore, when the QTL effect was small both characteristics did not identify the presence of the QTL probably due to more skewed distributions in both traits.

By applying the biochemical knowledge of the pathways that connect muscle glycogen and ultimate pH, it is possible to transform the actual pH data to the underlying glycogen. This new trait called predicted glycogen, presented a higher peak than pH indicating that it is possible to improve the significance in QTL searching based on the new approach.

The method proposed by Pleasants *et al.* (1999) discounts the information around the peak of ultimate pH in favour of the data contained in the tail of the distribution. The data in the tail reflects the differences among animals in the underlying muscle glycogen content. In the peak, the information about the individual differences has been lost due to the inhibition of the conversion of glycogen to lactic acid at pH=5.5.

The results obtained in scenarios 1 and 2 indicate that this method performed comparatively better when the QTL size was small. A smaller effect of the QTL affecting muscle glycogen determined comparatively lower levels of glycogen in both sub-populations (Q_1Q_1 and Q_2Q_1). Therefore, higher proportions of data in the tail of

pH distribution were observed in the sub-populations compared to scenario 1, which explains the higher power of predicted glycogen.

The calculation of predicted glycogen is based on some parameters of muscle glycogen and its relationship with ultimate pH. The magnitude of critical glycogen CG as well as the mean and standard deviation of muscle glycogen are important parameters whose values have to be assumed in order to back-transform the actual values of pH to the glycogen. The value of CG in conjunction with the characteristic of muscle glycogen distribution determines the expected proportions of observations in the tail and in the peak of ultimate pH distribution. These parameters are required to estimate the expected values of predicted glycogen from ultimate pH. Although this study did not analyse the effect of the accuracy of parameters assumed, they may have an important impact in the performance of the new approach. Beyond the potential influence of the accuracy of the parameters on the effectiveness of this approach, the utilisation of estimates of the real parameters of muscle glycogen will lead to predicted glycogen values that can be interpreted in biological terms. Taking into account that this approach would give more power to the investigations on ultimate pH, predicted glycogen expressed in real units would make the results understandable from a biological point of view and comparable to other studies.

The method proposed by Pleasants *et al.* (1999) captures the information about the underlying muscle glycogen from ultimate pH, assuming that pH is a mixture distribution. The back-transformation to predicted glycogen depends on the estimation of the parameters of the mixture distribution. The maximum likelihood algorithm used in this study, and by Pleasants *et al.* (1999), presents the problem that convergence is not guaranteed. However, there are other algorithms without this restriction, which should be considered in future applications of this novel approach.

In summary, the results obtained in this simulation indicate that applying the biochemical knowledge of the pathways that connect glycogen and ultimate pH it is possible to increase the power in the identification of a QTL affecting these traits. A comparative better performance of the new approach is expected in conditions of lower glycogen contents and consequently higher ultimate pH.

A comprehensive characterisation of muscle glycogen and its biochemical relationship with ultimate pH is considered relevant to obtain real values of the parameters required in the back-transformation of ultimate pH to the underlying muscle glycogen.

CHAPTER FOUR

MAPPING QUANTITATIVE TRAIT LOCI AFFECTING ULTIMATE pH IN A GENETIC MARKER EXPERIMENT

4.1 INTRODUCTION

Beef quality has become increasingly of economic importance to cattle breeders as a result of increasing market-orientation in the beef industry.

Ultimate pH is a characteristic of beef that influences other attributes that are relevant for consumers such as tenderness, colour and keeping quality. It is primarily determined by the content of glycogen in the muscles at the time of slaughter since it is the post-mortem catabolism of glycogen to lactic acid that determines the decrease of pH (Tarrant, 1989).

In spite of the relevance of meat quality traits, they have not been emphasised in genetic improvement programs because genetic evaluation for these attributes in breeding animals is generally time consuming, difficult and expensive. The identification of genetically superior breeding animals for beef quality usually requires progeny testing, which is a long-term and costly option. The development of marker-assisted selection (MAS) is an appealing alternative because it could allow direct assessment of breeding animals that would reduce the time required for the evaluation (Burrow *et al.*, 2001). The implementation of marker-assisted selection, based on the exploitation of quantitative trait loci associated with genetic markers, first requires the identification of QTL affecting beef quality attributes.

Genome-wide screening of DNA markers have revealed a number of putative QTL associated with beef quality characteristics, such as tenderness and fat colour (Hetzel *et al.*, 1997; Keele *et al.*, 1999; Morris *et al.*, 2001a), although in general few results have been published (Burrow *et al.*, 2001). However, no QTL for ultimate pH in beef has yet been reported.

The objectives of the present study are:

- Searching for QTL influencing ultimate pH in two chromosomes using experimental data collected by AgResearch. In 1995 a collaborative project was established by AgReseach and Adelaide University to identify DNA markers for carcass composition and meat quality (Morris *et al.*, 2001b). The trial is a reciprocal back-cross design between two extreme breeds, Limousin and Jersey.

- Comparing the performance of four traits in the detection of a QTL associated with ultimate pH. In addition to ultimate pH, three other related traits are analysed: logarithmic transformation of ultimate pH; glycogen content in the live animal that is biochemically related to ultimate pH; and the back-transformation of ultimate pH to the underlying glycogen levels (predicted glycogen) based on the approach proposed by Pleasants *et al.* (1999).

4.2 MATERIALS AND METHODS

4.2.1 Description of the experiment

The information used in this analysis arose from an AgResearch DNA-marker trial. The experiment was a double back-cross design between two divergent breeds Limousin (LL) and Jersey (JJ). The back-crosses were generated by mating three first-cross (F1) Jersey x Limousin bulls to cows of both parental breeds. Approximately 406 records were analysed in this study. Table 4.1 describes the number of observations per F1 sire in each back-cross.

The back-cross calves were born in 1996 and 1997 and reared in different conditions (Morris *et al.*, 2001b) (Appendix 2, Table A2.1). Steers and heifers were pre-allocated to slaughter groups of approximately 15 animals of the same sex, according to the criteria reported by Morris *et al.* (2001b) (Appendix 2, Table A2.2). The animals were killed in 1998 and 1999 at ages ranging from 22 to 28 months. The slaughter groups were killed at weekly intervals in both years in the Ruakura Experimental Abattoir.

Table 4.1: Number of observations for each F1 sire and by back-cross

FIRST-CROSS SIRES	BACK-CROSS		Total
	F1 x JJ	F1 x LL	
S1	86	53	**139**
S2	80	68	**148**
S3	93	26	**119**
Total	**259**	**147**	***406***

4.2.2 Analysed traits

Pre-slaughter muscle glycogen, ultimate pH, logarithmic transformation of pH and predicted glycogen were analysed. The first two characteristics were recorded in the genetic marker project established by AgResearch and Adelaide University. The other two traits were transformed from the actual pH data.

Muscle glycogen corresponds to the glycogen concentration measured in the live animals. A 300-mg biopsy sample was extracted under local anaesthetic from the *longissimus dorsi* muscle, frozen and analysed for the glycogen concentration using the technique described by Dreiling *et al.* (1987). The sampling was undertaken on 3 and 2 dates for the animals killed in 1998 and 1999, respectively. The average interval between the glycogen measurement and the slaughter were 56 days with a minimum of 32 and a maximum of 81 days (see Appendix 2, Table A2.2). This characteristic is expressed in mg of equivalent glucose per gram of muscle.

Muscle pH was periodically recorded from 30 minutes after slaughter and until the development of *rigor mortis* at the striploin in carcasses that were not electrically stimulated (Morris *et al.*, 2001b). The trait analysed in this study was ultimate pH, which was defined as the lowest pH value attained in the first 24 hours (Morris *et al.*, 2001b).

Predicted glycogen was computed from the actual ultimate pH based on the approach proposed by Pleasants *et al.* (1999). The calculations followed the steps detailed in Chapter 3 (section 3.2.2.2):

1. Estimation of the parameters of the mixture distribution of ultimate pH.
2. Computation of predicted glycogen as a function of the expected values for each distribution weighted for each ultimate pH record by the probability of belonging to each sub-population.

This procedure generates a set of look-up values relating ultimate pH to predicted glycogen. The look-up values are dependent on the estimated parameters of the mixture distribution.

4.2.3 Molecular information

Twenty-seven genetic markers located on two chromosomes were analysed. The distances among markers are in Table 4.2, as provided by AgResearch. The molecular data consisted of the genotypes at each marker for the three sires used in the experiment and their parents, as well as genotypes of the progeny when the sires were heterozygous for a particular marker.

For each marker, the most likely breed origin combination was derived for the F1 sires using the information of their parents. The numbers of informative and non-informative markers are described in Table 4.3 for the three F1 sires and both chromosomes. A marker is considered informative if it is possible to determine which parental allele was passed to the progeny (Weller, 2001). The markers were classified as non-informative when:

- the F1 sire was homozygous,
- its parents were not genotyped , or
- parents and F1 sire had the same genotype.

Table 4.2: Position of the genetic markers on chromosomes A and B

CHROMOSOME A		CHROMOSOME B	
Marker	**Position (cM)**	**Marker**	**Position (cM)**
AM1	0	BM1	1
AM2	8	BM2	13
AM3	12	BM3	22
AM4	57	BM4	26
AM5	76	BM5	35
AM6	88	BM6	36
AM7	91	BM7	66
AM8	92	BM8	67
AM9	107	BM9	73
AM10	109	BM10	90
AM11	122	BM11	94
AM12	123		
AM13	146		
AM14	151		
AM15	166		
AM16	174		

Table 4.3: Number of informative and non-informative markers per sire in chromosomes A and B

DESCRIPTION OF GENETIC MARKERS	F1 SIRES			TOTAL
	S1	S2	S3	
NON-INFORMATIVE	**6**	**7**	**10**	**23**
Chromosome A	3	4	7	14
Chromosome B	3	3	3	9
INFORMATIVE	**21**	**20**	**17**	**58**
Chromosome A	13	12	9	34
Chromosome B	8	8	8	24

The most likely breed origin was assigned to the marker haplotypes for each back-cross individual. Assuming a heterozygous sire for a particular marker with a genotype 'ab', the progeny were coded as having received one of the sire's allele ('a' or 'b') and one of the dam's alleles ('a', 'b' or other, coded as 'c'). The breed line combination for the possible genotypes in the offspring of a F1 sire x Jersey dam is presented in Figure 4.1. It was assumed that the derivation of the breed combination for the F1 sire indicated that the alleles 'a' and 'b' were inherited from Jersey and Limousin, respectively. For the genotypes 'ac' and 'bc', it is clear which alleles have been inherited from sire and dam, and the breed combination. For a 'aa' genotype, although it is not possible to define the inherence of each allele, there is only one possible breed combination because both alleles are J. Therefore, genotypes 'aa', 'ac' and 'bc' are clearly informative about the breed combinations in this example.

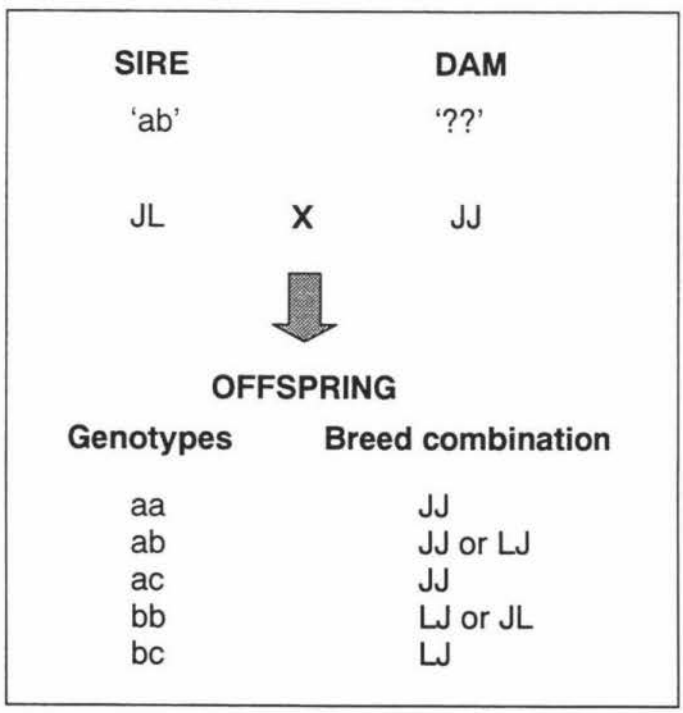


Figure 4.1: Possible breed origin combinations of marker alleles in back-cross progeny from a F1 sire and Jersey dam

If only one parent is genotyped and the progeny has the same genotype as its parent, the progeny could have received either allele from the sire or the dam. Thus, there are more than one possible breed combinations for genotypes 'ab'. Neither it is possible to determine which parental allele was passed to the progeny in the 'bb' genotype.

However, being JL or LJ does not modify the probabilities of carrying a particular QTL genotype within back-cross (Tables 4.4 and 4.5).

4.2.4 Interval mapping

Phenotypes of muscle glycogen, ultimate pH and logarithmic pH were adjusted for known fixed effects using SAS (2000), and the residuals stored. Different models were evaluated for the four traits considering the following fixed effects: year of birth, sex, rearing condition, breed composition, and days between glycogen sampling and slaughter (as a covariate). Slaughter group, which comprises year, sex and the difference of days to slaughter (Table A2.2), was also included. The final models were those that minimised the mean square error.

The fixed effects included in the selected model for muscle glycogen were breed composition and slaughter group, whereas for ultimate pH, logarithmic pH and predicted glycogen the models contained only slaughter group.

The flanking-markers analysis was undertaken based on a one-QTL model proposed by Haley and Knott (1992). The following model was separately fitted for muscle glycogen, ultimate pH, logarithmic pH or predicted glycogen:

$$y_i = \mu + \beta_1 x_{i1} + \beta_2 x_{i2} + e_i$$

where:

y_i is the residual for one of the analysed traits in the i^{th} observation;

μ is the overall mean;

β_1 is the difference between the homozygous QTL genotypes (Q_JQ_J or Q_LQ_L), defined as the additive effect (a) of the QTL;

x_{i1} is the difference between the conditional probabilities of carrying Q_JQ_J and Q_LQ_L for the i^{th} observation;

β_2 represents the dominance effect (d) of the QTL;

x_{i2} is the probability of a heterozygous QTL genotype, conditional on the marker genotypes for the i^{th} observation;

e_i is the random residual including random polygenic and non-genetic effects;

with $E(e_i) = 0$, $\sigma^2(e_i) = \sigma_e^2$ and $\sigma(e_i, e_j) = 0$, for $i \neq j$.

The assumption of independence among residuals is not strictly valid because 11% of experimental animals were full sibs via embryo transfer. For these animals, the covariance between residuals is: $\sigma(e_i, e_j) = 1/4\sigma_g^2$, with σ_g^2 the genetic variance. Unknown relationships among dams might also cause correlated residuals that may bias the estimation of regression parameters and standards error of these estimates (Lynch and Walsh, 1998).

The model was fitted at each 1-cM position on both chromosomes from the first to the last marker indicated in the linkage map. The analysis was undertaken by family of half-sibs and the following F-like-statistic stored for each 1-cM step:

$$\text{F-statistic} = \frac{R(\beta_1, \beta_2 | \mu)/2}{MSE}$$

where $R(\beta_1, \beta_2 | \mu)$ is the sum of squares of the regression corrected for the mean and *MSE* is the mean square error.

The phenotypic distribution in the marker classes may be a mixture distribution due to the presence of more than one QTL genotype. Consequently, the F-like-statistic may not follow an F distribution, although it was calculated as an F-statistic. A non-normal distribution also implies that tabulated critical values are not adequate. The comparisonwise values were calculated within families by permutation analysis (Churchill and Doerge, 1994). The phenotypes were randomly assigned to the marker genotypes and the F-statistic computed in each shuffle. This process was repeated 10,000 times, generating an empirical distribution for the test under the null hypothesis of no association between phenotype (residual) and QTL genotype. The 1%-, 5%- and 10%-critical values were calculated from the generated distribution

At the QTL positions with highest significant F-statistic, the significance of the regression coefficients, corresponding to additive and dominance effects, were evaluated by the *t*-statistic:

$$t\text{-statistic} = \frac{R(\beta_i | \mu, \beta_j)}{MSE}$$

where $R(\beta_i|\mu,\beta_j)$ is the reduced sum of squares of the regression coefficient β_i adjusted for the mean and β_j, with i=1,2 and j=2,1, and *MSE* is the mean square error.

4.2.4.1 CONDITIONAL PROBABILITIES OF QTL GENOTYPES

The probability of carrying a certain QTL genotype depends on the marker genotypes and the experimental design. Table 4.4 presents the conditional probabilities for the possible QTL genotypes in a double-back-cross design for the interval between two informative flanking markers (II in Figure 4.2), assuming no interference. The probabilities are a function of the recombination rates between the QTL and the markers (r_{MQ} and r_{QN}) and the recombination rate between markers (r_{MN}).

Table 4.5 shows the formulas applied when only the marker to the right or to the left was informative.

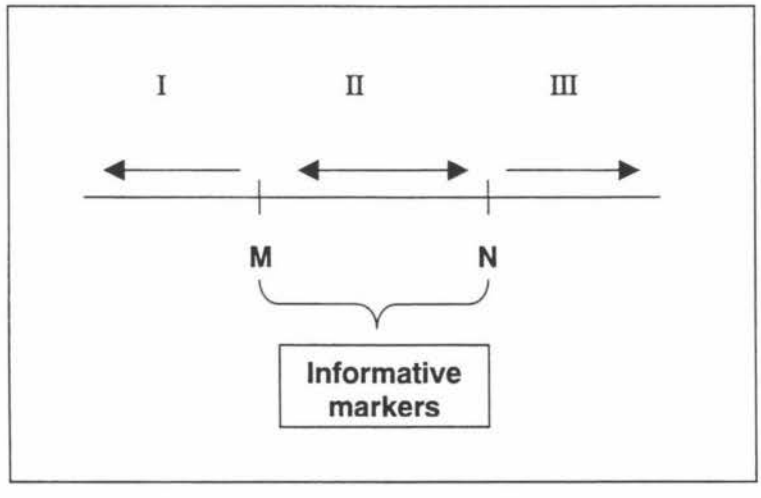


Figure 4.2: Illustration of the three possible situations considered in the calculation of the conditional probability of the QTL genoytpes: I: marker to the right is informative; II: flanking markers are informative; III: left marker is informative

Table 4.4: Probability for QTL genotypes conditional on the flanking markers genotypes

POSSIBLE GAMETES		FLANKING MARKERS		CONDITIONAL PROBABILITY OF:		
SIRE	DAMS	M	N	Q_JQ_J	Q_JQ_L	Q_LQ_L
Back-cross to Jersey						
M_JN_J	M_JN_J	JJ	JJ	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$	$r_{MQ}.r_{QN}/(1-r_{MN})$	0
M_JN_L	M_JN_J	JJ	LJ	$(1-r_{MQ})r_{QN}/r_{MN}$	$r_{MQ}(1-r_{QN})/r_{MN}$	0
M_LN_J	M_JN_J	LJ	JJ	$r_{MQ}(1-r_{QN})/r_{MN}$	$(1-r_{MQ})r_{QN}/r_{MN}$	0
M_LN_L	M_JN_J	LJ	LJ	$r_{MQ}.r_{QN}/(1-r_{MN})$	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$	0
Back-cross to Limousin						
M_JN_J	M_LN_L	JL	JL	0	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$	$r_{MQ}.r_{QN}/(1-r_{MN})$
M_JN_L	M_LN_L	JL	LL	0	$(1-r_{MQ})r_{QN}/r_{MN}$	$r_{MQ}(1-r_{QN})/r_{MN}$
M_LN_J	M_LN_L	LL	JL	0	$r_{MQ}(1-r_{QN})/r_{MN}$	$(1-r_{MQ})r_{QN}/r_{MN}$
M_LN_L	M_LN_L	LL	LL	0	$r_{MQ}.r_{QN}/(1-r_{MN})$	$(1-r_{MQ})(1-r_{QN})/(1-r_{MN})$

Table 4.5: Probability for QTL genotypes conditional on one informative marker genotype

POSSIBLE GAMETES		MARKERS	CONDITIONAL PROBABILITY OF:		
SIRE	DAMS	GENOTYPES	Q_JQ_J	Q_JQ_L	Q_LQ_L
Back-cross to Jersey					
$M_J\ Q_J$	M_JQ_J	JJ	$(1-r_{MQ})$	r_{MQ}	0
$M_J\ Q_L$					
$M_L\ Q_J$		LJ	r_{MQ}	$(1-r_{MQ})$	0
$M_L\ Q_L$					
Back-cross to Limousin					
$M_J\ Q_J$	$M_L\ Q_L$	JL	0	$(1-r_{MQ})$	r_{MQ}
$M_J\ Q_L$					
$M_L\ Q_J$		LL	0	r_{MQ}	$(1-r_{MQ})$
$M_L\ Q_L$					

The calculations of QTL probabilities, the interval regression and the estimation of comparisonwise critical values were programmed in A Programmers Language (APL, 1999).

4.3 RESULTS

The results obtained from chromosome B suggest the presence of a QTL affecting muscle glycogen. The F-statistic profile corresponding to sire 2 (S2) surpassed the comparisonwise critical values (5%-critical value=3.07) and reached a maximum at 66 cM (Figure 4.3). Regression coefficients were significant ($P<0.1$) with estimated values of -1.3 ± 0.8 and 2.2 ± 1.1 mg/g for the additive and dominance effects, respectively. The analysis of the other traits did not indicate a putative QTL on chromosome B. The F-statistic profiles are presented in Appendix 2 (Figures A2.1 to A2.4).

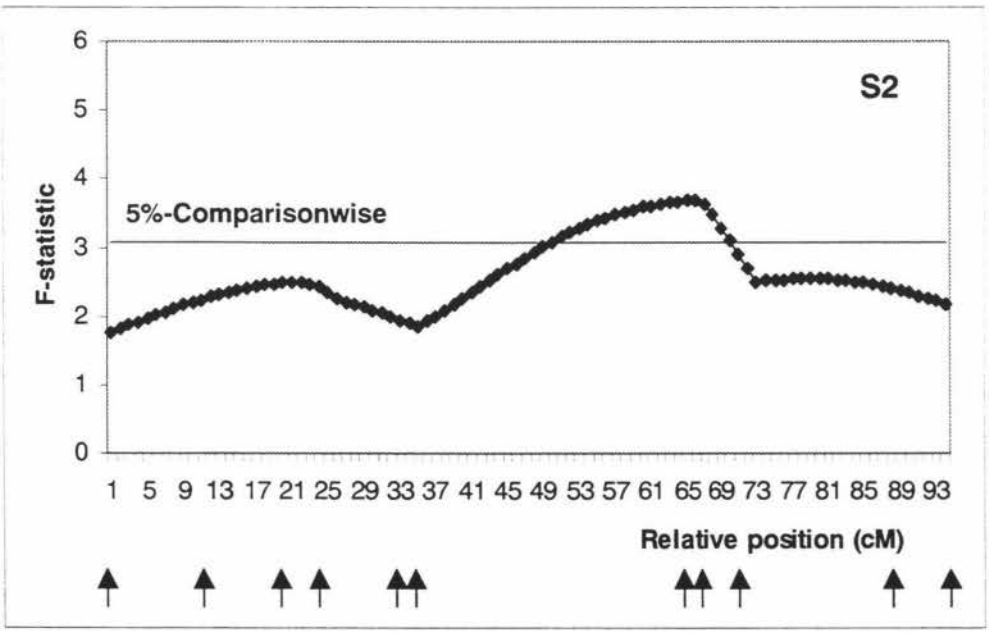


Figure 4.3: Muscle glycogen: curves of F-statistics in chromosome B for Sire 2 (Arrows indicate positions of markers. Comparisonwise value is the average for all positions)

The F-statistics curve of muscle glycogen did not show any QTL influencing this trait in chromosome A (Figure A2.5). However, a chromosomal region with effect on ultimate pH was identified on chromosome A. The peak of the F-statistic profile for

sire 1 (S1) indicates the presence of a putative QTL affecting ultimate pH at 140 cM (Figure 4.4)(5%-critical value = 3.06). The estimated regression coefficients of ultimate pH at the highest F-value were -0.0004±0.0035 and -0.0142±0.0054 units of pH for the additive genetic deviation (a) and dominance genetic deviation (d), respectively, although only the latter effect was significant (P<0. 01). Figure A2.10 shows the residuals of ultimate pH plotted versus the conditional probability of the heterozygous QTL at 140 cM, as well as the regression line. This data were also split into two groups in order to verify the magnitude of the significant effect. The first group includes the extreme values of the conditional probability of Q_JQ_L, whereas the intermediate values of the conditional probability of Q_JQ_L are included in the second one. Both groups were separately plotted and the regression coefficients re-calculated (Figures A2.11 and A2.12 in Appendix 2). The regression coefficients for the effect of dominance were similar indicating that the size of the effect was not determined by the extreme values of the probability of Q_JQ_L.

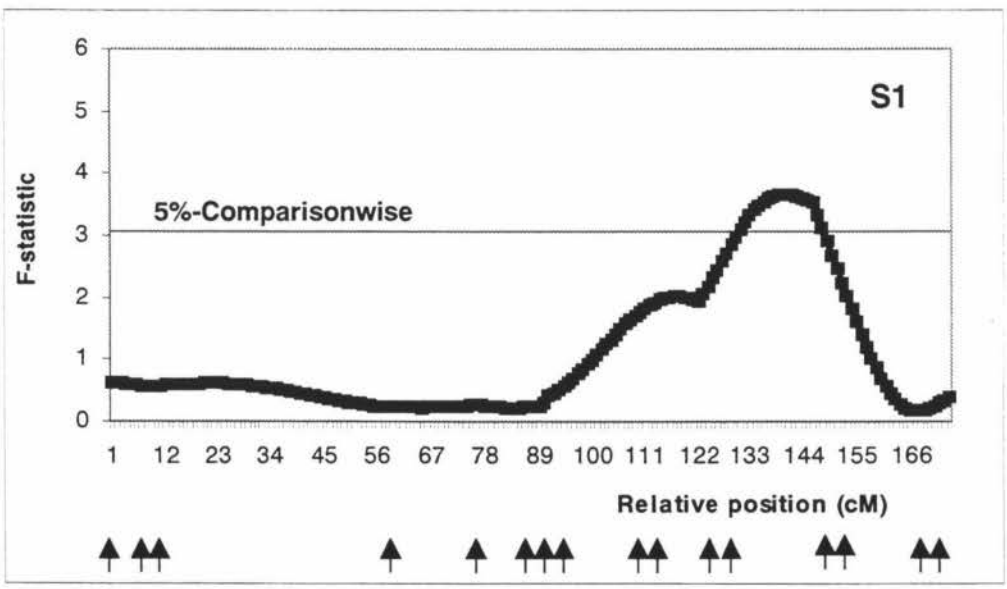


Figure 4.4: Ultimate pH: curves of F-statistics in chromosome A for Sire 1 (Arrows indicate positions of markers. Comparisonwise value is the average for all positions)

Logarithmic transformation of pH in chromosome A and sire one has a peak similar to that observed in ultimate pH. The logarithmic transformation presented the same F-statistic curves as ultimate pH in chromosomes B (Figures A2.2 and A2.3) and A (Figure 4.4 and Figures A2.6 and A2.7 in Appendix 2).

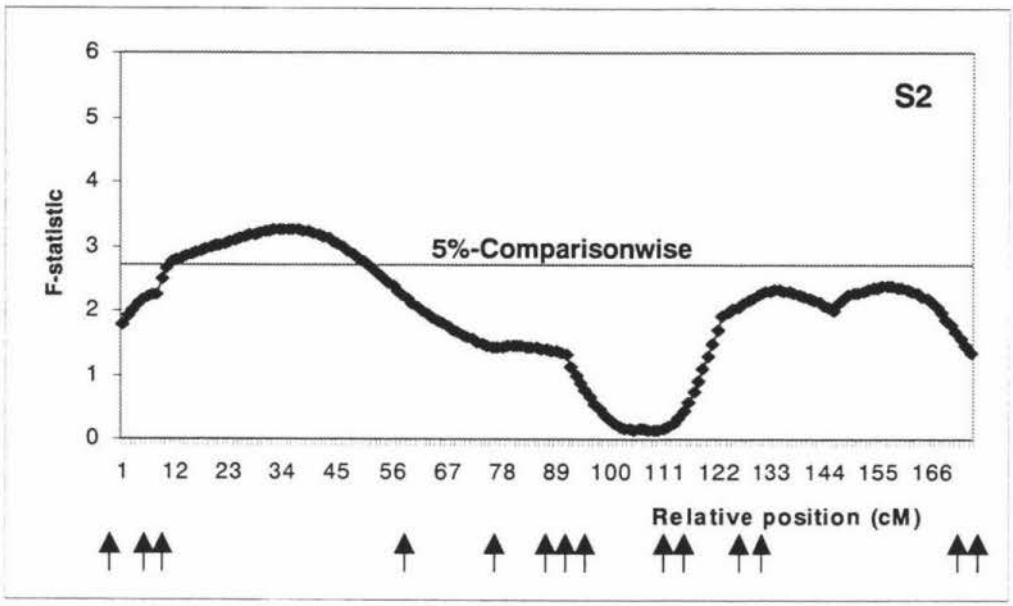


Figure 4.5: Predicted glycogen: curves of F-statistics in chromosome A for Sire 2 (Arrows indicate positions of markers. Comparisonwise value is the average for all positions)

A peak is observed in predicted glycogen in the same family (S1) and in a similar position (141 cM) as in ultimate pH. However another peak is located at 171 cM and the curve did not show a maximum (Figure A2.8).

The F-statistic profile of predicted glycogen for sire 2 (S2) showed a significant peak at 40 cM (Figure 4.5). The estimated additive and dominance coefficients were –0.74 ± 0.38 and 1.34 ± 0.77 units of predicted glycogen and both were significant at a significant level of 10%. Figures A2.13 and A2.14 show the existence of two outliers that may affect the results. These observations correspond to the two highest values of ultimate pH (5.6 and 6.2) that present the lowest values of predicted glycogen.

4.4 DISCUSSION AND CONCLUSIONS

Putative QTL affecting muscle glycogen and ultimate pH were identified on different chromosomes and in different families. Although QTL affecting beef quality traits have been reported (Hetzel *et al.*, 1997; Keele *et al.*, 1999; Morris *et al.*, 2001a), no information regarding QTL for ultimate pH or muscle glycogen in beef have been found in the published literature.

Ultimate pH is determined by the glycogen content of muscles at the time of slaughter. After slaughter, glycogen in the muscles is converted to lactic acid by glycolysis, which leads to acidification of beef from initial values of 7.0-7.2 to an ultimate pH value around 5.5, depending on the levels of glycogen (Tarrant, 1989). Therefore, it is expected that a putative QTL affecting the amount of glycogen may be also indicated by the study of ultimate pH. However, the possible QTL for muscle glycogen, located in chromosome B at 66 cM position, was not observed in ultimate pH in that chromosome. On the other hand, the analysis of chromosome A for muscle glycogen did not suggest a possible QTL, although a putative QTL affecting ultimate pH was identified at 140 cM in chromosome A.

This may be explained by the fact that in this case, muscle glycogen concentration was assessed 32 to 81 days before slaughter, whereas ultimate pH is associated with the content of glycogen at the time of slaughter. Although the glycogen level measured at the farm could be an indicator of the content at slaughter, it does not take into account the effect of pre-slaughter stress. Transporting and handling procedures imposed on cattle during the pre-slaughter period can induce stress that depletes muscle glycogen stores. The breakdown of glycogen into glucose is triggered by circulating adrenaline and physical activity, which limits the available substrate for post-mortem glycolysis, with a resultant higher ultimate pH (Tarrant, 1989; Warris, 1990).

The content of glycogen in the muscle immediately before the animals are killed is the result of the concentration at the time they are removed from feed and the magnitude of the depletion of glycogen due to pre-slaughter stress. The mean and standard deviation of ultimate pH were 5.44 and 0.06 and only one carcass presented a pH value higher than 5.8. These levels of ultimate pH indicate high contents of glycogen in the muscle at the moment of slaughter, with the exception of the one animal with high pH in which the glycogen concentration was not enough to achieve a normal value around 5.5.

Muscle glycogen assessed two months before slaughter suggest that if the animals were slaughtered at that moment, without the detrimental effect of pre-slaughter stress, the incidence of high ultimate pH would have been very low. To achieve a beef ultimate pH of 5.5 the concentration of glycogen must be greater than 8-10 mg/g (Warris *et al.*, 1984; Tarrant, 1989; Warris, 1990) and only 2% of the cattle presented muscle glycogen values lower than 10 mg/g.

The values recorded in the experiment for this trait suggest that the animals were subjected to low levels of stress. They were managed in small groups and killed in an experimental abattoir (Morris *et al.*, 2001b). These conditions might imply lower levels of stress than in commercial circumstances due to improved pre-slaughter handling of the animals. Furthermore, it is also possible that the responses to stress produced by fear were modulated through the previous experiences of the animals (Fergusson *et al.*, 2001). Experimental animals are routinely exposed to handling and human contact because of the regular measurements and other activities required by the research task.

Under conditions of low stress, high association can be expected between glycogen content in muscles before slaughter and the final concentration at the time of slaughter. However, the particular influence of stress, which determines the amount of substrate for glycolysis in the different individuals, is unknown. Even assuming a low intensity of stress, and a consequently low depletion, it is not possible to expect that its effect had the same magnitude for all the animals.

Ultimate pH did not present the typical skewed distribution that has been reported in other studies (Tarrant and Sherington, 1980; Graafhuis and Devine, 1994; Page *et al.*, 2001). In commercial populations of slaughtered animals, this non-normal distribution is characterised by a peaked primary mode around 5.5 and a long upper tail. The combination of high glycogen concentration in muscle and low pre-slaughter stress may explain that in this case the frequency distribution did not show the upper tail. This part of the distribution corresponds to those animals whose glycogen levels are limiting and arrest the decrease in pH at higher values than the normal 5.5.

Predicted glycogen was calculated by converting the ultimate pH data to the underlying glycogen levels based on the approach proposed by Pleasants *et al.* (1999). The mathematical model, which describes the conversion of glycogen to lactic acid in a manner suitable for statistical analysis, overcomes the difficulties in the studies of ultimate pH derived from the non-normal distribution and gives more power to the analysis (Pleasants *et al.*, 1999). Therefore, it is expected that clearer evidence of a putative QTL for ultimate pH can be obtained by analysing predicted glycogen.

The test-statistic curve for predicted glycogen showed significant values at a similar region of chromosome A and for the same sire in which the putative QTL for ultimate

pH was located. The F-statistic profile suggests the presence of QTL, however it did not reach a maximum in the analysed region. Although the F-statistic profile did not accurately reveal the presence of QTL, searching further positions from the last marker in the linkage map may clarify the presence of a putative QTL at the end of the mapped chromosome.

On the other hand, the analysis of predicted glycogen within the progeny of sire 2 indicated the presence of QTL in chromosome A. It was located in a similar position where the F-statistic for ultimate pH showed a non-significant peak. The higher values of the test-statistic for predicted glycogen are possibly explained by two outlying observations. They were the only two pH values higher than 5.5, which presented the lowest values of predicted glycogen. Although the higher peak of predicted glycogen could be interpreted as a higher power to detect differences in the underlying glycogen content, this result has to be interpreted with caution because they are explained by two observations.

The results obtained for ultimate pH and predicted glycogen do not allow definitive conclusions. The expected higher power of predicted glycogen is explained by the fact that the technique discounts the information in the peak in favour of the information in the tail of a skewed pH distribution (Pleasants *et al.*, 1999). However, in the present study pH distribution did not present a tail of high values. The failure to precisely detect the putative QTL affecting ultimate pH using predicted glycogen is then not surprising.

Suggestive significant peaks were only found in one of three sires used in the experiment, probably explained by the fact that not all sires were heterozygous for the putative QTL. In back-cross involving inbred lines, in which the alleles are fixed, all sires are heterozygous for the QTL. Because they all show evidence of the QTL, the analysis can be done over all offspring, regardless of their parents. With outbred populations, such as Limousin and Jersey, some F1 sires may not be QTL-informative, even being marker- informative. In this case, across-families studies may not detect a possible QTL, and each F1 parent has to be examined separately.

Coupled with MAS, exploitation of QTL for ultimate pH and/or muscle glycogen may provide a mechanism to reduce the incidence of high pH values and the consequent negative influences on beef quality. Despite the potential benefits of this knowledge, the implementation of MAS strategies depends on the genetic gain that can be

achieved (Weller, 2001). For example, the estimated parameters of the QTL affecting ultimate pH indicate a significant dominance genetic effect of -0.0142 units of pH that is equivalent to 0.25 phenotypic standard deviation. However, a change of 0.01 units of pH is less that 0.1% of the actual mean and would imply low benefit by MAS. Knowledge of QTL affecting muscle glycogen may be used as an alternative to increase glycogen levels and thus minimise the risk of high ultimate pH. Nevertheless, in the case of muscle glycogen the impact of MAS on ultimate pH is also affected by the non-linear association between muscle glycogen and ultimate pH. Muscle glycogen has a direct effect on ultimate pH for glycogen values that are below the amount of glycogen required to achieve a pH of 5.5. Above this level of glycogen, ultimate pH value will be around 5.5 independently of muscle glycogen because glycolysis is inhibited. Thus, there would be no gain in terms of ultimate pH for those populations with high glycogen contents.

Further studies are required prior to evaluating MAS implementation and its profitability. Primary genomic screen with markers located at 10- to 20-cM intervals indicates the presence of putative QTL and a secondary screen involving additional animals and markers is required to verify that QTL indicated by suggestive F-statistic peaks are real (Georges, 1998). The putative QTL have been identified in this study based on F-statistic curves that were greater than comparisonwise critical values for a significance level (α) of 5%. This indicates that for each test the probability of a false positive is 5%. However, analyses for marker-characteristic associations were performed at 1-cM positions along the chromosomes. This leads to a higher probability of identifying as positive a false QTL in the entire pool of analysis. For instance, for any trait the probability of at least one false positive in chromosome A is close to 1. Consequently, additional analysis to confirm the putative QTL are particularly relevant.

In the particular case of this study, only half of the final data set of the AgResearch/Adelaide trial has been considered and the analysis including all the information may confirm the results obtained. Furthermore, the re-evaluation of the most useful findings has been started in both institutions involved in this experiment (Morris *et al.*, 2001a).

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

Although ultimate pH is not directly assessed by consumers, it is an economically important trait due to its effect on other meat quality attributes. Values of ultimate pH above the "normal" 5.5 are associated with less tenderness, darker colours and shorter shelf life, with a consequent reduction of the acceptability of the final product.

The study of ultimate pH has been conditioned by the fact that elevated values determine a particular skewed distribution, which may affect the inferences that can be drawn. The typical frequency distribution is characterised by a peak around pH values of 5.5, which concentrates a high proportion of the observations, and a tail that comprises values in the range of approximately 5.7 to 7.0-7.2.

In the present study, a new approach that may overcome this problem was evaluated in the detection of QTL affecting ultimate pH through the analysis of: a simulated back-cross including two inbred lines and real data provided by AgResearch, which was collected in a back-cross experiment involving Limousin and Jersey cattle.

The new approach is considered innovative because it explicitly includes knowledge of the biochemical process that explains ultimate pH. By this method, ultimate pH is transformed to the underlying content of muscle glycogen, named in this study as predicted glycogen, based on the comprehension of the biological association between both traits. Ultimate pH is associated with the concentration of glycogen in the muscles when the animals are slaughtered. Glycogen is converted by glycolysis to lactic acid, which accumulates leading to the acidification of the muscle.

This new model was proposed by Pleasants *et al.* (1999), who pointed out that it would give more power to the study of beef ultimate pH. The effectiveness of predicted glycogen in QTL searching was analysed and compared to the performance of ultimate pH as well as to two other correlated traits: muscle glycogen, which determines ultimate pH, and the logarithmic transformation of ultimate pH. The former characteristic was included because this kind of transformation has been proposed to convert skewed distributions to normal distributions.

Results obtained in the analysis of the simulated data clearly indicate that the effectiveness on QTL detection depends on the analysed trait. The concentration of glycogen immediately before slaughter was the characteristic that more clearly detected the presence of the simulated QTL. In the analysis of the experimental data, although a putative QTL for ultimate pH was located, muscle glycogen did not provide evidence of this QTL. It was attributed to the fact that in the experimental data muscle glycogen corresponds to the content of glycogen assessed a couple of months before the animals were killed. Stress induced by the events that take place since the animals leave the farm until slaughter can deplete levels of glycogen, indicating that this trait may not be a good indicator of the actual content at the time of slaughter, and posterior ultimate pH. On the other hand, a putative QTL for muscle glycogen was found in another chromosome, which was not expressed in terms of ultimate pH, likely explained by the same reason.

The logarithmic transformation of ultimate pH had the same performance as ultimate pH, presenting very similar test-statistic curves in all analyses, because the transformation did not modify the original distribution of ultimate pH. Logarithmic transformation of pH suggested a putative QTL affecting ultimate pH, or failed in the identification, in the same analyses and with similar levels of significance as ultimate pH. Therefore, the utilisation of a logarithmic transformation does not represent any benefits compared to ultimate pH.

The study of the simulated back-cross indicated that clearer evidence of the presence of QTL can be obtained from predicted glycogen than from ultimate pH. When the QTL effect was small, the superiority of predicted glycogen was more noticeable. These findings agree with an expected higher power of predicted glycogen when a greater proportion of the observations belongs to the tail of the pH distribution.

The higher levels of significance showed by predicted glycogen compared to ultimate pH was not confirmed in the analysis of the real data. The fact that the ultimate pH did not present the characteristic skewed frequency distribution, which affects the performance of predicted glycogen, did not allow validation of the findings obtained by simulation. Predicted glycogen data did not clearly reveal the presence of a putative QTL influencing ultimate pH that was detected in one of the sires, probably due to the lack of high pH levels. On the other hand, it suggested a possible QTL in another family, which was not identified by ultimate pH, probably by the influence of the two

observations that presented pH values higher than 5.5. The results are in concordance with the inference that the comparative performances of ultimate pH and predicted glycogen in QTL detection depend on the existence of high pH values.

In conclusion, this study indicates that the new method increases the significance in QTL searching compared to ultimate pH and the logarithmic transformation of ultimate pH, when ultimate pH presents the typical skewed distribution. At the same time, the results suggest that the higher effectiveness of predicted glycogen decreases when ultimate pH data are concentrated around the "normal" value of 5.5.

Although the procedure was evaluated in the specific case of the detection of QTL with effects on ultimate pH, it can be applied in other investigations on this trait. For instance, the utilisation of this approach may be useful in the evaluation of the influence of pre-slaughter conditions, which have critical and irreversible effects on ultimate pH and beef quality.

The association between ultimate pH and muscle glycogen indicates that a better understanding of the factors affecting pH requires the presence of high ultimate pH values. It is in this context where the proposed procedure may play an important role, providing more power to these investigations.

Further studies focussed on a complete and holistic analysis of the biochemical association between ultimate pH and muscle glycogen are considered relevant. The re-evaluation of the factors involved in the calculation of predicted glycogen using actual parameters of glycogen would lead to results that are expressed in real magnitudes and more comprehensible from a biological point of view. Moreover, a future study may represent an opportunity to review the model and integrate new findings that may enhance the efficacy of this approach.

APPENDIX 1

Table A1.1: Description of muscle glycogen in the simulated parental lines, F1 and back-cross generations

MUSCLE GLYCOGEN	SCENARIO 1 QTL size = $1\sigma_a$			SCENARIO 2 QTL size = $0.5\sigma_a$		
Parental lines	**Both lines**	**Line 1 Q_1Q_1**	**Line 2 Q_2Q_2**	**Both lines**	**Line 1 Q_1Q_1**	**Line 2 Q_2Q_2**
Mean	30.6	38.0	22.8	30.3	33.6	26.7
SD[1]	16.3	14.7	14.0	15.1	14.7	14.7
First-crosses		**F1 Q_1Q_2**			**F1 Q_1Q_2**	
Mean		30.3			32.2	
SD		14.1			16.0	
Back-crosses	**Total**	**Q_1Q_1**	**Q_2Q_1**	**Total**	**Q_1Q_1**	**Q_2Q_1**
Mean	31.9	36.4	27.1	30.32	32.1	28.5
SD	15.7	15.2	14.8	14.7	14.6	14.5
% below CG[2]	26.2	18.2	34.5	26.0	20.4	31.7

[1] SD: standard deviation; [2] CG: critical glycogen.

Table A1.2: Description of ultimate pH, logarithmic transformation of pH and predicted glycogen in the back-cross generation

	SCENARIO 1 QTL size = $1\sigma_a$			SCENARIO 2 QTL size = $0.5\sigma_a$		
	Total	**Q_1Q_1**	**Q_2Q_1**	**Total**	**Q_1Q_1**	**Q_2Q_1**
ULTIMATE pH						
Mean	5.63	5.56	5.70	5.63	5.61	5.65
SD[1]	0.31	0.22	0.37	0.30	0.29	0.31
Frequency distribution:						
≤ 5.39	14.8	16.7	12.7	15.0	16.4	13.6
5.4 – 5.49	23.5	25.1	21.8	24.3	24.9	23.6
5.5 – 5.59	25.5	28.6	22.3	27.3	28.4	26.1
5.6 – 5.69	15.0	17.7	12.2	9.5	11.9	7.0
5.7 - 5.79	3.3	3.5	3.1	6.5	5.0	8.5
≥ 5.8	18.0	8.4	27.9	17.5	13.4	21.2
LOGARITHMIC TRANSFORMATION OF pH						
Mean	1.73	1.72	1.74	1.73	1.72	1.73
SD	0.05	0.04	0.06	0.05	0.05	0.05
PREDICTED GLYCOGEN						
Mean	29.80	32.30	27.22	29.70	30.66	28.70
SD	9.94	6.82	11.82	9.93	9.32	10.45
% below CG[2]	17.6	7.8	27.4	18.03	13.44	22.61

[1] SD: standard deviation; [2] CG: critical glycogen.

Table A1.3: Estimated comparisonwise threshold values for muscle glycogen, predicted glycogen, ultimate pH and logarithmic pH in scenario 1

SCENARIO 1 Size QTL = 1 σ_a	GLYCOGEN	PREDICTED GLYCOGEN	pH	LOG Ph	TABULATED *t*-VALUES [1]
α = 1%					
5 cM	2.54	2.62	2.58	2.64	
15 cM	2.52	2.47	2.50	2.68	
30 cM	2.56	2.57	2.64	2.61	**2.58**
45 cM	2.64	2.55	2.60	2.62	
60 cM	2.61	2.65	2.66	2.51	
Average	**2.58**	**2.59**	**2.58**	**2.58**	
α = 5%					
5 cM	1.90	1.99	2.00	1.98	
15 cM	1.96	1.92	1.96	2.02	
30 cM	1.97	1.95	1.96	1.96	**1.96**
45 cM	1.96	1.94	1.97	1.95	
60 cM	1.95	2.03	1.97	1.95	
Average	**1.96**	**1.96**	**1.96**	**1.97**	
α = 10%					
5 cM	1.62	1.66	1.69	1.67	
15 cM	1.62	1.60	1.63	1.66	
30 cM	1.66	1.63	1.63	1.66	**1.65**
45 cM	1.66	1.62	1.61	1.63	
60 cM	1.64	1.67	1.63	1.61	
Average	**1.64**	**1.65**	**1.65**	**1.65**	

[1] Assuming normality

Table A1.4: Estimated comparisonwise threshold values for muscle glycogen, predicted glycogen, ultimate pH and logarithmic pH in scenario 2

SCENARIO 2 Size QTL = 0.5 σ_a	GLYCOGEN	PREDICTED GLYCOGEN	pH	LOG pH	TABULATED *t*-VALUES [1]
α = 1%					
5 cM	2.58	2.58	2.65	2.60	
15 cM	2.66	2.61	2.59	2.59	
30 cM	2.65	2.60	2.54	2.54	**2.58**
45 cM	2.63	2.59	2.53	2.61	
60 cM	2.60	2.59	2.58	2.61	
Average	**2.59**	**2.59**	**2.58**	**2.58**	
α = 5%					
5 cM	2.01	1.97	1.98	1.97	
15 cM	1.96	1.98	1.97	2.00	
30 cM	1.95	2.00	1.96	1.94	**1.96**
45 cM	1.95	1.94	1.98	1.98	
60 cM	2.02	1.98	1.94	1.95	
Average	**1.96**	**1.97**	**1.96**	**1.96**	
α = 10%					
5 cM	1.66	1.66	1.65	1.66	
15 cM	1.65	1.66	1.63	1.69	
30 cM	1.68	1.65	1.64	1.60	**1.65**
45 cM	1.66	1.63	1.66	1.66	
60 cM	1.71	1.63	1.64	1.65	
Average	**1.65**	**1.65**	**1.65**	**1.65**	

[1] Assuming normality

Table A1.5: Estimated comparisonwise threshold values for predicted glycogen analysed by WLS in scenarios 1 and 2

PREDICTED GLYCOGEN – WLS			
Significance level	**Position**	**SCENARIO 1** Size QTL = 1 σ_a	**SCENARIO 2** Size QTL = 0.5 σ_a
	5 cM	3.36	3.33
	15 cM	3.11	3.30
α = 1%	30 cM	3.38	2.95
	45 cM	3.54	3.25
	60 cM	3.31	3.40
	Average	**3.25**	**3.27**
	5 cM	2.45	2.30
	15 cM	2.46	2.74
α = 5%	30 cM	2.48	2.27
	45 cM	2.64	2.51
	60 cM	2.56	2.43
	Average	**2.49**	**2.51**
	5 cM	2.03	2.03
	15 cM	2.13	2.13
α = 10%	30 cM	2.18	2.04
	45 cM	2.13	2.16
	60 cM	2.13	2.04
	Average	**2.10**	**2.12**

APPENDIX 2

Table A2.1: Number of observations by back-cross and year of birth and distribution of the data for the different rearing conditions

	BACK-CROSS		TOTAL
	F1 x JJ	F1 x LL	
YEAR 1996	**159**	**94**	**253**
Farm A: single	159	47	
Farm A: twins		47	
YEAR 1997	**100**	**53**	**153**
Farm B: single	100		
Farm C: single		26	
Farm D: single		27	
TOTAL	**259**	**147**	***406***

The F1xJJ animals were born on dairy farms as singles and were bucket-reared. In 1996, the F1xLL calves were born by embryo transplant as singles or twins to HerefordxFriesian dams and were reared on them. The F1xLL animals in 1997 were born from LL dams and reared on them (Morris *et al.*, 2001a).

Table A2.2: Number of observations by sex, back-cross and period between glycogen sampling and slaughter per slaughter group

SLAUGHTER GROUP	DAYS BETWEEN SAMPLING AND SLAUGHTER	NUMBER OF OBSERVATIONS			
		SEX		CROSS F1 x	
		Heifers	Steers	JJ	LL
Year 1998	**Average: 59**	**124**	**129**	**159**	**94**
1	32	12		7	5
2	39	13		10	3
3	53	13		10	3
4	60	15		10	5
5	67	14		8	6
6	33		13	10	3
7	81	14		11	3
8	47		15	12	3
9	54	15		7	8
10	61		14	10	4
11	68	15		6	9
12	75		15	11	4
13	82	13		5	8
14	47		15	10	5
15	54		14	9	5
16	61		14	8	6
17	68		13	7	6
18	75		16	8	8
Year 1999	**Average: 53**	**75**	**78**	**100**	**53**
19	34	15		9	6
20	41	15		9	6
21	48	15		9	6
22	55	15		9	6
23	62	15		9	6
24	40		16	11	5
25	47		16	12	4
26	61		14	10	4
27	68		16	11	5
28	75		16	11	5

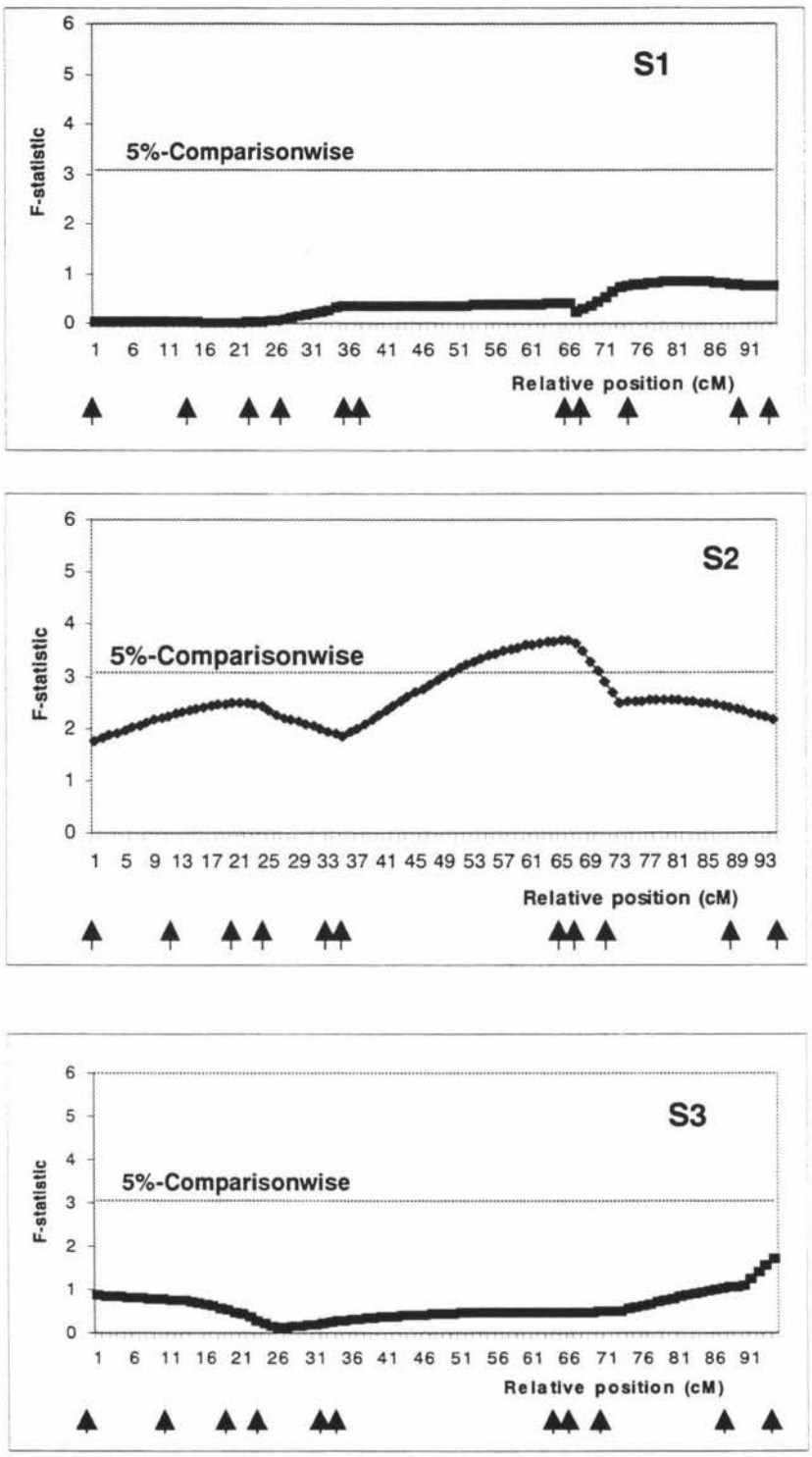
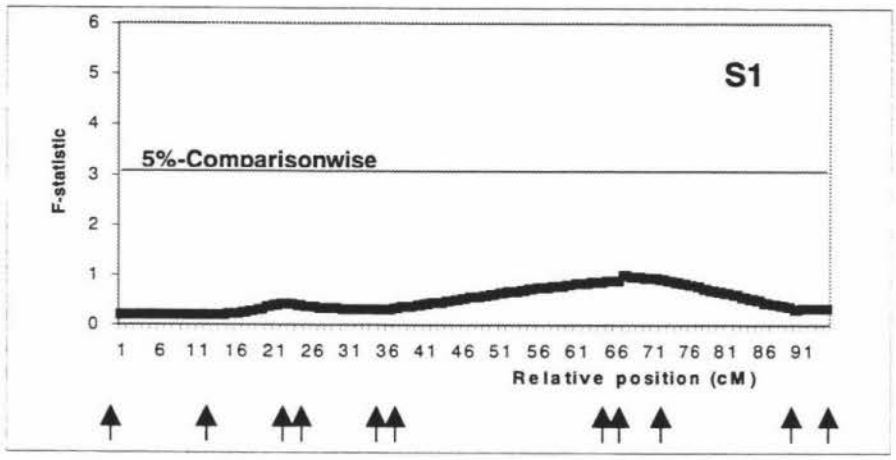
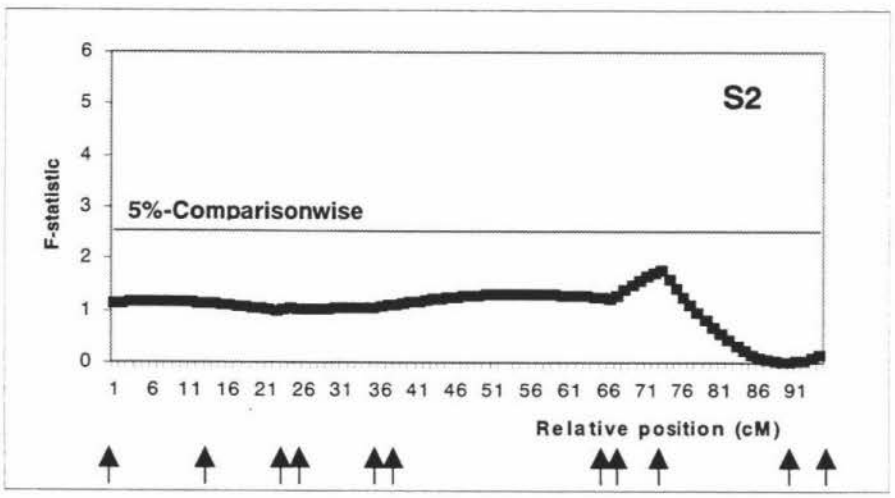


Figure A2.1: Muscle glycogen: curves of F-statistic in chromosome B by sire (S1, S2, S3) (Arrows indicate positions of markers)

NOTE: The comparisonwise critical value illustrated in Figures A2.1 to A2.8 is the average of the critical values calculated by permutation test in each position for a significance level of 5%.





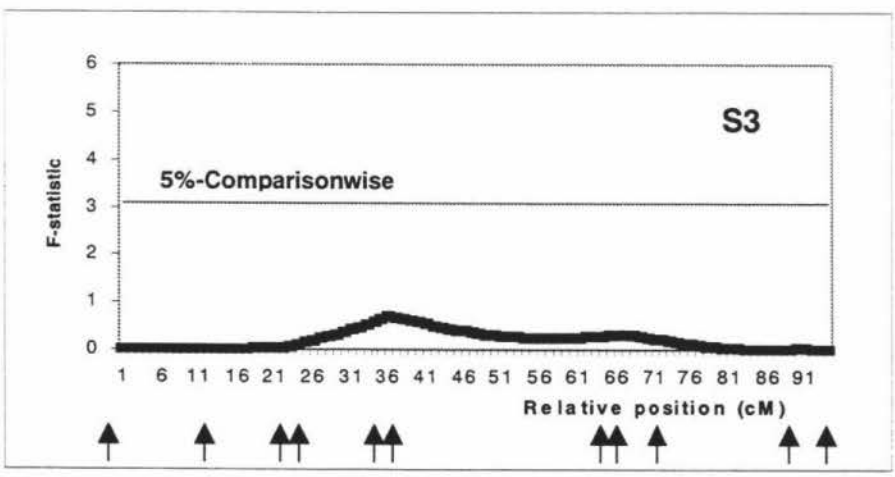
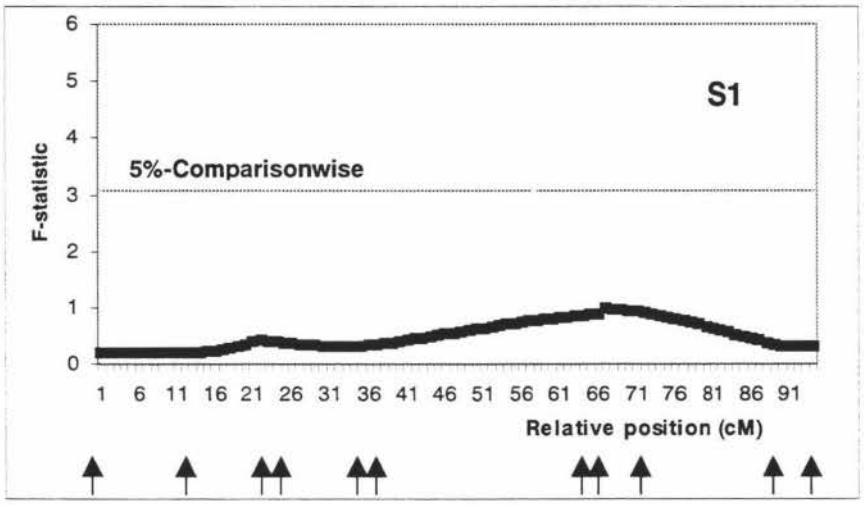
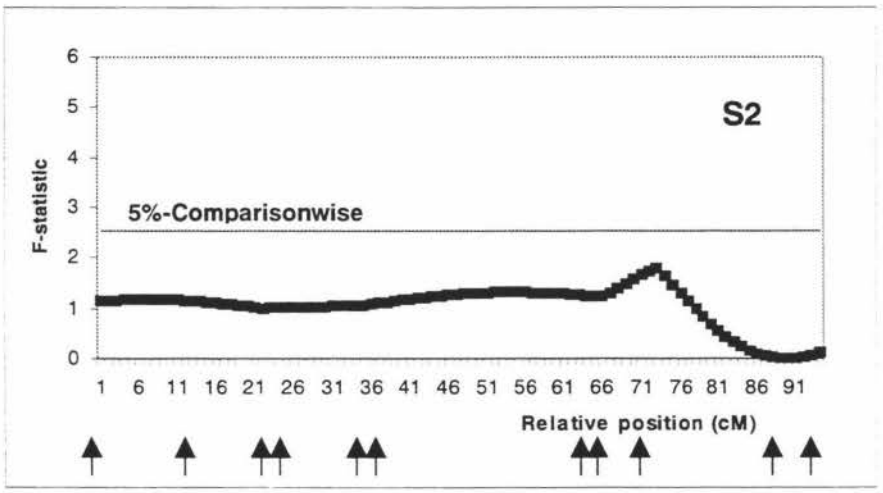


Figure A2.2: Ultimate pH: curves of F-statistic in chromosome B by sire (S1, S2, S3) (Arrows indicate positions of markers)





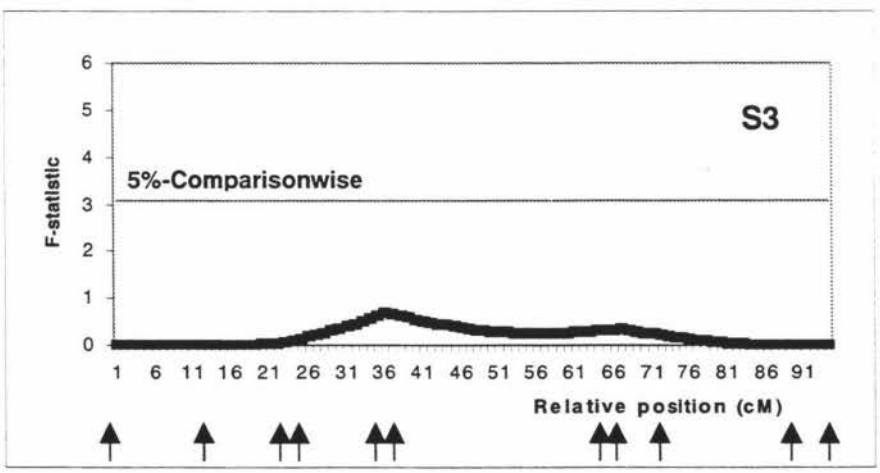


Figure A2.3: Logarithmic transformation of pH: curves of F-statistic in chromosome B by sire (S1, S2, S3) (Arrows indicate positions of markers)

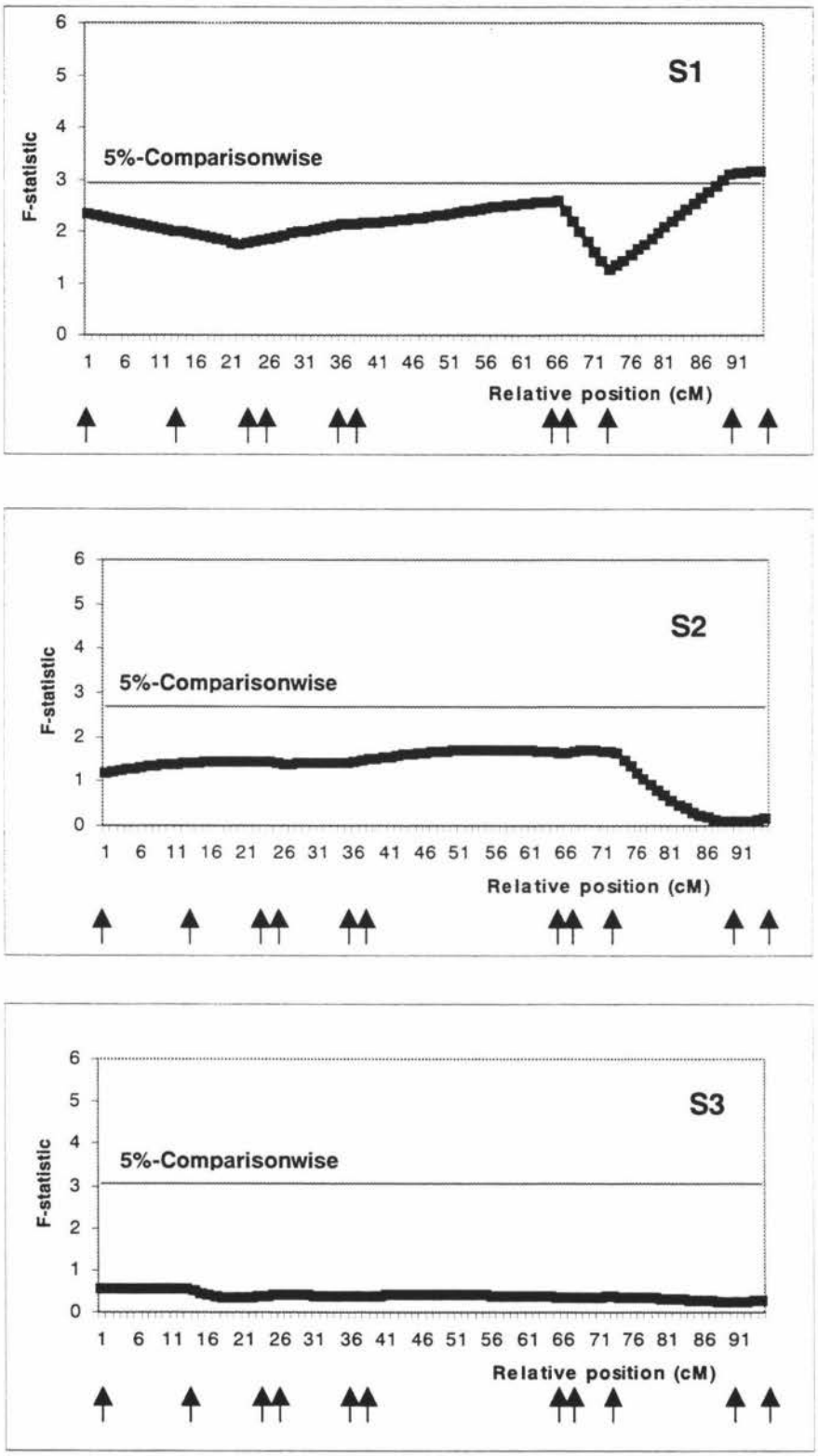


Figure A2.4: Predicted glycogen: curves of F-statistic in chromosome B by sire (S1, S2, S3) (Arrows indicate positions of markers)

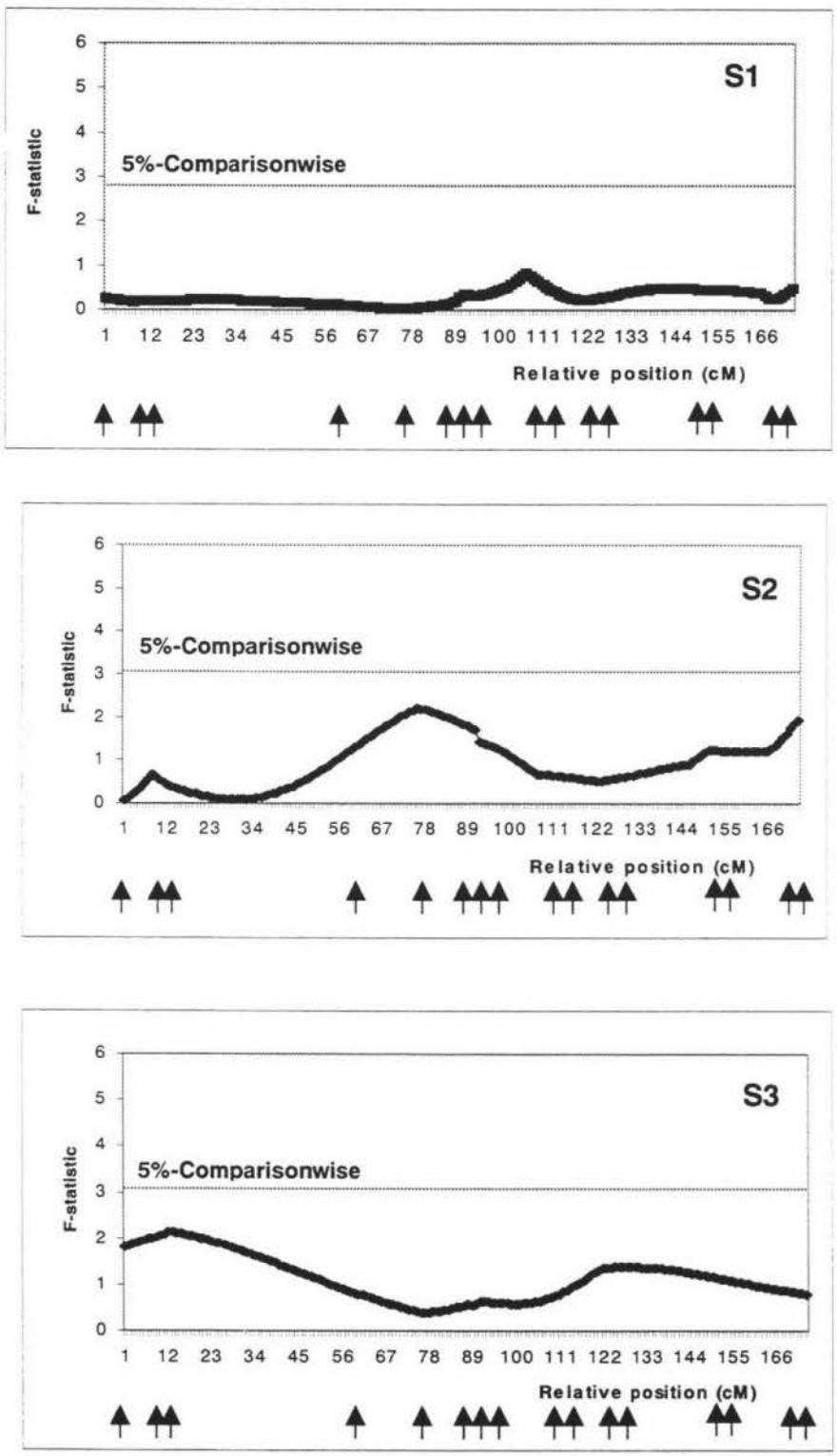


Figure A2.5: Muscle glycogen: curves of F-statistic in chromosome A by sire (S1, S2, S3) (Arrows indicate positions of markers)

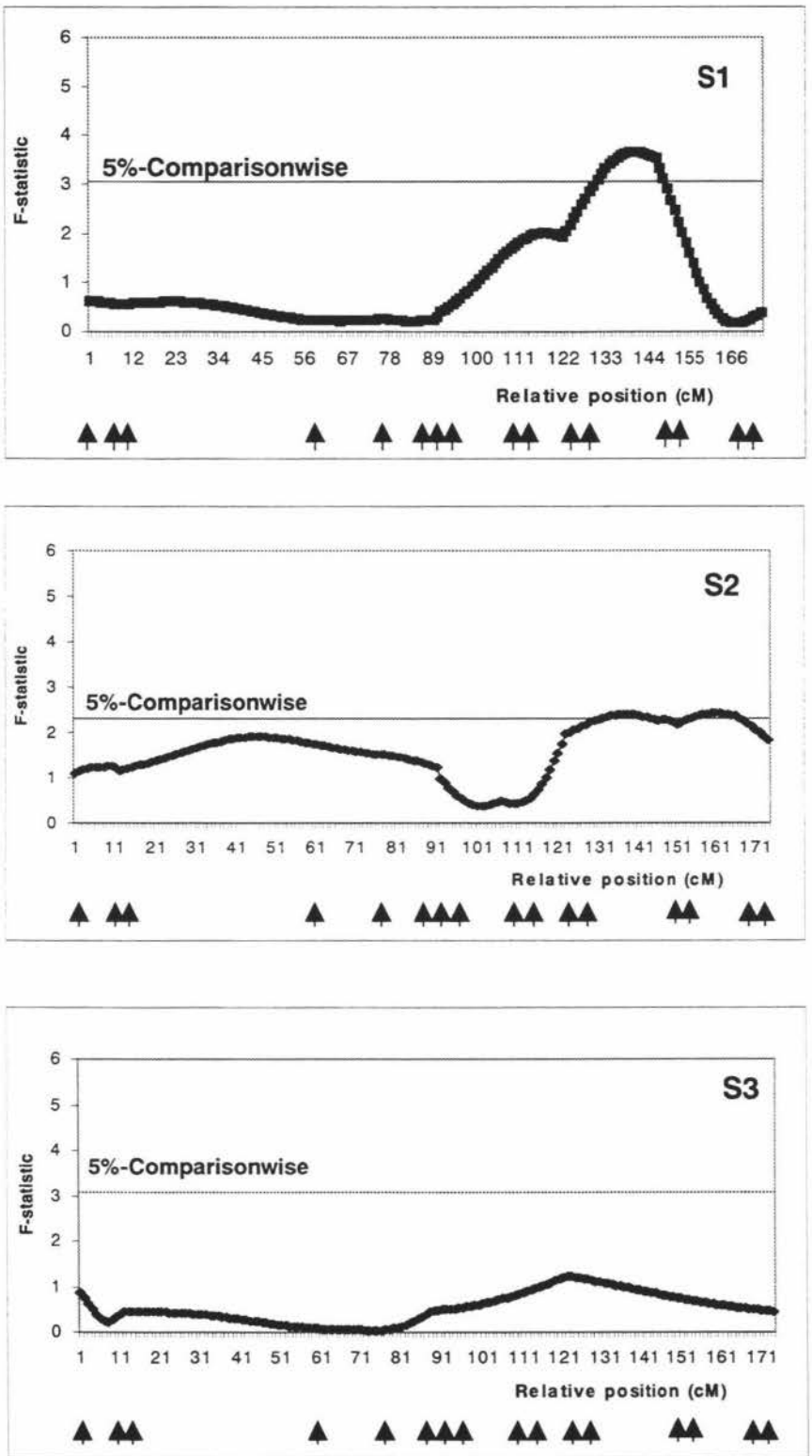


Figure A2.6: Ultimate pH: curves of F-statistic in chromosome A by sire (S1, S2, S3)
(Arrows indicate positions of markers)

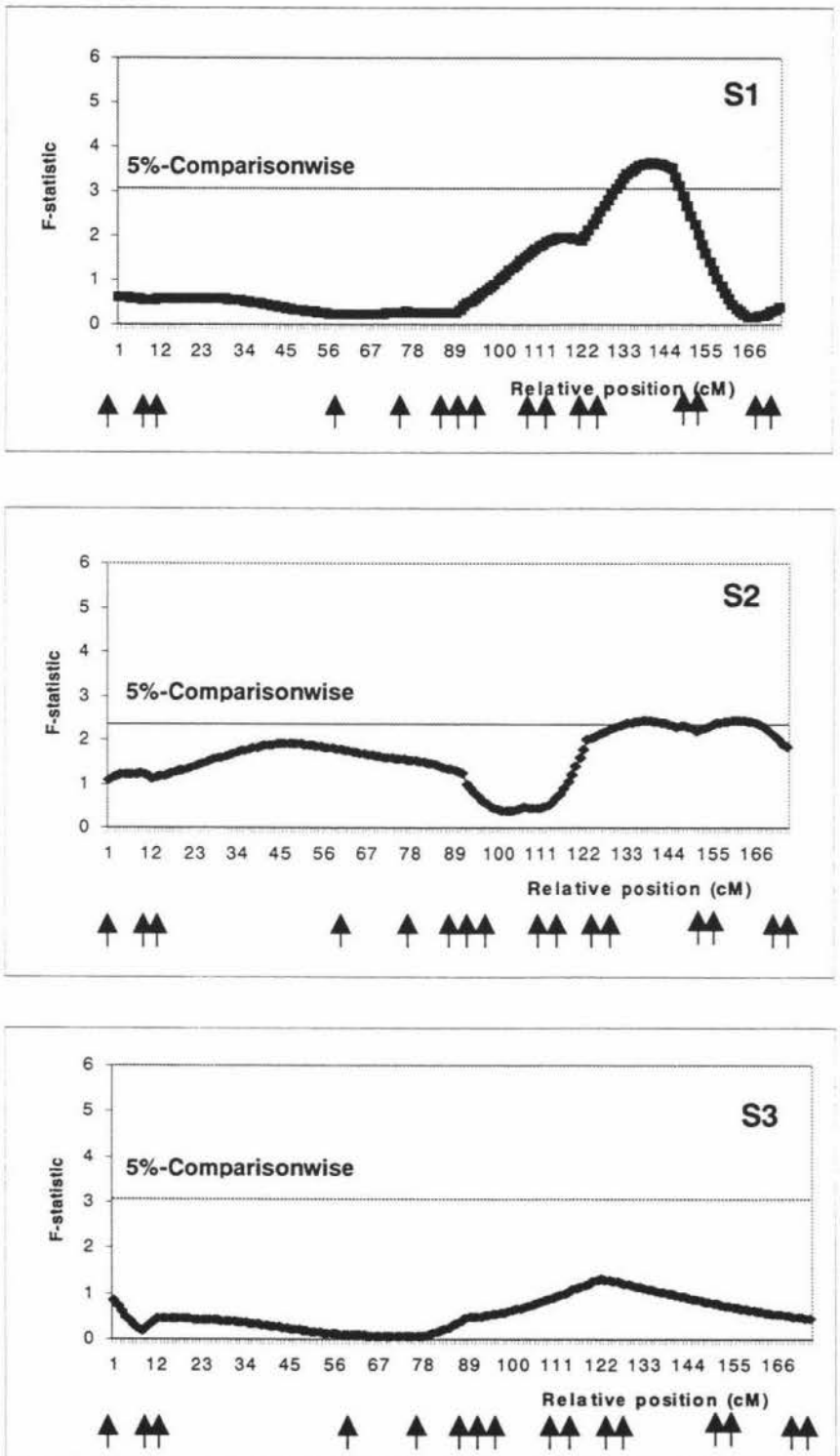
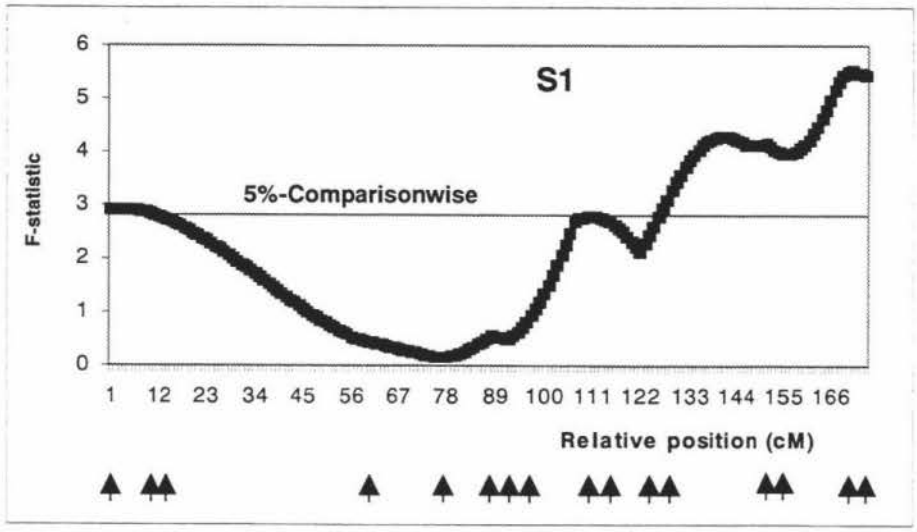
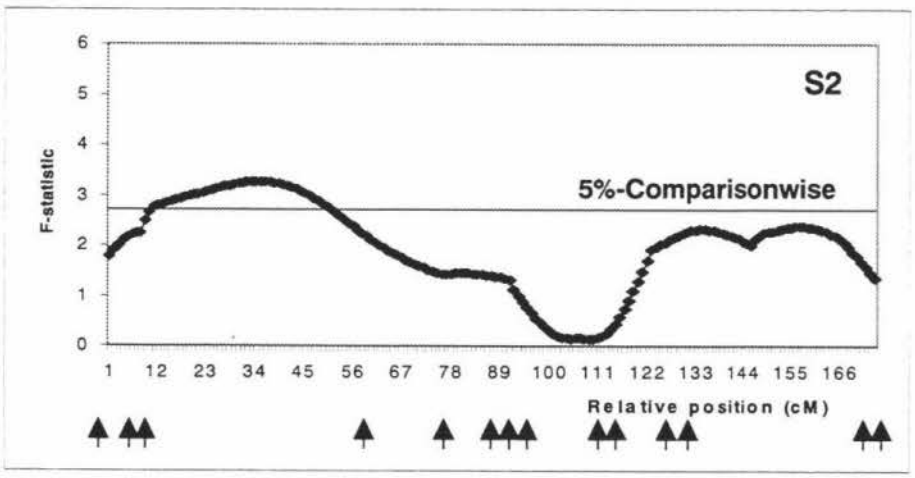


Figure A2.7: Logarithmic transformation of pH: curves of F-statistic in chromosome A by sire (S1,S2, S3) (Arrows indicate positions of markers)





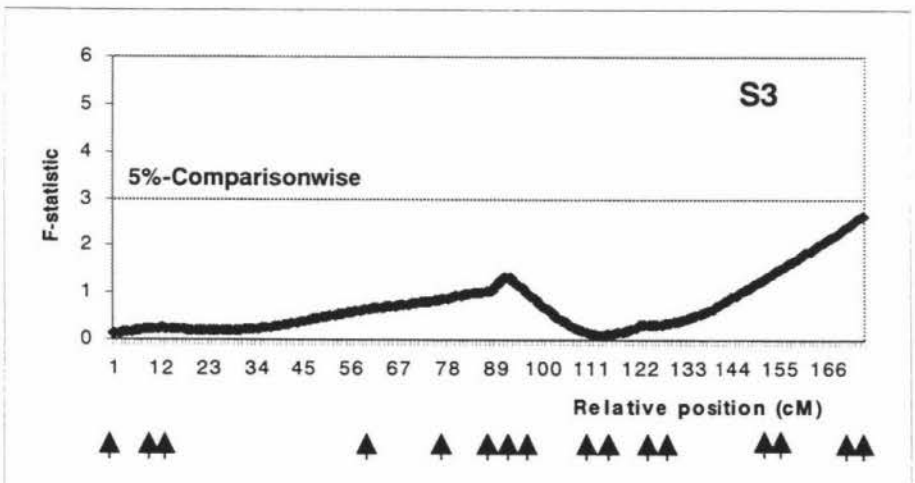


Figure A2.8: Predicted glycogen: curves of F-statistic in chromosome A by sire (S1, S2, S3) (Arrows indicate positions of markers)

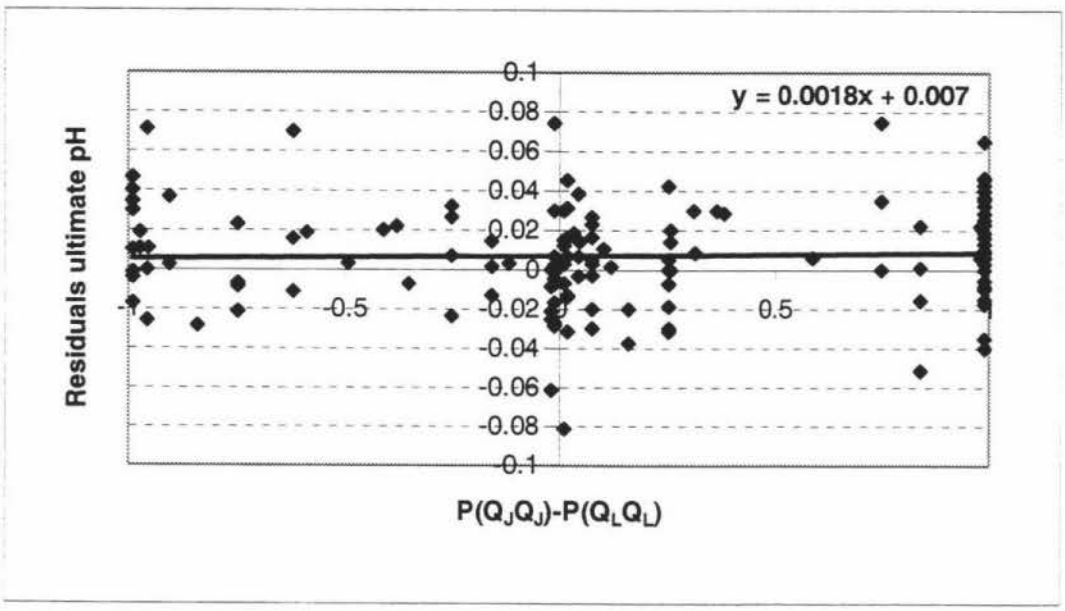


Figure A2.9: Scatterplot of ultimate pH residuals against the difference between the conditional probability of carrying the homozygous genotypes (Q_JQ_J; Q_LQ_L), at 140 cM in chromosome A

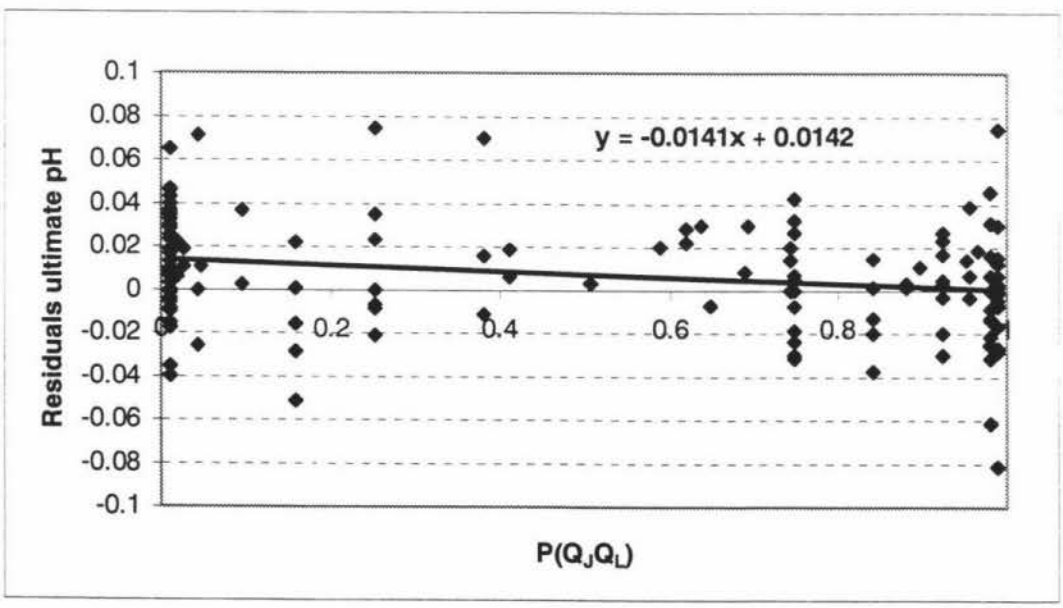


Figure A2.10: Scatterplot of ultimate pH residuals against the conditional probability of carrying the heterozygous genotype (Q_JQ_L), at 140 cM in chromosome A

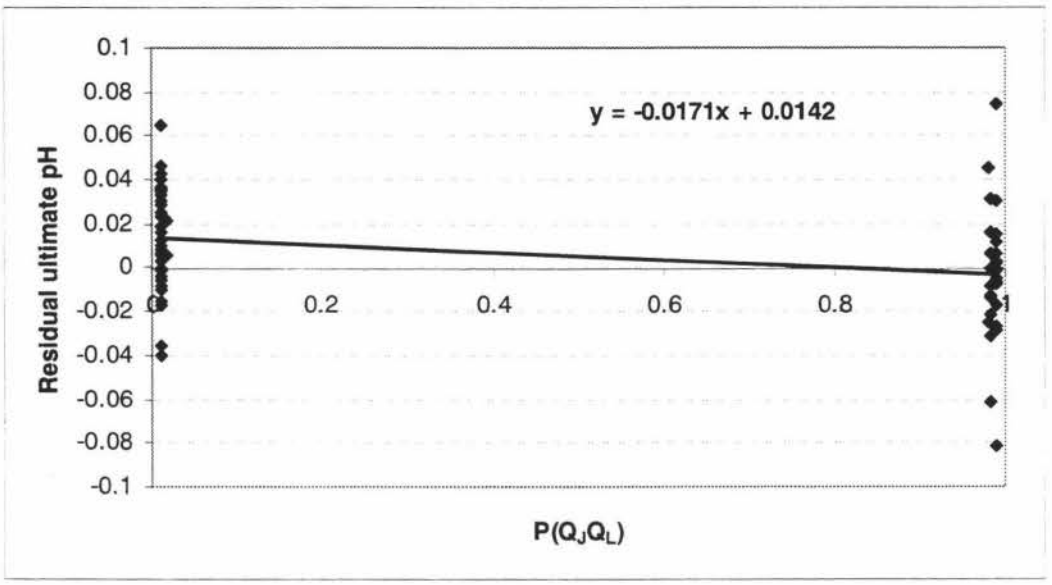


Figure A2.11: Scatterplot of ultimate pH residuals against the extreme values of the conditional probability of carrying the heterozygous genotype (Q_JQ_L), at 140 cM in chromosome A

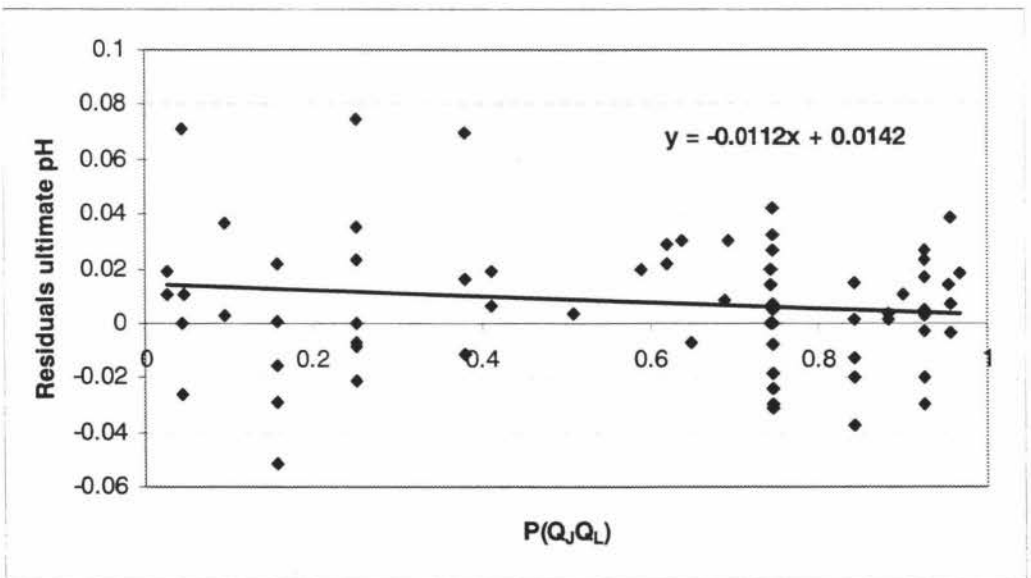


Figure A2.12: Scatterplot of ultimate pH residuals against the intermediate values of the conditional probability of carrying the heterozygous genotype (Q_JQ_L), at 140 cM in chromosome A

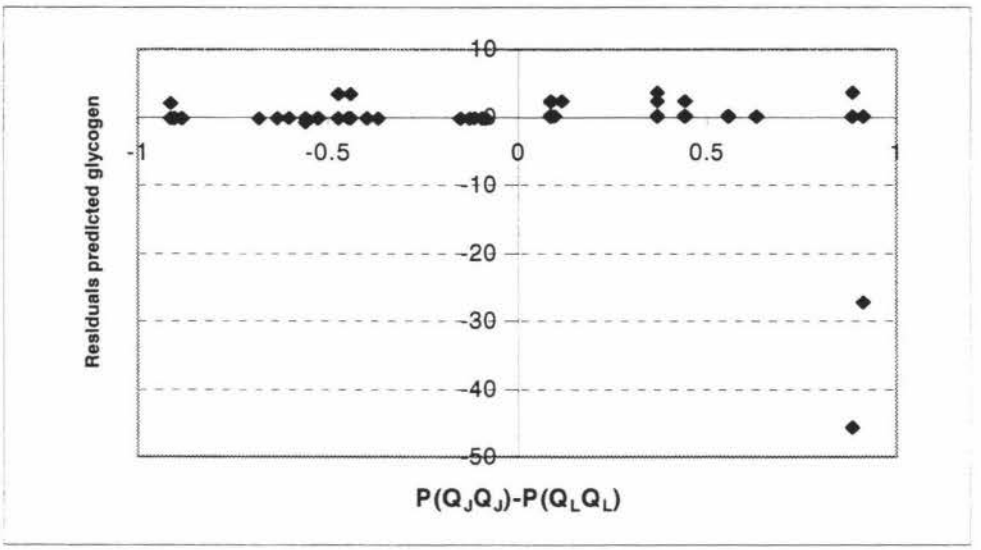


Figure A2.13: Scatterplot of predicted glycogen residuals against the difference between the conditional probability of carrying the homozygous genotypes (Q_JQ_J; Q_LQ_L) at 40 cM in chromosome A for the progeny of sire 2

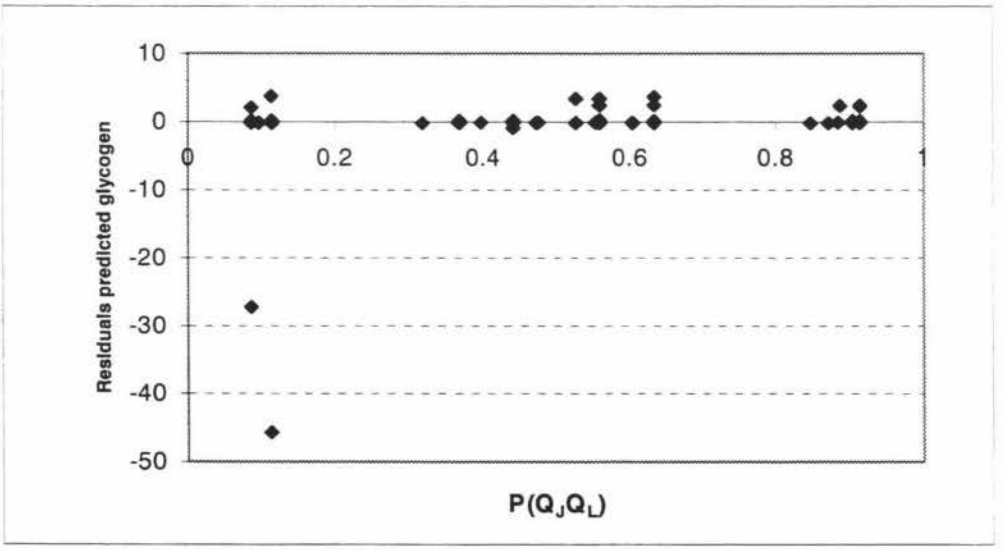


Figure A2.14: Scatterplot of predicted glycogen residuals against the conditional probability of carrying the heterozygous genotype (Q_JQ_L), at 40 cM in chromosome A for the progeny of sire 2

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