Glycogen Metabolism and Meat Quality

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Summary

This paper summarises the role of glycogen as a determinant of meat quality and then proceeds to discuss factors which affect glycogen metabolism. High levels of glycogen at slaughter assure the production of lactic acid during the post mortem phase such that the ultimate pH (pHu) of meat reaches 5.5. A low pHu of meat is an index of high quality since it is associated with a bright red colour, increased tenderness, increased flavour and increased keeping and cooking qualities. Few studies have examined glycogen level and/or metabolism directly in meat producing animals. Instead most studies have examined the pHu of meat as an index of glycogen metabolism. This is unfortunate since the pHu of meat is a very insensitive measure of glycogen status because it only starts to increase when glycogen is heavily depleted. Data is presented to show that glycogen level in muscle is highly sensitive to nutrition and exercise in sheep and cattle. In addition a model for studying glycogen metabolism involving the sampling of muscle 'on farm' and at the abattoir is discussed to show its effectiveness in determining management procedures which will optimise glycogen level in the muscle of meat producing ruminants.

Introduction

Glycogen is defined in the dictionary as "the store of body sugar" and it occurs widely throughout the mammalian body. The chemical structure is a relatively simple branched chain polymer of glucose with a small protein core. Quantitatively the liver and muscle account for most of the body's glycogen stores. As a store of carbohydrate, glycogen plays an important biochemical role for the homeostatic control of blood glucose and energy balance of animals. Hepatic glycogen reserves act as a source of blood glucose during energy deprivation or during times of increased glucose demand. The role of glycogen in skeletal muscle is thought to be less influenced by nutrition and more by the effects of stress or by the energy demands of muscle (Harriss 1992).

In farm animals the glycogen level of tissues has important commercial and practical implications. First, the glycogen content of muscle at slaughter has an important influence on meat quality. In particular, the dark-firm-dry meat syndrome is a direct result of low glycogen levels at the time of slaughter and this problem causes a reduction in meat quality. The incidence of dark cutting meat is sufficient to cause a significant financial loss for the sheep and cattle industries (Warriss 1990; Fabiansson *et al.* 1989). Second the glycogen content of tissues is of great importance for adaptation to environmental stressors such as lack of food especially during pregnancy and lactation, hypothermia, exercise and various management procedures. For example, low glycogen levels would greatly reduce the capacity of shorn sheep to combat hypothermia (Martineau and Jacobs 1988).

The aim of this paper is to discuss the role of glycogen in meat quaility and to present some recent research from our laboratories which is designed to understand the regulation of glycogen metabolism by nutrition and stress.

Biochemistry of Glycogen in Muscle

Classically glycogen is readily mobilised during physical activity. During prolonged exercise such as a marathon, glycogen in muscle is heavily depleted and it is common for depletion to coincide with fatigue or exhaustion. During the sprint glycogen is also rapidly lost and converted to lactic acid. It is the build up of lactic acid and the subsequent increase in acidity which limits the middle distance runner. During the recovery phase the lactic acid is removed chiefly via direct oxidation in muscle and heart or else by the liver where it is converted back into glycogen and/or glucose. Muscle glycogen can also be lost during periods of psychological stress due to the elevation of similar hormones that drive the tissue response to exercise i.e. adrenalin and ACTH (Tarrant 1989)

Skeletal muscle is not a uniform tissue but instead consists of several different fibre types i.e. type I and type II fibres. Type I fibres are slow acting and type II more rapid. The type II fibres are further split into type IIa and type IIb. Type IIa fibres are fast acting but also have good aerobic activity and give muscle a red colour

'(along with type I fibres). Muscles which produce high quality cuts tend to have higher levels of type IIa fibres while an increased ratio of type IIb fibres is associated with an increase in toughness. The metabolism of glycogen is different in the various fibres - type I fibres have low levels of glycogen. Type IIa fibres have high levels of glycogen, a high rate of glycogen resynthesis and are least affected by stress. Type IIb fibres have lower glycogen levels, slow rates of glycogen synthesis and are therefore the most susceptible to stress induced glycogen depletion (Monin, 198 1). These differences can be largely explained by the different enzyme compliment of each fibre type as shown in table 1. Thus the very high activity of glycogen phosphorylase in combination with low activities of glycogen synthase and hexokinase mean that type IIb muscle fibres rapidly deplete and slowly replete glycogen levels.

Table 1 Enzyme activities for carbohydrate metabolism in rat skeletal muscle (Adapted from Saltin and Gollnick (1983)

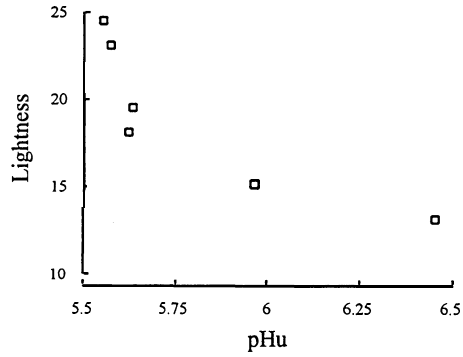
Enzyme	Activity for each Fibre type (μmol/min/gm muscle)		
	Type I	Type IIa	Type IIb
Glycogen phosphorylase	14	115	171
Glycogen synthase	6	10	5
Hexokinase	2	2	0.8

Most muscle bundles are mixtures of all fibre types; however the *m. semimembranosis* (SM) and *m. longissimus dorsi* (LD) tend toward type IIa while the *m. semitendinosis* (ST) has more type IIb fibres (Monin, 198 1; Conlee *et al.*, 1978). The SM and ST muscles represent ideal sites for sampling, in order to study glycogen metabolism.

Glycogen and Meat Quality

During the post slaughter period the muscle cells are still capable of converting glycogen to lactic acid - the process takes about 48 hours for completion (at refrigeration temperatures) - electrical stimulation of carcasses speeds this process so as the reactions are complete by 24 hours (Kastner *et al.* 1993). If the level of glycogen is adequate (approx. 40-50 mmol glucose/ kg muscle, see fig. 2) the ultimate pH of muscle (pHu) reaches 5.5 after 48 hours. At this point the muscle cells become "pickled" and accordingly some functions are lost i.e. the ability of mitochondria to use oxygen. This loss of function allows myoglobin, a red pigment in meat, to remain in the oxygenated form and so give meat a bright red/pink colour. Low levels of muscle glycogen at the time of slaughter leads to meat with a high pHu and a dark colour due to the presence of deoxymyoglobin (Moss, 1992). Meat with a high pHu (³5.7-6.0) is classified as dark-firm-dry beef (DFD). The lightness (inverse of darkness) of meat is very sensitive to change in pHu in the range of 5.5-5.8 (Fig. 1).

Figure 1 The relationship between lightness of beef (LD) and the ultimate pH (pHu) (adapted from Warriss *et al* 1984)



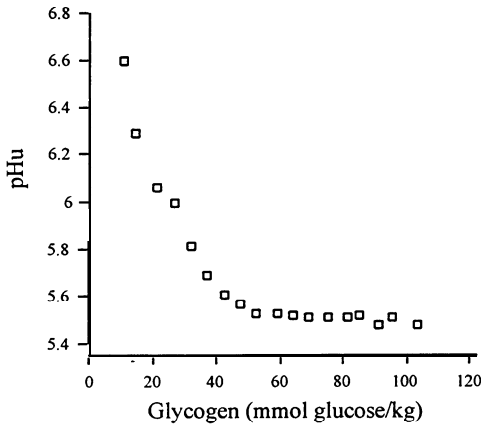
The role of glycogen in the formation of acidic meat interacts with other factors in determining meat quality. A low pH promotes increased meat flavour and palatability of beef through mechanisms which are poorly understood (Lawrie 1985). The flavour of lamb is less influenced by the pHu of muscle (LD). However, tenderness of sheep and beef meat increases as the pHu declines from 5.8 to 5.5 (Devine *et al* 1993) indicating that some muscle groups with a moderate pHu will be unacceptable to the consumer. Additionally the low acidity assures a bright attractive colour as discussed above. Finally a low pHu slows bacterial growth and so helps to guard against spoilage (Newton and Gill, 1981). It is clearly no coincidence that the pioneering work of the Qantus Flight catering division has achieved enormous gains in consumer acceptability of meat by including a pHu²5.7 as one of their specifications for meat purchases.

When meat contains high levels of glycogen at slaughter the resulting low pHu prevents complete conversion of glycogen to lactic acid leaving behind residual glycogen. The residual glycogen improves meat quality in several ways. Firstly it allows for improved keeping qualities since the microbial population utilises glycogen as a fuel rather than protein. Utilisation of protein by bacteria results in the production of ammonia and "off' odours and flavours (Newton and Gill, 198 1). Secondly glycogen is a very hydrophilic (water loving) molecule (3-4gm water/gm glycogen; Olsson and Saltin, 1970) and so contributes to the moisture content of meat. Meat with a high pHu and so low residual glycogen is not only dark in colour but also dry in texture (i.e. DFD). Indeed a loss of glycogen preslaughter implies a significant loss of carcass weight. Finally residual glycogen is thought to undergo browning reactions with protein during the cooking process and so further contribute to flavour - however the later has not yet been scientifically tested.

The relationship between the ultimate pH of muscle and the glycogen content is shown in fig. 2. The pHu of muscle does not increase above 5.5 until muscle glycogen reserves are heavily depleted. For example the

glycogen levels of a typical high quality muscle (LD) in a well fed, unstressed feedlot steer should be around 100 mmol glucose/kg and over half of this must be lost before the pHu will increase. The critical level for glycogen in muscle to assure a pHu²5.7 is around 40-50 mmol glucose/kg muscle with depletion past this point likely to result in a marked increase in pHu. Clearly the best way to understand and predict the pHu of a meat sample is to understand the biochemical control of glycogen level in the skeletal muscle of the live animal; however, there have been no systematic studies of glycogen level in farm animals in Australia.

Figure 2 The relationship between ultimate pH (pHu) of meat and the concentration of glycogen in muscle (LD) immediately post slaughter (adapted from Warriss 1990).



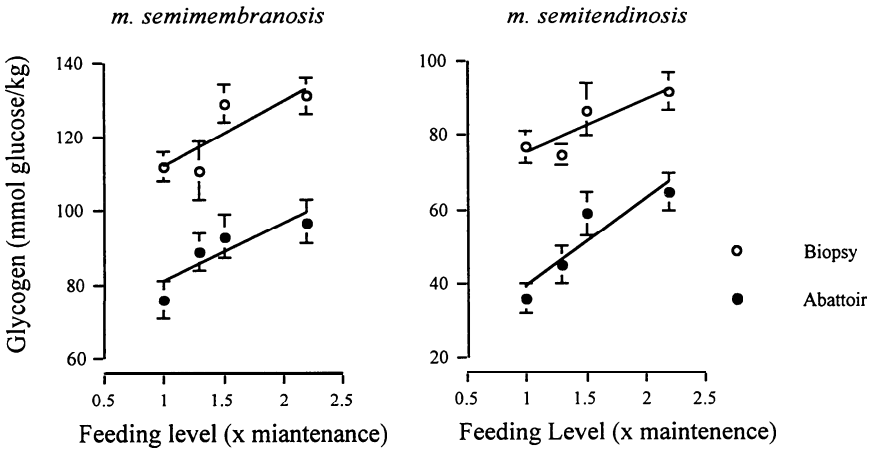
Nutritional Control of Muscle Glycogen Level

The effect of nutrition on meat quality is thought to be relatively minimal. Thus Lawrie (1985) showed no effect of extended starvation on the pHu of meat and McVeigh and Tar-rant (1982) found relatively small changes in the level of glycogen in the LD of heifers fed a barley based ration compared to hay. To further evaluate the effects of nutrition we fed 12 month old merino wethers a pelleted hay:barley:lupin (20:53:26) feedlot type diet at 4 levels of intake representing 1, 1.3,1.5 and 2.2 times maintenance (8 sheep per treatment). Samples of the SM and ST were obtained by biopsy in the sheep shed and within 10 minutes post slaughter at the abattoir and then analysed for glycogen content (Fig 3). The sheep were transported for 60min and then slaughtered within 2 hours (Pethick and Rowe, 1995).

There was a strong linear relationship between feed intake and glycogen level that was similar for both muscle types and at both sampling times (fig 3.). The effects of nutrition could again be seen when the level of residual glycogen in meat was analysed (fig 4.).

The level of residual glycogen in meat can be thought of as a buffer against a tendency for high pHu. When the level drops to below 10-20 mmol glucose/kg of meat a pHu³5.7 is very likely. The changes in pHu of meat derived from sheep fed at the different nutritional levels is shown in fig 5. The pHu is very unresponsive to nutrition in the SM and LD as shown by previous workers but more sensitive to feed intake for the ST. This is closely associated with the inherently low level of glycogen in the ST.

Figure 3 The effect of feed intake on the glycogen level of skeletal muscle taken by biopsy from sheep in pens and then later at the abattoir. (Note the different scales for the two muscle types, however the y-axis span is similar).



To assess the influence of nutrition 'on farm' the level of glycogen in finished cattle grazing dry pasture in December (south west, Western Australia) was compared to the level found in finished steers consuming a feed lot ration (hay:barley:lupin ration similar to the diet fed to the sheep cited above). In addition steers were compared eating their ration in individual pens at a research station versus those housed in a commercial feedlot (fig 6.).

The level of glycogen was dramatically reduced in those steers grazing dry pasture when compared to the feedlot ration. There was a smaller although significant reduction in glycogen in the commercial feedlot versus the individually housed animals.

This group of experiments has clearly shown that nutrition has a powerful effect on the level of glycogen in skeletal muscle. Further studies are needed to understand the metabolic basis of this control.

Figure 6 The level of glycogen in skeletal muscle of finished steers (~440kg) fed a feedlot ration in individual pens (n=22), a feedlot ration in a commercial feedlot (n=22) and in steers grazing dry summer pasture (n=24). (a-significantly different to individual pens; b-significantly different to Individual pens and Feedlot, $P<0.05$).

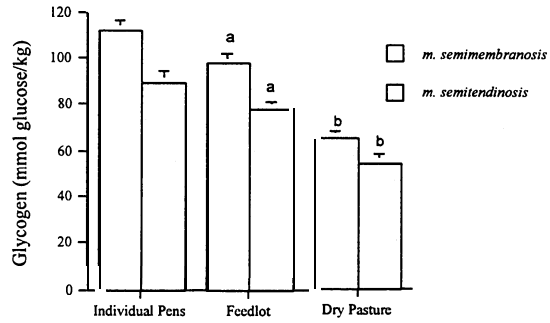


Figure 4 The effect of fed intake and regular exercise on the level of residual glycogen (glycogen 48 hours post slaughter) in meat.

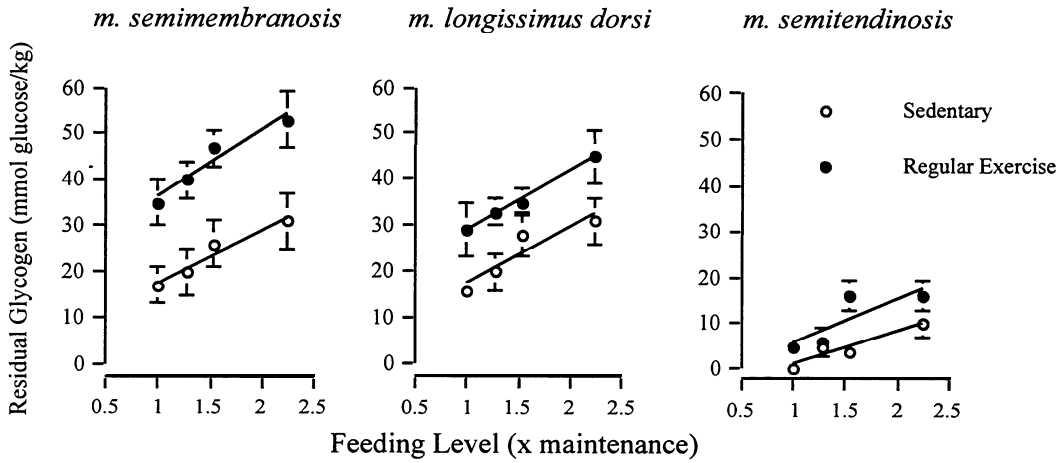
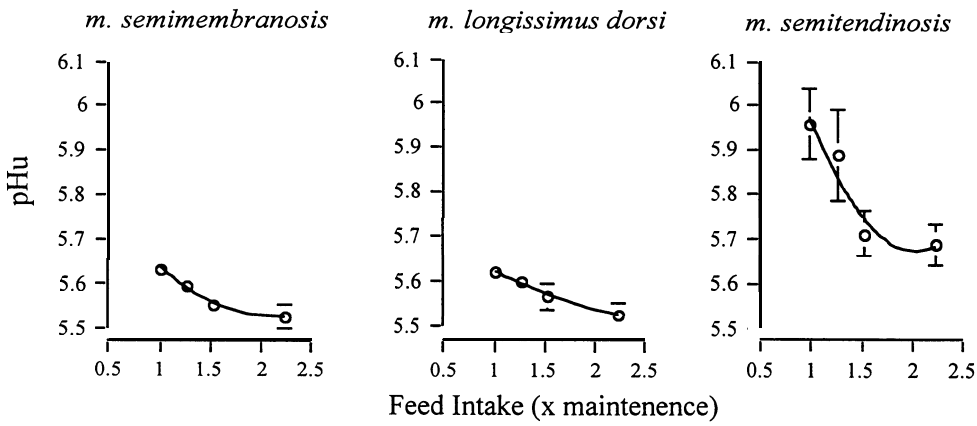


Figure 5 The effect of feed intake on the ultimate pH (pHu) of meat derived from sedentary sheep fed at different levels of intake.



The Effects of Exercise on Muscle Glycogen Level

Regular exercise (training) is known to increase the level of glycogen in the muscle of a variety of animals including the rat (Tan et *al.* 1984), the pig (Essen-Gustavsson *et al.* 1988) and the horse (Topliff *et al.* 1985). In preliminary studies with growing steers (Pethick *et al.* 1994) we have shown that regular exercise in cattle can increase glycogen levels. In this case exercise was a more powerful stimulus for increased glycogen level than was nutrition.

In the experiment testing the effect of nutrition on merino sheep cited above an additional treatment included regular exercise (i.e. 4 levels of nutrition and 2 levels of exercise, 8 sheep per treatment). The group receiving regular exercise were run for 1 hour at 8-9 km/h (trot) three times per week for 6 weeks. Regular exercise resulted in a significant elevation of glycogen and residual glycogen (fig 4). The effects were most pronounced for the SM and LD (predominance type IIa fibres) and smaller for the ST (predominance of type IIb fibres) which is to be expected since the exercise regime operated at around 60-70%VO_2max and so would have a greater influence on the aerobic muscle groups.

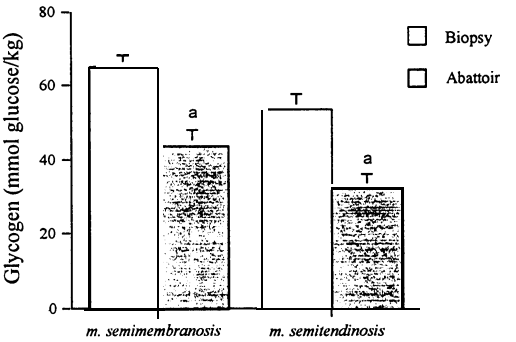
The practical significance of these results is not clear but it may imply that well fed animals in an extensively managed situation are at less risk of producing meat with an elevated pHu. Perhaps more importantly the results show that glycogen level in the muscle of ruminant animals can be manipulated and further studies are needed to understand the metabolic basis of this control.

Loss of glycogen Between Farm and Abattoir

Few studies have attempted to quantify the losses of glycogen from muscle during the various activities associated with the 'post farm gate' phase. Most studies have centred on the measurement of pHu as an indicator of meat quality and it should be remembered that the pHu will only change as a result of extreme depletion of glycogen. Consequently the role of more subtle stressors as contributors to an elevated pHu are poorly understood.

Using the model of muscle biopsy 'on farm' and subsequent collection of muscle soon after slaughter we have undertaken preliminary studies to quantify the losses associated with 'post farm gate' procedures. The losses of glycogen from the muscle of sheep between the animal house and slaughter were about 30mmol glucose/kg or 30-40% depending on muscle type (fig 3). This loss is not associated with glycogen degradation post slaughter since we and others (Tarrant and McVeigh, 1979) have shown that the level of glycogen does not change within 30 minutes post death. This level of loss was unexpected since these sheep were subjected to apparently minimal stress i.e. 1 hour of transport and 0.5-2 hours of lairage. In a similar study the level of glycogen in the muscle of steers grazing dry summer pasture was compared 'on farm' and immediately post slaughter after the steers had been 'off feed' for 48 hours (24 hours fast on farm and 18 hours lairage) and transported for 4 hours to the abattoir. The losses are shown in fig 7 and amount to some 20 mmol glucose/kg muscle or 30-40% depending on muscle type.

Figure 7 The level of glycogen in the muscle of finished steers (~440kg) sampled both at dry pasture (taken by biopsy) and at the abattoir immediately post slaughter (a-significantly different to biopsy, $P<0.05$)



The factors that contribute to low glycogen levels are additive and so if we consider the finished steers grazing dry pasture (fig 7) then a combination of low nutrition and the apparently 'normal' losses associated with the post farm gate procedures resulted in levels of glycogen in the SM (and therefore LD) on the threshold (i.e. 40mmol glucose/kg) of causing a pHu>5.7. The final level of glycogen in the ST would have certainly resulted in an elevated pHu since we have found the relationship between pHu and glycogen content to be influenced mainly by glycogen level.

The effects of mixing of unfamiliar animals on glycogen level in the muscle and the subsequent development of DFD beef has been well studied in the northern hemisphere by Tarrant and colleagues (Tarrant, 1989; Warriss 1990). Their work used mainly bulls and the resulting agonistic behaviour in the form of butting, pushing, mounting and chin-resting induced a physical stress which heavily depleted glycogen in muscle. In this case the physical stress was the most important reason for depletion since the researchers prevented mounting activity in bulls with overhead electrical grids and greatly reduced the incidence of DFD beef. If animals have to be mixed; a large group size seems to cause less problems. The role of growth promotant implants is unclear but those which increase the "bully" behaviour of steers (i.e. Revalor") could promote more trouble if unfamiliar animals are mixed together. Heifers in oestrous are also susceptible to glycogen depletion, again due to the characteristic mounting activity.

Factors contributing to meat with an elevated pHu have not been so clearly defined but have been

summarised by Warriss (1990) and Tarrant (1989). Transportation has been shown to cause only small increases in the pHu of beef unless animals are "floored" in which case DFD beef is almost guaranteed. Cold exposure in unacclimatised animals should theoretically increase the pHu of beef as the shivering reflex is known to deplete glycogen (Martineau and Jacobs, 1988) - despite this it is not commonly reported as a significant contributor to DFD beef. The study of Bray et *al.* (1989) highlights the additivety of various stressors as causes of high pHu meat. They found that undernutrition, shearing or swimming of sheep alone produced no significant response but when combined they had a dramatic effect on pHu of meat. This study again highlights the need to study glycogen level of muscle and not the pHu of meat.

The role of psychological stress? The extent to which placing an animal in unfamiliar surroundings (sale yards, lairage or cattle trucks) causes glycogen depletion is poorly defined. In an extreme case, restraining of sheep (by leg tying) alone in an unfamiliar room for 6 hours each day for 3 days caused heavy depletion (Apple *et al.* 1993). Certainly in our hands the stress susceptible animals (i.e. turning around in crush, sweating, trembling) consistently have low glycogen levels. In a more relevant study of bulls penned in their own producer lots overnight, followed by lairage for 27 hours without food reduced glycogen levels by 20% resulting in an increase in pHu from 5.6 to 5.7 (Campbell, 1984). Few studies have been performed to examine the effects of psychological stress.

Surprisingly exercise does not always deplete glycogen. Low level exercise (slow-brisk walk) does not cause glycogen loss from muscle (Harman and Pethick, 1994) and even high intensity exercise (gallop) may not always be a problem since it allows for unusual and rapid resynthesis of glycogen from lactate during recovery. However sustained medium-high (trot-canter) intensity exercise will definitely deplete glycogen.

Glycogen Resynthesis

One general problem with all ruminants seems to be a very slow rate of repletion of muscle glycogen, typically some 3-l 1 days (see Warriss, 1990; Warriss *et al.* 1984). The reason for the slow rates are unclear and may pertain to the nature of the studies. Repletion rates have only been reliably measured in the Northern Hemisphere where the experimental animals were tethered. Tethering via stocks is a common production system for the EEC countries and may predispose cattle to low rates of repletion since the animals are rarely exercised and therefore are not adapted to repleting glycogen. There is much work in experimental animals which shows that a sedentary state slows the rate of glycogen synthesis. We have shown rates of glycogen repletion some 3 times faster in exercised trained sheep when compared to published data (Harman and Pethick, 1994). The rates of repletion in sheep and cattle in Australian conditions are unknown - however they are likely to be considerably faster. Certainly the processing sector in Western Australia strongly believe that repletion is possible since they are convinced that 36 hours lairage is better than 12-24 hours for cattle that have travelled relatively long distances (10 hours travel). It is important to understand the mechanisms and rate of glycogen repletion so as appropriate lairage can be designed.

Animal Welfare

This a difficult and complex subject as welfare is often based on subjective criteria. Even objective approaches have problems as they often are based on acute changes (i.e. hormone levels) which can be difficult to interpret. It is possible that glycogen will represent an excellent integrated estimate of the stresses placed on animals during the production cycle. Glycogen level in muscle and liver will reflect a balance between substrate supply and the hormonal environment of the animal. Thus the ratio of anabolic:stress hormones (or signals in the case of the nervous system) will influence the activity of the glycogen degradation (glycogen phosphorylase) and synthesis (glycogen synthase) pathways.

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