Nutritional influences on muscle glycogen recovery following exercise in sheep and cattle

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Summary

This paper investigates the nutritional regulation of glycogen concentration in skeletal muscle of sheep and cattle following exercise, with particular emphasis on a comparison between these species. Four experiments are summarized, indicating a clear relationship between metabolizable energy (ME) intake and rate of muscle glycogen repletion following exercise. Sensitivity to ME intake appeared to differ between species, with sheep demonstrating higher rates of glycogen repletion on lower levels of ME intake than cattle. Roughage diets appeared to produce maximal rates of muscle glycogen repletion in sheep. The use of a glycerol and propylene glycol supplement to enhance muscle glycogen repletion following exercise had marginal success as compared with normal rations, but the efficiency of conversion of the ME of the supplement into muscle glycogen was far higher.

Keywords: metabolisable energy, glycerol, propylene glycol, hyperglycaemia, fluid intake

Introduction

Following slaughter, muscle glycogen fuels the gradual acidification of muscle via anaerobic production of lactic acid, reducing the pH from about 7.2 to an ultimate pH (pHu) of about 5.5. Factors such as poor nutrition or stress can reduce muscle glycogen at slaughter resulting in elevated pHu which, when exceeding pH 5.8–5.9, can lead to a problem of meat quality known as dark, firm and dry (DFD). DFD causes significant financial loss to cattle and sheep industries globally (Fabiansson *et al.* 1989; Warriss 1990), and work is in progress to identify animals potentially at risk of DFD before they are slaughtered. The 'at risk' animals could be withheld from slaughter and fed to allow recovery of muscle glycogen. Thus nutritional regimens are required to enhance muscle glycogen recovery in depleted animals. Previous work has investigated the rates of recovery of muscle glycogen following stress but, to date, there is little information on the effect of different levels of nutrition on rate of recovery. The aim of this paper is to describe our recent experiments on the effect of nutrition on the recovery of muscle glycogen in the skeletal muscle of cattle and sheep following exercise stress. Particular emphasis is placed on between species comparisons of the nutritional responses.

A model to study the metabolism of muscle glycogen

To study the regulation of muscle glycogen in live animals a simple muscle biopsy procedure was developed (Gardner *et al.* 2000); samples are taken from the *M. semimembranosis* (SM, topside) and *M. semitendinosis* (ST, eye round). These muscles were chosen for ease of sampling and representation of a range in fibre types; the SM is classified as a fast red muscle, and the ST a fast white muscle (Braind *et al.* 1981). The SM has high levels of glycogen and is less sensitive to stress–induced depletion of glycogen, and the ST has lower glycogen levels and is more sensitive (Monin 1981). For the work described in this paper, glycogen concentration was measured enzymatically and represents total muscle glucose plus lactate. Further details of methodology and sampling procedures are described in Gardner *et al.* (2000).

To study glycogen repletion, a model based on exercise was utilised to elicit significant levels of glycogen depletion (approximately 50%), from which rates of muscle glycogen repletion could be determined. The exercise regimen involved trotting cattle at 9 km/h for 5 x 15 min periods, or sheep at 8 km/h for 4 x 15 min periods, in both cases with a 15 min rest between each period and with an exercise intensity equivalent to about 65% VO_2 max (Gardner *et al.* 2000; Pethick and Rowe 1996). Muscle biopsies were taken immediately before and after exercise, and 36 and 72 h post exercise, enabling both muscle glycogen depletion and the subsequent rates of repletion to be determined.

The aims of this study were to use repeated muscle biopsy and the exercise model to determine the ability of cattle and sheep on (i) roughage and cereal grain based diets and (ii) glycerol and propylene glycol water supplements, to achieve repletion of muscle glycogen.

Glycogen repletion on roughage/ grain rations

A total of 40, 18 month old Angus steers (average liveweight 376 kg) were allocated randomly to 4 dietary treatments: pasture hay, fresh silage, barley, and maize (Table 1).

In the sheep experiment 90, 6 month old Merino wethers (average liveweight 29.4 kg) were allocated randomly to three dietary treatments: hay, maize, and barley (Table 1).

In both experiments sheep and cattle were housed individually and had *ad libitum* access to their dietary treatments for 6 weeks prior to, and immediately after being subjected to the exercise regimen. Feed intakes were measured daily.

In cattle both the SM and ST of the animals on the hay diet, and the ST of the animals on the silage, barley, and maize diets, showed very little repletion of glycogen 72 h following exercise, but the higher energy grain rations produced marked levels of glycogen repletion in the SM ($P<0.001$) (Table 2). In sheep there was significant glycogen repletion in both the SM and ST for all diets, although the level of repletion in the ST was only half that seen in the SM (Table 2). This emphasises the importance of muscle type and confirms that muscle groups classified as fast white are relatively unresponsive to short term nutritional change. Given this contrast between muscles, the assessment of

Table 1 Composition of diets for cattle and sheep exercise/glycogen repletion experiments.

Component of diet (%)	Cattle dietary treatments				Sheep dietary treatments		
	Hay	Silage	Barley	Maize	Hay	Barley	Maize
Lupin			10	12		20	25
Pasture hay	100		15	15	45	15	15
Lucerne hay					55		
Silage		100					
Maize				64.2			57.6
Barley			66.3			62.8	
Urea			0.9	1		0.2	0.4
Molasses			5	5			
Minerals + Vitamins	2.8			2.8		2	2
				Ration analysis			
ME (MJ/kg DM)	8.0	10.2	11.3	11.3	8.1	10.9	11.4
CP (%)	8.0	15.9	13.3	12.3	13.1	16.9	16.8
Intake (kg DM/head	6.98	7.81	10.76	11.77	1.13	1.17	1.14

Table 2 Effect of experimental rations on change in muscle glycogen concentration (g/100 g) in cattle and sheep 72 h after exercise.

		Dietary treatment				
	Muscle	Hay	Silage	Barley	Maize	Significance of effect (*P*)
Cattle						
Repletion	SM	0.10 ± 0.083a	0.32 ± 0.089b	0.49 ± 0.052bc	0.57 ± 0.084c	**
72 h post–exercise	ST	0.08 ± 0.082	0.04 ± 0.053	–0.05 ± 0.109	–0.02 ± 0.088	n.s.
Sheep						
Repletion	SM	1.01 ± 0.100	–	0.82 ± 0.099	0.76 ± 0.068	n.s.
72 h post–exercise	ST	0.41 ± 0.057	–	0.35 ± 0.075	0.35 ± 0.055	n.s.

Values are mean ± SEM. Values within rows followed by different letters are significantly different at $P = 0.05$
n.s., not significant; ** $P<0.01$

nutritional impacts on muscle glycogen repletion for the remainder of this paper will focus on the more nutritionally responsive SM.

In contrast to the SM of cattle the level of muscle glycogen repletion in sheep did not differ between rations (Table 2). This difference is further highlighted in Figure 1 which demonstrates a positive linear relationship (P<0.001) between SM glycogen repletion after 72 h and metabolizable energy (ME) intake per $kgW^{0.75}$ in cattle, whereas no such relationship was evident in sheep. Thus glycogen repletion in sheep following exercise appears to be independent of ME intake over the range studied, though the range was smaller than for cattle. Consequently the experimental rations in the sheep experiment may not have properly tested the effect of ME intake on rate of muscle glycogen repletion. Would glycogen repletion in sheep be independent of ME at much lower levels or across a broader range of ME intake? However, the results do clearly show that the absolute rate of muscle glycogen repletion in sheep appears to be higher than in cattle on similar ME intakes per $kgW^{0.75}$.

Therefore the conclusions from this initial experiment were that (i) glycogen repletion in cattle was dependent on ME intake, (ii) the absolute rate of glycogen repletion in sheep was higher, and (iii) the range of ME intakes fed to sheep was smaller when expressed in terms of metabolic body weight—that is we did not really test the effect of ME intake on rate of muscle glycogen repletion in sheep.

Glycogen repletion on glycerol and propylene glycol in water supplements

Glycogen repletion in the muscle of human athletes is maximised by dietary induced hyperglycaemia, particularly when carbohydrates are ingested soon after exercise during the rapid post exercise repletion phase (Sherman 1991). Hence sports drinks containing soluble carbohydrate, such as Powerade® and Gatorade®, will enhance an athlete's recovery post exercise. Given that ruminants extensively ferment carbohydrates, these drinks are unlikely to promote hyperglycaemia in sheep and cattle. However there are some recognised hyperglycaemic agents, such as glycerol, which are used widely within the dairy industry to treat bovine ketosis. Thus studies were carried out to identify the best hyperglycaemic agent and to assess its impact on muscle glycogen repletion post exercise in cattle and sheep. If it promoted glycogen repletion in muscle, it might be of use during the curfew/lairage period immediately pre–slaughter.

Hyperglycaemic response to substrate delivered as a drench

The initial work compared the effect of a range of hyperglycaemic agents drenched directly into the rumen of sheep. These included glycerol, propylene glycol, glucose, and a combination drench of 70% glycerol plus 30% propylene glycol; water was used as a control drench. The glucose drench included 150 g of glucose dissolved in water and made up to 300 ml. The other drenches were diluted to the same volume, and contained an equivalent amount of total carbon (excluding the water control). Blood samples were collected from indwelling jugular catheters over a 9 h period following the initial drench, for the determination of plasma glucose concentrations.

Area under curve analysis was carried out for each of the treatments (indexed against the water control), demonstrating that the 70% glycerol and 30% propylene glycol combination, and glucose drenches, produced the greatest hyperglycaemic responses (P<0.001, Figure 2). The glycerol/propylene glycol response confirms earlier observations (Buswell *et al.* 1986; Rodriguez Iglesias *et al.* 1996) that a mixture of these

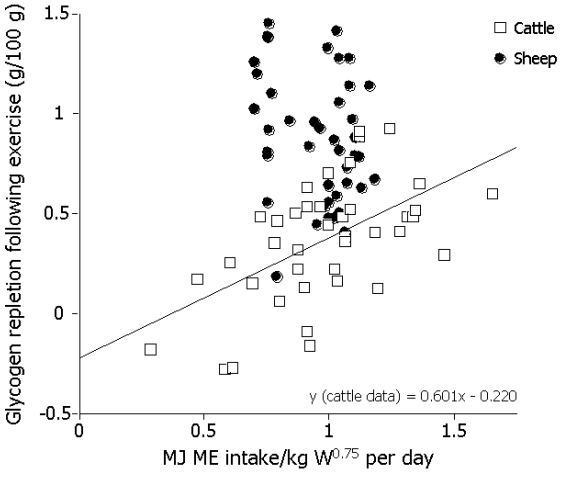

Figure 1 Relationship between SM glycogen repletion 72 h after exercise and ME intake, MJ/day/kg metabolic body weight ($kgW^{0.75}$), for cattle and sheep. Cattle demonstrated a positive linear relationship (R^2 = 0.37, P<0.001).

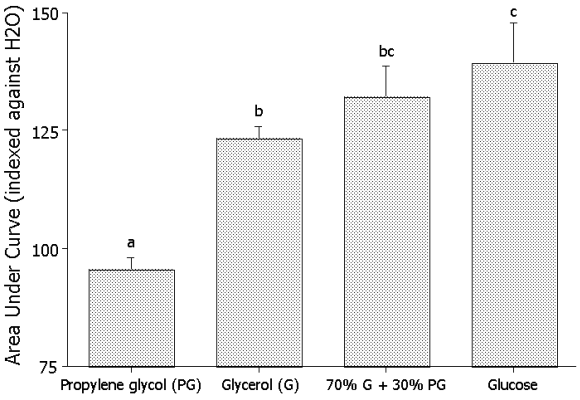

Figure 2 Effect of administration of gluconeogenic substrates on plasma glucose concentration. Values are mean area under curve ± SEM indexed against H_2O; water was given an index value of 100 and all other treatments were indexed proportionately against this value.

substances is a potent hyperglycaemic agent when drenched into the rumen of sheep. The hyperglycaemic effect of glucose may indicate the possibility of direct glucose transport across the rumen wall, although practical concerns associated with attracting insects preclude the use of glucose in drinking water. Additionally, the propylene glycol component of the glycerol/propylene glycol drench may have benefits as a preservative (Baurle *et al.* 1985; Kinnunen and Koskela 1991), potentially improving the keeping quality of water trough contents. Therefore the combination glycerol–propylene glycol treatment was chosen as a drinking water supplement for use in the subsequent muscle glycogen studies.

Hyperglycaemic response to substrate delivered in drinking water

Having established the effectiveness of the glycerol/propylene glycol supplement, we tested its hyperglycaemic impact when offered in drinking water at the rate of 5%, mimicking our intended application of the product. Thus sheep maintained on either low energy (roughage) or high energy (pelleted) rations, were offered either water (control) or water containing 3.5% glycerol and 1.5% propylene glycol over a 24 h period. Blood samples were collected from indwelling jugular catheters over a 9 h period following commencement of water supplementation, with a final sample taken after 24 h, for the determination of plasma glucose concentrations. The low energy ration consisted of 50% lucerne hay and 50% oaten hay (ME 10.0 MJ/kg, CP 15.3%, intake 0.69 kg DM), and the pelleted ration consisted of 7% straw, 46% barley, 15% wheat, 15% oats and 11% lupins (ME 12.1 MJ/kg, CP 15.5%, intake 1.08 kg DM). These rations were divided into 9 equal portions, with one portion given hourly immediately after each collection of blood, and consumed by the lambs within 10 min, and the final portion given after the 9 h sample.

The rates of water intakes, L/h, appeared to be relatively constant over the full 24 h treatment/sampling period and did not differ between treatment groups (Figure 3). Plasma glucose concentrations demonstrated a steady increase over the first 9 h after water supplementation began, but had declined to the same levels as the control by 24 h. This increase was more pronounced in the roughage fed sheep than the pellet fed sheep ($P<0.01$) (Figure 4). Thus the hyperglycaemic response was still evident when the substrates were offered in diluted form in the drinking water, although this response appeared to decline at some point between 9 and 24 h after the initial supplementation commenced. The greater response in the roughage fed animals may indicate there would be a greater effect of these supplements in pasture fed than in feedlot animals.

Muscle glycogen repletion response to substrate delivered in drinking water

To test the effect of glycerol/propylene glycol inclusion in the water on muscle glycogen metabolism we again used the exercise depletion/repletion model in sheep and cattle. In the cattle study 40, 18 months old Hereford cross heifers (average liveweight 349 kg) were maintained on a high energy mixed ration *ad libitum* (similar to the diets used to generate the data shown in Figure 1) in individual pens for 6 weeks. On the day prior to exercise, half of these animals were randomly allocated to the treatment group and offered drinking water with inclusions of glycerol and propylene glycol at the rates of 3.5% and 1.5% respectively. The water for the control group did not contain either substance. The following day all animals were subjected to the exercise regimen, with biopsies taken pre and post exercise and 36 h post exercise. Cattle were maintained on their experimental water treatments throughout the repletion period.

In the sheep experiment 30 Merino wether lambs, average liveweight 32 kg, were housed in individual

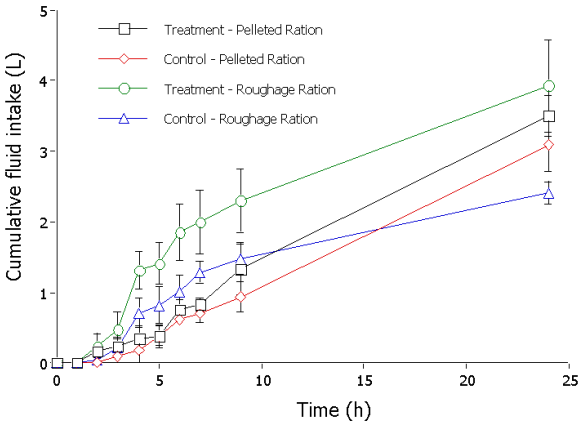

Figure 3 Effect of supplementing drinking water with 3.5% glycerol and 1.5% propylene glycol on cumulative fluid intake (L). Values are means ± SEM.

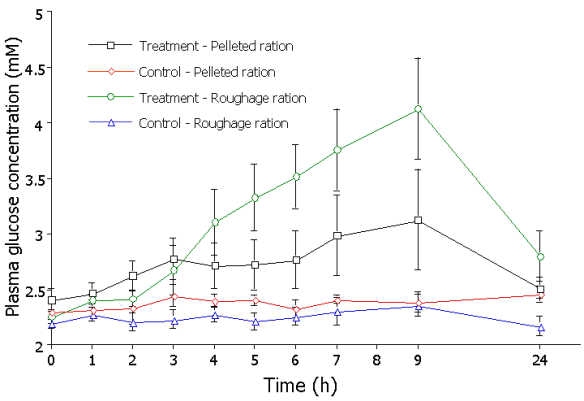

Figure 4 Effect of supplementing drinking water with 3.5% glycerol and 1.5% propylene glycol on plasma glucose concentration (mM). Values are means ± SEM.

pens and fed a high energy total mixed ration *ad libitum*. They were subjected to the exercise protocol, with biopsies taken pre and post exercise, and then 24 h and 48 h post exercise. During the post exercise repletion period, half of the animals were offered water, and half were offered water containing 3.5% glycerol and 1.5% propylene glycol.

All animals in both sheep and cattle experiments were maintained on their rations prior to exercise. Following exercise feed was withheld for the duration of the repletion phase.

The results from the control treatments of both sheep and cattle experiments demonstrate that during the post exercise phase there was no significant repletion of muscle glycogen in the absence of food, contradicting the previous indication that muscle glycogen repletion in sheep is independent of ME intake (Table 3). Furthermore, glycogen repletion in both the SM and ST of sheep was increased after 48 h by the supplementation with glycerol and propylene glycol ($P<0.05$). There was a similar yet smaller trend evident in cattle although the response was not significant (Table 3). The glycogen repletion promoted by the glycerol and propylene glycol supplement was quite small in comparison to the levels evidenced in these species when offered full rations, demonstrating that substrate supplied through the water is not an adequate replacement for a high energy ration. This is hardly surprising, when assessing the relative ME intakes. There are no values for the ME of glycerol and propylene glycol, but the gross energy (GE) of the supplement is 16.8 MJ/L (Blaxter 1962). If GE were taken to equal ME, the ME intake of each lamb offered the glycerol and propylene glycol supplement was equivalent to 1.05 MJ/day. In contrast, lambs maintained on the barley ration following exercise (described in the experiment above) consumed 12.7 MJ ME/head/day, this difference facilitating faster rates of repletion in the barley fed animals.

The effectiveness of the two energy forms also differed. The associated rate of muscle glycogen repletion for the glycerol plus propylene glycol substrate over 48 h was equivalent to 0.068 g/100g per h, for every MJ of ME intake per kg metabolic liveweight per day. The sheep offered the high energy barley ration repleted at 0.011 g/100 g per h, for every MJ of ME intake per kg metabolic liveweight per day, demonstrating a six–fold increase in the efficiency of conversion of the glycerol and propylene glycol supplement into muscle glycogen compared with high energy rations.

Using the assumed ME intake values for the glycerol and propylene glycol supplement, ME intake per kg metabolic body weight per day correlates strongly with increased rates of glycogen repletion in both sheep ($P<0.001$) and cattle ($P<0.01$) (Figure 5), dispelling the indication from Figure 2 that glycogen repletion in sheep is not responsive to ME intake. Furthermore, the slope of the glycogen response in sheep, shown in Figure 5, was greater than that in cattle, suggesting that at the lower ME levels the glycogen repletion in sheep was more sensitive to ME intake than in cattle. Thus the amount of ME provided by the hay ration was sufficient to maximise the rate of repletion in sheep in the first experiment, and additional ME provided by the grain rations was surplus to the repletion demands of the muscle glycogen depot following exercise in the 'red' type muscles.

Therefore the conclusions from the hyperglycaemic substrate experiments were that (i) glycerol and propylene glycol produced the largest hyperglycaemic response, (ii) when administered

Table 3 Effect of 3.5% glycerol + 1.5% propylene glycol (G+PG) on change in muscle glycogen concentration (g/100 g) after exercise in cattle and sheep.

		Treatment		
	Muscle	**Control**	**G + PG**	**Significance of effect (*P*)**
Cattle				
Glycogen repletion	SM	–0.02 ± 0.076	0.200 ± 0.116	n.s.
36 h post–exercise (g/100g)	ST	–0.08 ± 0.052	0.072 ± 0.062	n.s
Liquid intake during 36h				
post–exercise (L/day)		32.80 ± 1.52	28.90 ± 2.56	n.s.
Sheep				
Glycogen repletion	SM	–0.02 ± 0.061	0.37 ± 0.128	**
48 h post–exercise (g/100g)	ST	–0.12 ± 0.059	0.17 ± 0.095	*
Total liquid intake during 48h				
post–exercise (L/day)		1.25 ± 0.179	1.54 ± 0.261	n.s.

Values are means ± SEM
n.s., not significant; * $P<0.05$; ** $P<0.01$

in the drinking water the impact on rate of glycogen recovery following exercise was small although it did correlate with ME intake in both sheep and cattle, and (iii) when given no access to either food or water borne substrates both sheep and cattle demonstrated no muscle glycogen repletion following exercise.

Proposed model for muscle glycogen responses to energy intake

A proposed model for the response in muscle glycogen repletion relative to ME intake in sheep and cattle is shown in Figure 6. Although the data are limited, the results from the sheep and cattle experiments described in this paper can be explained by an exponential response, with approximate estimates of energy supplied by the grain and hay rations and the glycerol and propylene glycol treatments indicated on the x–axis. Of less certainty is whether the absolute rate of muscle glycogen repletion (Vmax) is higher in sheep than in cattle, more data over the higher levels of intake in both species being required for elucidation.

Although fluid intakes in the post exercise phase were not affected by the glycerol and propylene glycol treatments, in the lairage scenario prior to slaughter the fluid intakes were at least doubled in all then trials with cattle and sheep. Thus the inclusion of glycerol/ propylene glycol in the drinking water of sheep and cattle does not have adverse effects on fluid intake, and may actually increase intake.

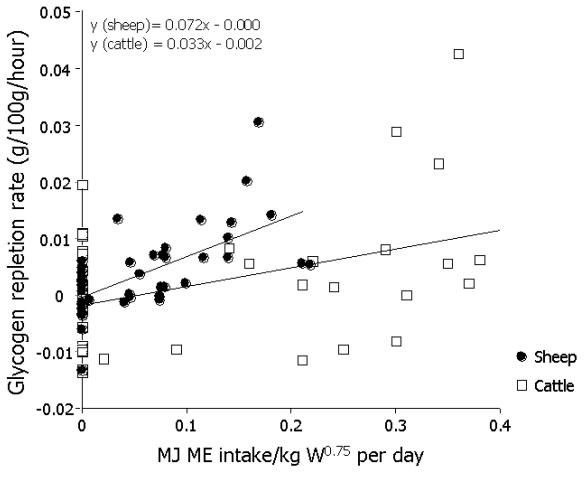

Figure 5 Rate of SM glycogen repletion following exercise versus ME intake per kg metabolic body weight per hour in sheep and cattle consuming water containing 3.5% glycerol and 1.5% propylene glycol. Both sheep (R^2 = 0.40. P<0.001) and cattle (R^2 = 0.15 P<0.01) demonstrated a positive linear relationship.

Conclusion

This work suggests that glycogen repletion in sheep may be more sensitive to ME intake than cattle; sheep respond at lower levels of ME intake, and at the higher levels provided by grain rations there was no further increase in the rate of muscle glycogen repletion. Muscle type was also shown to affect rate of glycogen repletion following exercise, with the red type muscles repleting faster than white type muscles. Lastly, although the efficiency of glycerol and propylene glycol substrates for enhancing muscle glycogen repletion appears to be greater than for high energy ruminant rations, it does not provide the same level of ME intake and therefore is not an adequate replacement for normal rations when animals are repleting glycogen stores.

The practical implications of this work are that glycerol and propylene glycol supplements may marginally enhance the repletion of muscle glycogen following a marked level of depletion, but they are no match for the much greater amounts of substrates supplied by normal rations which result in much higher rates of muscle glycogen repletion. Additionally, the results suggest that feeding roughage rations to sheep will maximise rates of glycogen repletion, but that cattle require high energy rations to potentiate maximal rates of recovery.

References

Baurle, G., Bieber, S., Hornstein, O.P., Nurnberg, E. and Muckenschnabel, R. (1985). The suitability of propylene glycol (1,2–propanediol) as an active antimicrobial adjuvant in ointments. *Zeitschrift für Hautkrankheiten* 60, 1198–1213.

Blaxter, K.L. (1962). *The Energy Metabolism of Ruminants*. Hutchinson Scientific and Technical, London.

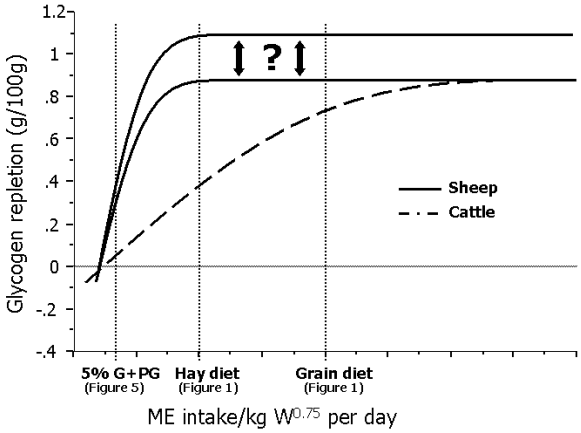

Figure 6 Hypothetical glycogen repletion curves as influenced by ME intake per metabolic body weight in sheep and cattle.

Braind, M., Talmant, A., Braind, Y., Monin, G. and Durand R. (1981). Metabolic types of muscle in sheep: I. Myosin ATPase, glycolytic, and mitochondrial enzyme activities. *European Journal of Applied Physiology* 46, 347–358.

Buswell, J.F., Haddy, J.P. and Bywater, R.J. (1986). Treatment of pregnancy toxaemia in sheep using a concentrated oral rehydration solution. *Veterinary Record* 118, 208–209.

Fabiansson, S.U., Shorthose, W.R. and Warner, W.R. (1989). *Dark cutting in Cattle and Sheep.* Australian Meat and Live–stock Research Corporation, Sydney, Australia.

Gardner, G.E., McIntyre, B.L., Tudor, G. and Pethick, D.W. (2000). The impact of nutrition on bovine muscle glycogen metabolism following exercise. *Australian Journal of Agricultural Research* 52 (in press).

Kinnunen, T. and Koskela, M. (1991). Antibacterial and antifungal properties of propylene glycol, hexylene glycol, and 1,3–butylene glycol *in vitro*. *Acta Dermatologica—Venereologica* 71, 148–150.

McVeigh, J.M. and Tarrant, P.V. (1982). Glycogen content and repletion rates in beef muscle, effect of feeding and fasting. *Journal of Nutrition* 112, 1306–1314.

McVeigh, J.M. and Tarrant, P.V. (1983). Effect of propranolol on muscle glycogen metabolism during social regrouping of young bulls. *Journal of Animal Science* 56, 71–80.

Monin, G. (1981). Muscle metabolic type and the DFD condition. In: *The Problem of Dark–cutting in Beef*, pp. 63–85 (eds. D.E. Hood and P.V. Tarrant), Martinus Nijhoff, The Hague, Netherlands.

Pethick, D.W. and Rowe, J.B. (1996). The effect of nutrition and exercise on carcass parameters and the level of glycogen in skeletal muscle of Merino sheep. *Australian Journal of Agricultural Research* 47, 525–537.

Rodriguez Iglesias, R.M., Ciccioli, N.H., Irazoqui, H. and Giglioli, C. (1996). Ovulation rate in ewes after single oral glucogenic dosage during a ram–induced follicular phase. *Animal Reproduction Science* 44, 211–221.

Sherman, W.M. (1991). Carbohydrate feedings before and after exercise. In: *Perspectives in Exercise Science and Sports Medicine Volume 4: Ergogenics—Enhancement of Performance in Exercise and Sport*, pp. 1–34 (eds. D.R. Lamb and M.H. Williams), Wm. C. Brown Publishers, USA.

Warriss, P.D. (1990). The handling of cattle pre–slaughter and its effects on carcass and meat quality. *Applied Animal Behaviour Science* 28, 171–186.