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The regulation of glycogen level in the muscle of ruminants by nutrition


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ABSTRACT

This paper discusses recent work by the authors which has investigated the nutritional regulation of glycogen concentration in skeletal muscle of sheep and cattle. Several experiments are summarised which show a clear relationship between the level of glycogen in muscle and the intake of metabolisable energy. This translates into strong seasonal effects on the level of muscle glycogen in pasture fed cattle that correlate with live weight change. The clear message is that animals destined for slaughter should be on a high plane of nutrition as this will contribute to an increased level of muscle glycogen at slaughter and so help alleviate the problem of dark cutting meat. Acute regulation of glycogen is more problematical since the rate of glycogen repletion in skeletal muscle is relatively slow and the scope for rapid dietary change in ruminants is constrained by the need to allow rumen adaptation to high starch/sugar diets. However the sudden introduction of a high energy diet (based on cereal grain) in the presence of a ‘rumen modifier’ to reduce ruminal acidosis can increase muscle glycogen concentration within 1 week of feeding. The ability to further modify glycogen level in skeletal muscle using carbohydrate and electrolyte products is discussed. In particular the possibility of using oral glycerol/propylene glycol as a means for increasing blood glucose and so glycogen synthesis is proposed. Experiments to examine the effectiveness of MgO as a means for reducing the stress response are also discussed.

Keywords: energy intake; muscle glycogen; ruminants

INTRODUCTION

The rate and extent of post mortem change in the pH of meat is considered an important cause of variation in beef and sheep meat quality. For example if the pH exceeds 5.8-5.9, the meat appears dark, firm and dry (DFD) and is significantly tougher in the zone of pH 5.8-6.2. The incidence of DFD meat is sufficient to cause a significant financial loss for the sheep and cattle industries (Warriss, 1990; Fabiansson et al., 1989). The post mortem change in the pH of muscle is largely based on the degradation of glycogen to lactic acid using the metabolic pathways of glycogenolysis and glycolysis. The aim of this paper is to describe our recent experiments, which have focused on the nutritional regulation of glycogen level in skeletal muscle.

We have developed an experimental system based on a simple muscle biopsy procedure to study the regulation of glycogen concentration in the muscle of live animals. The two muscles sampled in this study, m. semimembranosus (SM, topside) and m. semitendinosus (ST, eye round – part of the silverside) were chosen for ease of sampling and representation of a range in fibre types. The SM is particularly useful as it is biochemically similar to high value muscles like the m. longissimus dorsi (LD). The SM and LD are classified as fast red (Braind et al., 1981) with a ratio of type I:IIa:IIb muscle fibres in the LD of 50:40:10 (Suzuki, 1971; see also Aalhus and Price 1991). These muscle groups have high levels of glycogen, and are less sensitive to stress induced depletion of glycogen (Monin, 1981). The ST is classified as a fast white muscle (Braind et al., 1981) with a ratio of type I:IIa:IIb of 34:56:10 (Suzuki, 1971; see also Aalhus and Price, 1991). The ST has lower glycogen levels and is more sensitive to stress induced depletion of glycogen. For the work described in this paper glycogen concentration was measured enzymatically and represents total muscle glucose plus lactate (Gardner et al., 1999).

Chronic effects of nutrition

To evaluate the effects of nutrition 12 month old Merino wethers (n=8 per treatment) were fed 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. Samples of the SM and ST were obtained by biopsy in the live animal and within 10 minutes post slaughter at the abattoir, and were then analysed for glycogen content. The sheep were transported for 60min and then slaughtered within 2 hours (Pethick and Rowe, 1996). There was a linear relationship between feed intake and glycogen level that was similar both for the biopsy and immediate post slaughter sample (Figure 1) and for different muscles (Pethick and Rowe, 1996). The effects of nutrition could again be seen when the level of residual glycogen (48 hours post-slaughter) in meat was analysed. The level of residual glycogen in meat can be thought of as a buffer against a tendency for a rise in the ultimate pH. When the level drops to below about 0.2g/100g in muscle a pHu≥5.7 is very likely.

FIGURE 1. The effect of feed intake on the level of glycogen in the m. semimembranosus in the live animal and in the carcass 10 minutes and 48 hours post slaughter.

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In a second experiment we evaluated the effects of changing the nutritional state of steers (Tudor et al. 1996). At the time there was a concern in the Western Australian beef industry that silage-fed steers were more likely to have dark coloured meat than animals fed other diets. The aim of this experiment was to investigate the effects of metabolisable energy (ME) and dietary composition on muscle glycogen concentration in steers previously fed poor quality dry pasture and then transferred to diets containing either silage or hay with or without a barley grain supplement. Forty, 10 month old Angus x Friesian or Limousin x Angus x Friesian cross steers were weaned and grazed on dry standing improved pasture (DM 88%, ME 6.8 MJ/kg and crude protein 8.6%). At 12 months of age, they were stratified on initial live weight of 304 ± 6 kg (± sem) and allocated, within breed type, at random to 4 dietary treatments: 1. Silage ad lib. (DM 22%, ME 9.6 MJ/kg DM and crude protein 13.5%), 2. Hay ad lib (86, 10.8 and 16.2, respectively), 3. silage ad lib. + 3 kg cracked barley grain (89, 11.1 and 10, respectively) and, 4. hay ad lib + 3 kg cracked barley grain. Urea at 2% was added to the barley grain. The animals were individually fed to appetite for 7 weeks and live weight was recorded weekly. Biopsy samples were collected from the SM and ST at the start and end of the feeding period and analysed for glycogen.

The change in muscle glycogen content between the initial pasture value versus the value seven weeks later after consuming the dietary treatments was directly related to the intake of metabolisable energy (Figure 2). The source of the ME was not important but rather the total intake. A similar graph could have been generated if the change in glycogen content was plotted against liveweight change with no response in muscle glycogen at 0.6 kg/day liveweight change (approx. 55 MJ ME/day) to the largest response when cattle were gaining at 1.2 kg/day (approx. 102 MJ ME/day).

The occurrence of dark-cutting in beef carcasses in Australia has been reported to have a seasonal effect although the peak months of dark-cutting vary between years and region. Our studies in Victoria have investigated the effect of season and stocking rate on muscle glycogen levels in cattle and found that there was little difference in SM or ST glycogen concentration between cattle grazed at 1.5 hd/ha and those grazed at 2.5 hd/ha (Table 1). There was a strong seasonal influence on the concentration of glycogen in muscle with consistently low levels in winter and summer and high levels in spring. This drop in muscle glycogen concentration is partly explained by declining animal growth rate, which is driven by changes in pasture availability and quality. Using the data is Table 1 there is a significant relationship between live weight change and muscle glycogen concentration (r² = 0.69, P = 0.04). Given that the ultimate pH of muscle begins to increase when the immediate pre-slaughter glycogen concentration is less than about 1%, then a growth rate of above 1kg/day is needed to assure a level of muscle glycogen that is sufficient to help reduce the incidence dark cutting.

**Acute effects of nutrition**

Given the seasonal influence on muscle glycogen content in pasture fed cattle an experiment was initiated to test the effect of high-energy supplements. Sixty yearling steers (Hereford, Angus and Hereford Angus cross) were randomly allocated to six equally sized paddocks at a stocking rate of 1.5 steers/ha. During March, the cattle were introduced to troughs and small quantities of grain feeding, to familiarise them with the supplementation program. Grain feeding was then withdrawn and the steers were grazed on pasture to ensure that glycogen concentrations would be relatively low in winter when the experiment commenced. After three months on pasture, the steers were randomly allocated to either an intensive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Winter</th>
<th>Spring</th>
<th>Season</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Δ in LW (kg/day)</td>
<td>0.7</td>
<td>0.3</td>
<td>1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST Glycogen (g/100g)</td>
<td>0.91</td>
<td>1.04</td>
<td>1.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SM Glycogen (g/100g)</td>
<td>0.99</td>
<td>1.01</td>
<td>1.09</td>
<td>&lt;0.001</td>
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**TABLE 1:** Effect of season and stocking rate (SR; Low = 1.5 hd/ha, High = 2.5 hd/ha) on changes in daily liveweight change (Δ in LW) and glycogen levels in the m. semimembranosus (SM) and m. semitendinosus (ST) of cattle.
grain feeding treatment, whilst grazing on the same pastures, or a control treatment of no supplementary feeding. The supplemented groups were introduced to a ration comprised of 65% cracked triticale, 15% cracked lupins, 20% hammermilled straw, 2% molasses and Eskape® (Ridley Agri Products, Australia; a mineral premix containing virginiamycin & salinomycin to reduce rumen acidosis) fed at a daily rate of 3 kg, 3 kg, 3 kg, 5 kg and 5 kg per head respectively for the first five days with ad libitum access for a subsequent 18 days. Weekly muscle glycogen concentration for the SM and ST are shown in Table 2.

The supplemented groups showed significantly higher concentrations of muscle glycogen in both the SM and ST at Week 1, Week 2 and Week 3 compared to control groups. This study confirms the muscle glycogen concentration can be increased substantially within one week of supplementation, and achieve concentrations equivalent to lot-fed steers. However it is essential that the high energy grain feeding is carefully introduced or that it includes rumen modifiers to minimise the risk of acidosis. In addition the cattle would have to have some acquaintance with grain feeding.

Finally, a trial was run to estimate the repletion rate of muscle glycogen utilising an exercise depletion/repletion model. Twenty 18 month old Angus steers of average live weight 376kg were allocated to three dietary treatments, hay and maize or barley. The hay diet consisted solely of pasture hay (ME 8 MJ/kg, CP 8% & intake 7.5 kg/hd in DM); the maize diet consisted of 64.2% steam flaked maize, 12% lupin, 15% hay, 1% urea, 5% molasses and 2.8% min-vits (ME 11.3 MJ/kg, CP 14% & intake 12.8 kg/hd in DM) and the barley diet was 66% barley, 10% lupin, 15% hay, 0.9% urea, 5% molasses and 2.8% mineral premix (ME 10.9 MJ/kg, CP 14.11% & intake 11.5 kg/hd/d in DM). All animals were subjected to a 5 x 15 min exercise regime, with muscle biopsies taken immediately pre and post-exercise, and 36 and 72 hrs post exercise. Animals were housed individually and had access to their dietary treatments throughout the post-exercise period. A summary of the results is shown in Figure 3.

TABLE 2: The effect of feeding a high energy supplement (supplement versus control) to cattle for three weeks on the change in muscle glycogen for the m. semimembranosus (SM) and the m. semitendinosus (ST).

<table>
<thead>
<tr>
<th></th>
<th>Supplemented</th>
<th>Control</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>SM Week 0</td>
<td>11.3</td>
<td>10.2</td>
<td>0.374</td>
</tr>
<tr>
<td>SM Week 1</td>
<td>17.3</td>
<td>12.9</td>
<td>0.073</td>
</tr>
<tr>
<td>SM Week 2</td>
<td>16.2</td>
<td>12.1</td>
<td>0.034</td>
</tr>
<tr>
<td>SM Week 3</td>
<td>20.3</td>
<td>12.1</td>
<td>0.005</td>
</tr>
<tr>
<td>ST Week 0</td>
<td>10.7</td>
<td>9.7</td>
<td>0.466</td>
</tr>
<tr>
<td>ST Week 1</td>
<td>15.2</td>
<td>11.9</td>
<td>0.012</td>
</tr>
<tr>
<td>ST Week 2</td>
<td>15.1</td>
<td>10.79</td>
<td>0.015</td>
</tr>
<tr>
<td>ST Week 3</td>
<td>18.8</td>
<td>11.3</td>
<td>0.001</td>
</tr>
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Both the SM and ST of the animals on the hay diet, and the ST of the animals on the maize and barley diet, showed no significant repletion of glycogen following exercise. This indicates the importance of a high-energy diet for driving glycogen repletion in ‘red’ type muscle. It also emphasises the importance of muscle type and confirms that muscle groups with an increased proportion of type II fibres are relatively unresponsive to short term nutritional change. Fortunately, most high value muscle groups in the carcass are biochemically similar to the SM and so are more responsive to short term nutritional change.

There was also no significant repletion of glycogen in the SM of the cereal grain fed animals during the first 36hrs post-exercise, however between 36 and 72hrs, glycogen was repleted to within 80-90% of the pre-exercise glycogen concentration. This rate of repletion when calculated between 0-72 hrs post exercise, was 0.008 g/100g muscle/hr, falling within the published range of values in previous papers (Tarrant, 1989). However there also appears to be a carry-over effect of the stress involved in the exercise protocol, which may explain why repletion in the SM did not begin until after 36 hrs. The rate of repletion during the second 36 hrs (36-72hrs) was considerably higher at 0.012 g/100g muscle/hr suggesting higher repletion rates are possible.

FIGURE 3. Glycogen repletion in the m. semimembranosus following exercise, and metabolisable energy intake over the repletion period. Values are means ± sem. (Bars with different letters are significantly different, P<0.05)

Hyperglycemic agents

The results of the previous glycogen repletion trial clearly demonstrate that ruminants are relatively slow (compared to monogastric animals e.g., human) at repleting glycogen even in response to high energy diets. The key to glycogen repletion in the muscle of human athletes is dietary sources which induce hyperglycemia (soluble carbohydrate drinks) given soon after exercise as this is when the greatest rates of repletion are seen (Sherman, 1991). This presents a problem for ruminant nutrition since the intake of high energy diets does not result in hyperglycemia due to extensive fermentation of carbohydrate in the rumen. Given this, we initiated studies to explore the best hyperglycemic agents in ruminants to see if they might promote more rapid glycogen repletion in muscle and so be of use in the curfew/lairage period immediately pre-slaughter.

Our preliminary confirmed earlier observations (Buswell et al. 1986; Rodriguez Iglesias et al. 1996) that a mixture of glycerol and propylene glycol is a potent hyperglycemic treatment when drenched into the rumen of sheep (Figure 4).
The results show firstly that in the absence of food intake there is no significant repletion of muscle glycogen following exercise in the SM. Secondly there is a significant increase in the glycogen concentration of the SM due to the inclusion of the carbohydrate supplement in water. However the extent of glycogen resynthesis is relatively small compared with complete repletion within 48 hours that we have found in similar experiments where the sheep are allowed to consume a high energy diet after exercise (Gardner and Pethick, 1998). There was no significant repletion of glycogen in the ST on either treatment.

**Electrolytes**

Various electrolyte preparations containing a variety of ingredients have long been available to help ruminants cope with the stress of transport and lairage pre-slaughter. Phillips (1997) showed a positive response to a relatively simple water based electrolyte/sugar supplement on meat colour in cows undergoing long haulage (1,500km). Schaefer et al. (1997) reviewed the use of electrolyte preparations for cattle and concluded that they reduced the incidence of dark cutting carcasses. The work of Schaefer and colleagues has led to the development of a new generation patented electrolyte/carbohydrate preparation called Nutricharge. We have tested two commercial electrolyte preparations; Glucotrans (Pfizer Animal Health) a water based product and a prototype ‘in feed’ based Nutricharge (AgResearch, New Zealand) product. The results of 5 trials are shown below in table 4 where the level of glycogen in the SM was compared in cattle post-slaughter of treated and untreated groups (typically 45-50 cattle per group). There was a small positive effect on muscle glycogen level due to the inclusion of Glucotrans. The effectiveness of Nutricharge was severely hampered by problems of poor intake in lairage. On the one occasion where the product was consumed (Nutricharge 2a, Table 4) there was a large increase in muscle glycogen compared to the control group.

We conclude that further work with electrolytes is warranted. Importantly ‘in feed’ preparations should be restricted to use ‘on farm’ and delivery via the water is considered the best option for the curfew/lairage period.

**Magnesium**

Magnesium supplementation has been shown to reduce the stress response in sheep suffering hypothermia (Terashima et al., 1996). The stress response in pigs before...
slaughter was also reduced, leading to higher muscle glycogen concentrations (DeSousa et al., 1998). We therefore designed an experiment to test the influence of supplemental magnesium oxide (MgO) on muscle glycogen concentration in sheep exposed to exercise and the commercial slaughter process (Gardner and Pethick, 1998).

Sheep supplemented with either 0.5% or 1% MgO for 7 days prior to exercise stress lost between 12-24% less muscle glycogen in the ST during exercise. In the period up to 72 hrs post-exercise, those animals supplemented with 1% MgO repleted about 55% more muscle glycogen in the SM than the 0% MgO group. This indicates that MgO supplementation reduced the response to exercise stress and increased the rate of glycogen repletion. When the lambs were slaughtered it was found that feeding MgO (0.5 or 1%) for four days pre-slaughter significantly increased the muscle glycogen concentration in the ST at slaughter by about 20%. We have subsequently completed a further 3 slaughter experiments using lambs in the commercial environment where MgO was added at 1.0% to a feedlot ration for 4 days pre-consignment. In 2 of these experiments there was a significant increase in the glycogen content of muscle (either the SM or ST) immediately post-slaughter in the lambs offered MgO in the diet pre-slaughter. In the one experiment where no response was seen the level of muscle glycogen was relatively high immediately post-slaughter indicating that the animals did not experience a particularly high stress load and hence there was little opportunity for MgO to have an effect. We conclude that commercially MgO appears to reduce the response to stress, leading to a subsequent reduction in muscle glycogen loss.

**CONCLUSION**

This paper has highlighted the importance of nutrition as a determinant of the glycogen concentration in muscle. The data indicates that ruminants destined for slaughter should be on a high plan of nutrition so as adequate levels of muscle glycogen are present to act as a buffer against the various stressors apparent in the post farm gate period leading up to slaughter. Short term feeding for at least 1 week can substantially raise muscle glycogen concentration but adaptation of the rumen to the ‘new’ high energy feed is probably the greatest constraint for determining how quickly high energy diets can boost muscle glycogen content in ruminants consuming a poor quality basal diet. The ability of ‘in feed’ or oral carbohydrate sources which cause hyperglycemia (i.e. glycerol/propylene glycol mixes) to stimulate glycogen repletion in muscle is significant but not as effective as a high energy diet. Evidence is put forward to suggest that electrolyte/carbohydrate preparations can stimulate muscle glycogen levels when given 24-72 hours pre-slaughter. However further work is needed to verify the practical worth of electrolytes. Finally pre-dosing animals with MgO will reduce the stress response during the ‘post farm gate’ period pre-slaughter.

**REFERENCES**


