The regulation by nutrition of glycogen in the muscle of ruminants

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Summary

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 muscle glycogen in pasture fed cattle. The clear message is that animals destined for slaughter should be on a high plane of nutrition as this will contribute to an increased muscle glycogen at slaughter and so help alleviate the problem of dark cutting meat. Short–term 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 one 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 meaning for increasing blood glucose and so glycogen synthesis is proposed. An experiment to examine the effectiveness of MgO as a means for reducing the stress response is also discussed.

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 excedes 5.9, the meat appears dark, firm and dry (DFD) and is significantly tougher. 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. semimembranosis* (SM, topside) and *M. semitendinosis* (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.

Chronic effects of nutrition

To evaluate the effects of nutrition we fed 12 month old Merino wethers (n = 8 per treatment) a pelleted hay:barley:lupin (20:53:26) feedlot–type diet at four 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 60 min and then slaughtered within 2 h (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 residual glycogen

(48 hours post–slaughter) in meat was analysed. This glycogen can be thought of as a buffer against a tendency for a rise in the ultimate pH, and when it drops to below about 0.2 g/100g in muscle a pHu = 5.7 is very likely.

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 liveweight of 304 ± 6 kg (± sem) and allocated, within breed type, at random to four dietary treatments: (i) silage *ad lib.* (DM 22%, ME 9.6 MJ/kg DM and crude protein 13.5%); (ii) hay *ad lib*. (86, 10.8 and 16.2, respectively); (iii) silage *ad lib.* + 3 kg cracked barley grain (89, 11.1 and 10, respectively); and (iv) 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 liveweight 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/d liveweight change (approx. 55 MJ ME/d) to the largest response when cattle were gaining at 1.2 kg/d (approx. 102 MJ ME/d).

The occurrence of dark–cutting in beef carcasses in Australia has been reported to vary with season although the peak months vary between years and regions. Our studies in Victoria have investigated the effect of season and stocking rate on muscle glycogen in cattle and found that there was little difference in SM or ST glycogen concentration between cattle grazed at stocking rates of 1.5/ha and those grazed at 2.5/ha (see Table 1). The cattle on the lower rate were gaining an average of 0.55 kg/d over autumn and winter whereas those on the higher rate had no weight gain over the same period. There was a strong seasonal influence on the concentration of glycogen in muscle with consistently low levels in summer and high levels in spring for both muscles. The drop in summer probably indicates a period of nutritional stress (reduced pasture availability and quality) following abundant pasture availability in spring. It coincided with a significant decline in the available feed in January. It is possible that there is a greater negative response, in terms of glycogen concentration in muscle, to the combined effects of heat stress and reduced quality of available feed that often occurs in summer when compared to other forms of chronic stress 'on farm'. The seasonal effect was consistent in both muscles. The SM muscle exhibited a decline in glycogen from autumn to winter in both stocking rate groups, reflecting the longer–term stressors of colder weather and low pasture availability persisting into winter. During winter and summer, the

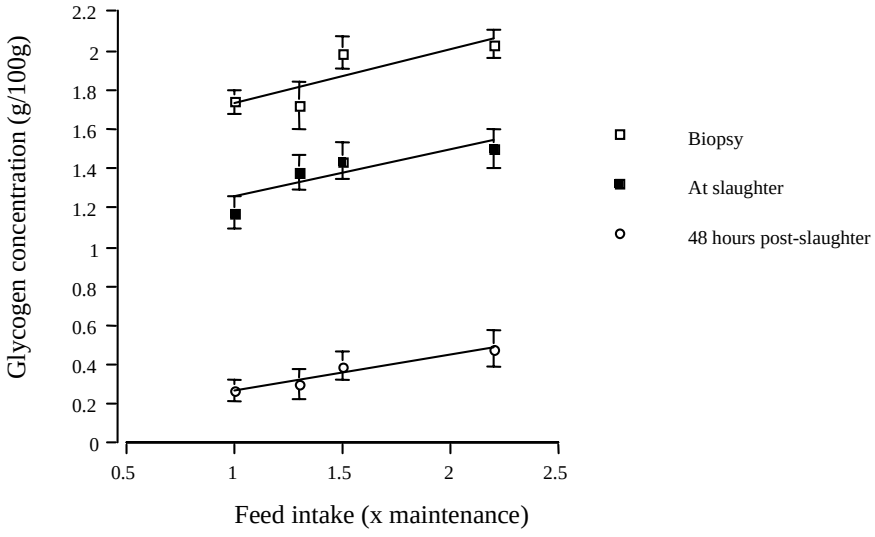

Figure 1 The effect of feed intake on glycogen concentration in the *M. semimembranosis* in the live animal and in the carcass 10 minutes and 48 h post–slaughter.

low muscle glycogen levels would have put the cattle at risk of producing dark–cutting beef. Over winter, the high and low stocking rate treatments were estimated to supply about 40 and 60 MJ/d per head respectively. Thus, it would appear that in winter, 60 MJ/d is insufficient to maintain muscle glycogen levels. Feedlot cattle typically consume about 120 MJ/d in their ration and their muscle glycogen levels are consistently high. Thus it would appear that pasture–fed cattle in Victoria need supplementary feeding with grain to achieve higher muscle glycogen levels in winter and in summer.

Short–term effects of nutrition

The effect of high energy supplementation with grain for four weeks on muscle glycogen levels of cattle grazing at low (1.5/ha) and high (2.5/ha) stocking rates was investigated in a subsequent experiment. The supplement was offered *ad libitum* and consisted of 75% cracked triticale, 15% cracked lupins and 10% hammer–milled straw mixed with virginiamycin (ESKAPE, Ridley Australia)and molasses (CP = 12%, ME = 12 MJ/kg DM). By the end of the first week, muscle glycogen levels had risen by 53% in the SM and 30% in the ST for supplemented cattle (consuming 1.5% of liveweight as DM) compared to controls (see Table 2). The subsequent three weeks showed a gradual increase in muscle glycogen levels in the supplemented group (as intake increased to 2.2% of liveweight as DM) but nowhere near the large increase seen in the first week. The muscle glycogen response to grain feeding was similar at both stocking rates. Thus short–term high energy grain feeding was successful in increasing muscle glycogen and is considered an effective and practical method to reduce the occurrence of dark–cutting during problem periods. It is essential, though, that the high–energy grain feeding includes a modifier of rumen fermentation to minimise the risk of acidosis and that the cattle have some acquaintance with grain feeding.

The repletion rate of muscle glycogen was examined in a trial utilising an exercise depletion/ repletion model. Twenty 18 month old Angus steers of average liveweight 376 kg were allocated to three dietary treatments, hay alone and with maize or barley. The hay diet consisted solely of pasture hay (ME 8 MJ/kg, CP 8% and intake 7.5 kg, all values in DM); the maize diet

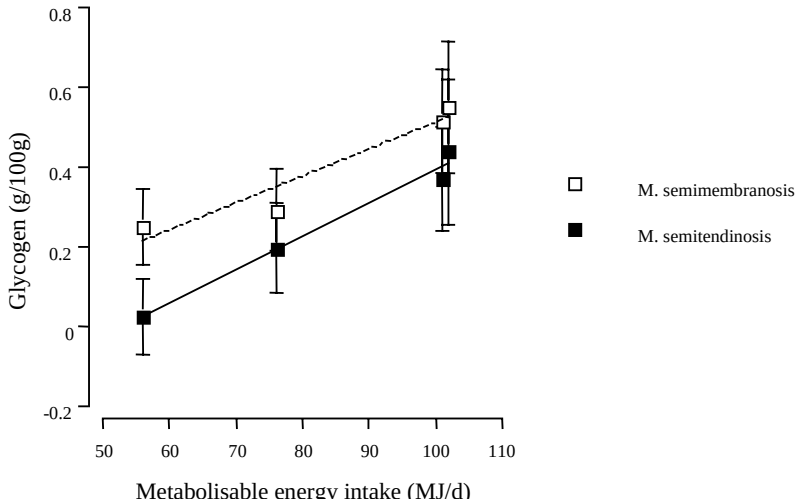

Figure 2 The effect of dietary metabolisable energy intake on the response of muscle glycogen concentration to nutritional change.

Table 1 Effect of season and stocking rate of cattle (SR: Low = 1.5/ha, High = 2.5/ha) on daily changes in liveweight and muscle glycogen concentration.

		Season				P value		
	SR	Autumn	Winter	Spring	Summer	Season	SR	SED
Liveweight	Low	0.4	0.7	1.0	0.4	<0.001	0.01	0.21
change (kg/d)	High	–0.2	0.3	1.3	0.4			
SM glycogen	Low	1.29	0.99	1.09	0.87	<0.001	0.68	0.43
(g/100g)	High	1.19	1.01	1.21	0.78			
ST glycogen	Low	0.92	0.91	1.04	0.80	<0.001	0.06	0.61
(g/100g)	High	0.79	0.91	1.05	0.73			

consisted of 64.2% steam flaked maize, 12% lupin, 15% hay, 1% urea, 5% molasses and 2.8% mineral–vitamin mix (ME 11.3 MJ/kg, CP 14% and intake 12.8 kg); the barley diet was 66% barley, 10% lupin, 15% hay, 0.9% urea, 5% molasses and 2.8% mineral premix (ME 10.9MJ/kg, CP 14.11% and intake 11.5 kg). 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 h 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 Table 3.

Both the SM and ST of the animals on the hay diet, and the ST of the animals on the maize and barley diets, 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 grain–fed animals during the first 36 h post–exercise, but between 36 and 72 h glycogen was repleted to within 80–90% of the pre–exercise concentration. This rate of repletion when calculated between 0–72 h post exercise was 0.008 g/100g muscle per hour, 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 h. The rate of repletion during the second 36 h (to 72 h) was considerably higher at 0.012 g/100g muscle per hour, suggesting higher repletion rates are possible.

Table 2 The effect of feeding a high–energy supplement (supplement versus control) to cattle for four weeks on the average change in total muscle glycogen for the *M. semimembranosus* (SM) and the *M. semitendinosus* (ST).

	Control	Supplement	F–ratio	SED
SM initial glycogen (g/100g)	0.876	0.76	0.081	0.35
Δ glycogen, week 1	+0.02	+0.40	0.069	1.06
Δ glycogen, week 2	+0.30	+0.63	0.305	2.39
Δ glycogen, week 3	+0.33	+0.61	0.160	1.30
Δ glycogen, week 4	+0.34	+0.76	0.105	1.46
ST initial glycogen (g/100g)	0.84	0.81	0.792	0.94
Δ glycogen, week 1	–0.003	+0.25	0.037	0.49
Δ glycogen, week 2	+0.17	+0.40	0.199	1.20
Δ glycogen, week 3	+0.05	+0.50	0.091	1.45
Δ glycogen, week 4	+0.13	+0.63	0.129	1.99

Table 3 The effect of diet on muscle glycogen concentration, g/100 g, post–exercise.

Treatment	Muscle[x]	Sampling time (relative to exercise) [y]				P
		–1h	+1h	+36h	+72h	value[z]
Hay diet	SM	1.54±.07[a]	1.10±.08[b]	1.04±.05[b]	1.20±.07[b]	**
	ST	1.26±.15	1.03±.11	0.82±.11	1.11±.06	ns
Barley diet	SM	2.04±.08[a]	1.23±.04[b]	1.35±.08[b]	1.72±.06[c]	***
	ST	1.70±.10[a]	1.42±.11[b]	1.16±.09[b]	1.37±.02[b]	***
Maize diet	SM	1.90±.064[a]	1.11±.078[b]	1.15±.068[b]	1.68±.052[c]	**
	ST	1.76±.143[a]	1.36±.076[b]	1.23±.11[b]	1.35±.058[b]	**

[x] SM = *M. semimembranosis*; ST = *M. semitendinosis*. [y] Values with different superscripts are different. [z] ns, not significant; *P<0.05; ** P<0.01

Hyperglycaemic agents

The results of the glycogen repletion trial clearly demonstrate that ruminants are relatively slow compared to monogastric animals (e.g. humans) at repleting glycogen, even in response to high energy diets. The key to glycogen repletion in the muscle of human athletes is a diet that induces hyperglycaemia, such as a soluble carbohydrate drink, given soon after exercise because 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 hyperglycaemia due to extensive fermentation of carbohydrate in the rumen. We have therefore initiated studies to explore the most effective hyperglycaemic agents in ruminants to see if these might promote more rapid glycogen repletion in muscle and so be of use in the curfew/lairage period immediately pre–slaughter.

Our preliminary work has confirmed earlier observations (Buswell *et al*. 1986; Rodriguez Iglesias *et al.* 1996) that a mixture of glycerol and propylene glycol is a potent hyperglycaemic treatment when administered into the rumen of sheep (Figure 3). Furthermore our initial observations suggest that a mixture of glycerol and propylene glycol (3.5% and 1.5% respectively) doubles the water intake of lambs. Currently further studies are being made to determine the extent of hyperglycaemia and glycogen repletion in muscle post–exercise after glycerol/propylene inclusion in the water.

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 (1500 km). 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' Nutricharge (Agresearch, New Zealand). The results of five trials are shown in Table 4 where glycogen in the SM post–slaughter of treated and untreated groups of cattle (typically 45–50 per group) was examined. There was a small positive effect on muscle glycogen 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. It appears that 'in feed' preparations should be restricted to use 'on farm', and that delivery via water is 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

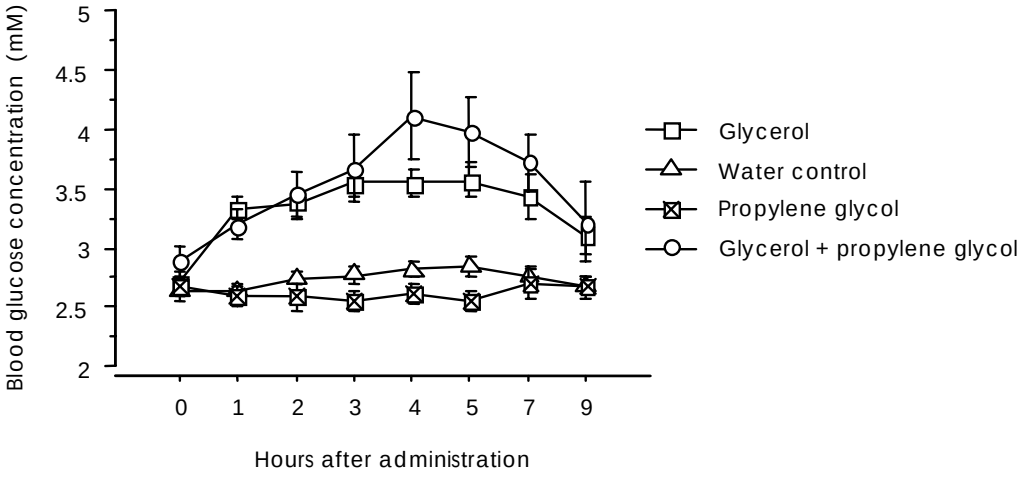

Figure 3 The effect of oral glycerol (150 g), propylene glycol (150 g) and a mixture of glycerol/propylene glycol (105/45 g) on blood glucose concentration.

muscle glycogen concentration in sheep exposed to stress by exercise and from the commercial slaughter process (Gardner and Pethick 1998).

Sheep supplemented with either 0.5% or 1% MgO for 10 d prior to exercise stress lost between 12–24% less muscle glycogen in the ST during exercise. In the period up to 72 h post–exercise, in those animals supplemented with 1% MgO there was about 55% more repletion of glycogen in the SM than in the group given no MgO. 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 glycogen concentration in the ST at slaughter by about 20% (Figure 4). We conclude that MgO appears to reduce the response to stress, leading to a subsequent reduction in glycogen loss, and increases the rate of glycogen repletion in skeletal muscle following stress.

Conclusion

This paper has highlighted the importance of nutrition as a determinant of the glycogen concentration in muscle. The data indicate that ruminants destined for slaughter should be on a high plane of nutrition so that 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 one week can substantially raise muscle glycogen concentration but adaptation of the rumen to a '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 awaits further testing. Evidence is put forward to suggest that electrolyte/carbohydrate preparations can stimulate muscle glycogen levels when given 24–72 h pre–slaughter. However further work is needed to verify the practical worth of electrolytes. There is a possibility that pre–dosing animals with MgO will reduce the stress response during the 'post–farm gate' period pre–slaughter.

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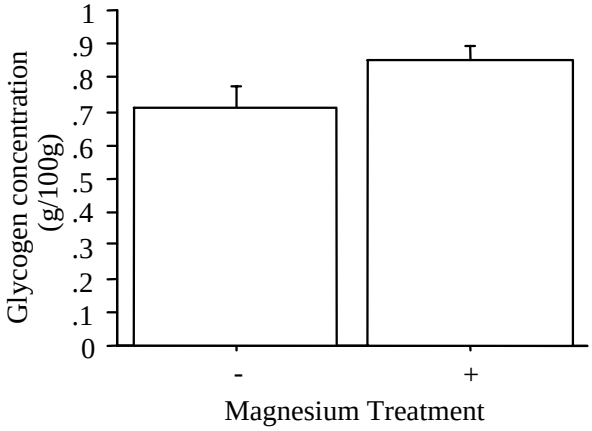

Figure 4 Effect of magnesium oxide treatment on glycogen concentration in the *M. semitendinosis* at slaughter.

Table 4 The effect of commercial electrolyte preparations on glycogen in the *M. semimembranosis* (SM). Summary of trials with 'in water' (Glucotrans) and 'in feed' (Nutricharge) preparations.

Experiment	Species/diet	Consumed on farm[a]	Hours in lairage	Consumed in lairage	Offered in lairage[b]	Extent of glycogen increase (SM)
Glucotrans	Cattle/feedlot	yes	40	yes	yes	+
Nutricharge 1	Cattle/feedlot	yes	20	no	yes	none
Nutricharge 2a	Cattle/feedlot	yes	40	yes	yes	+++
Nutricharge 2b	Cattle/feedlot	yes	17	no	yes	none
Nutricharge 3a	Cattle/feedlot	yes	1	no	no	+
Nutricharge 3b	Cattle/feedlot	yes	32	no	yes	none
Nutricharge 4a	Lambs/hay	yes	20	no	no	+
Nutricharge 4b	Lambs/feedlot	yes	20	no	no	none

[a]consumed 1 kg/hd of Nutricharge pellets; [b]offered 2 kg/hd of Nutricharge pellets
[b]significant effect P<0.1

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