DIETS HIGH IN RUMEN DEGRADABLE NITROGEN REDUCE BOVINE MUSCLE GLYCOGEN CONCENTRATION AT SLAUGHTER

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Abstract - High levels of muscle glycogen prior to slaughter are essential for avoiding dark cutting meat. In this study we assessed the impact of diets high in rumen degradable nitrogen on muscle glycogen metabolism after exercise and at slaughter in cattle. Variation in nitrogen content was achieved by using rations containing either 1%, 2%, or 3% urea, or 17%, 35%, 50% or 70% lupins. Following the exercise regimen there were no differences in the rate of repletion of muscle glycogen between dietary treatments, however at slaughter there was a negative relationship between increasing dietary urea inclusion and muscle glycogen concentration. Furthermore, only the 3% urea ration significantly elevated plasma ammonia levels, suggesting that not only nitrogen content but also the rate of availability is important for impacting muscle glycogen concentration.

Key Words – Urea, Lupins, Exercise, Stress

I. INTRODUCTION

Dark cutting beef is one of the key meat quality problems affecting the beef industry globally, and is characterized by its darker meat colour, shorter shelf life, bland flavour, variable tenderness and its impact on degree of doneness [1, 2]. A major cause of dark cutting is low levels of muscle glycogen at slaughter which is a function of the initial muscle glycogen 'on-farm' minus the amount lost due to stressors during the pre-slaughter period.

Nutrition is an important factor determining muscle glycogen concentrations, with increasing levels of metabolisable energy shown to increase basal muscle glycogen concentrations in cattle [3] and sheep [4]. Balanced ruminant rations also require enough rumen degradable protein along with the metabolisable energy to optimise the growth of rumen micro-organisms. However, when a ration contains high levels of readily degradable nitrogen and low levels of fermentable carbohydrate, this imbalance can lead to extensive production and absorption of ammonia from the rumen [5]. Thus rations containing high levels of urea and/or rapidly fermented grains rich in rumen degradable protein such as lupins could lead to increased ammonia absorption.

Under most physiological circumstances ammonia absorbed from the rumen is converted by the liver to urea, which is less toxic. However, if the rate of ammonia absorption exceeds the livers detoxification capacity this will result in increases in systemic ammonia concentrations [6] which can become toxic when plasma concentrations reach 1.0 mmol/L [7]. However it also appears to have an impact at subclinical levels resulting in hyperglycaemia in cattle [8], and reduced glycogen concentrations in brain, liver and muscle tissue of rats [9]. It remains to be seen whether this also causes reduced muscle glycogen levels in cattle.

Therefore we hypothesised that increasing levels of rumen degradable protein supplied in the form of urea or lupin grain will reduce muscle glycogen concentration in cattle at slaughter.

II. MATERIALS AND METHODS

Animals and Diet

Seventy 18 month old Angus heifers were maintained in individual pens which were part of an outdoors feedlot, with each pen partially covered and the feeding area fully covered. These animals were randomly allocated across a live-weight strata to one of 7 dietary treatment groups. Three of the experimental rations consisted of 1%, 2%, or 3% urea mixed with 60-62% barley, 15% hay, 20% canola meal and 2% minerals. These rations had similar levels of metabolisable energy (10.4 MJ/kg in dry matter (DM)), and crude protein ranged between 19.6% - 24.3% in DM. There were 4 lupin rations consisting of 17%, 35%, 50% or 70% lupins. In all cases the lupins were

mixed with 15% hay, 0.75% - 0.5% urea and 2% minerals, with barley making up the remainder of each ration. The metabolisable energy and crude protein ranged from 10.8 MJ/kg and 17.7% to 11.9 MJ/kg and 25.2% in DM for the 17% and 70% lupin rations respectively. The average weight of the steers on entry into the feedlot was 356 kg.

Experimental design

After an initial 20 day acclimation period, the cattle were maintained on their experimental rations for a further 46 days allowing basal glycogen levels to assimilate. On day 66 of the experimental period the cattle were subjected to an exercise regimen consisting of 5 x 15 min intervals, run at 9km/h, with a 15 min rest between each interval. Previous work in this laboratory has shown that trotting cattle at 9km/h is equivalent to an exercise intensity of about 70% VO2 Max, leading to an approximate 50% depletion of muscle glycogen stores [10]. Muscle biopsies were taken immediately before (-1 h) and within 1 hour of completing the exercise regime (+1 h), with further samples taken at 36 h (+36 h) and 72 h (+72 h) post exercise. At all biopsies samples were taken from the *M. semimembranosus* (SM) and *M. semitendinosus* (ST) for the determination of glycogen and lactate concentration. Animals had access to their dietary treatments throughout the post-exercise period.

After the final post-exercise biopsy, the cattle were maintained on the same dietary treatment until slaughter. On day 92 of the experimental period blood samples were taken from the tail vein of all cattle for the determination of plasma ammonia and urea concentrations. Five days later the cattle were trucked for 2 h to a commercial abattoir where they were held in lairage for 15 hours lairage until slaughter. Muscle samples were taken from the SM and ST within 1 hour of slaughter for the determination of glycogen and lactate concentration, and muscle ultimate pH for the SM, ST and *M. longissimus thoracis et lumborum* (LD) was measured 48 hours after slaughter.

Statistical analysis

Muscle glycogen concentration was analysed using a linear mixed effects model (SAS). For the exercise data, fixed effects included dietary treatment, muscle, and biopsy time (pre-exercise, post-exercise, and 36 and 72 hours post exercise), with animal ID used as a random term. The same model was used for the slaughter data except that the time term was removed. Interactions between fixed effects were tested and removed if non-significant ($P>0.05$).

III. RESULTS AND DISCUSSION

Muscle glycogen concentrations at slaughter were markedly different ($P<0.01$) between dietary treatments in both the SM (Figure 1(i)) and ST (Figure 1(ii)). This was particularly evident between the urea diets, with the animals on the 1% urea diet having 16% more glycogen in the SM and 24% more in the ST than the animals on the 3% urea diet. These results align well with our initial hypothesis that increasing levels of rumen degradable nitrogen would reduce muscle glycogen concentration. One explanation for this could be associated with glucose storage. Fernandez et al [8] demonstrated that hyper-ammonaemia results in hyperglycaemia, most likely due to a reduced molar insulin:glucagon ratio leading to an under-utilisation of glucose by insulin sensitive tissues. This notion is further supported by Milano et al [11] who found hepatic glucose production to be unaffected after administration of NH_4HCO_3. Thus the glycogen response evidenced at slaughter may reflect the basal glycogen levels on farm, with differences caused by reduced glucose uptake in the muscles. Yet contrary to this assertion, basal muscle glycogen concentrations (glycogen concentration – 1 h relative to exercise) were the same across all dietary treatment groups, with an average concentration of 1.75±0.093 and 1.54±0.079 g/100g in the SM and ST. Alternatively, catecholamine concentrations increase during hyperammonaemia [8, 12-14], implying heightened sensitivity to stress and therefore greater rates of glycogen catabolism during pre-slaughter lairage.

The impact of dietary urea did not translate into an ultimate pH response in this study which was the same across all treatment groups with an average of 5.44±0.011, 5.50±0.009, and 5.50±0.009 within the SM, ST and LD. This can be explained by the muscle glycogen concentrations which were above 1g/100g in all treatment groups, a level recognised as adequate for reaching an ultimate pH of 5.5 [15]. None-the-less, these results imply that cattle

maintained on diets containing 3% urea are likely to be more prone to dark cutting.

Contrary to our hypothesis muscle glycogen concentration did not reduce with increasing levels of dietary lupins. The only difference evident was in the ST of the 70% lupin treatment which had about 26% more muscle glycogen (P<0.01; Figure 1(i) & (ii)) than the 35% and 50% treatment groups. Plasma urea concentrations increased linearly with increasing levels of either dietary urea (P<0.01) or lupins (P<0.01), yet compared to the 3% urea diet, animals consuming the 70% lupin diet had similar CP intakes and plasma urea levels (Figure 2(ii)). This suggests a similar ammonia detoxification load placed on the liver. However the 70% lupin diet did not lead to an elevation of plasma ammonia (Figure 2(i)), possibly due to lower rates of ammonia absorption. This is in contrast to the 3% urea dietary treatment (Figure 2(i)), which had ammonia levels 14% higher than the other treatments (P<0.01). This may implicate rate, rather than amount of ammonia absorption as the key determinant of the muscle glycogen response.

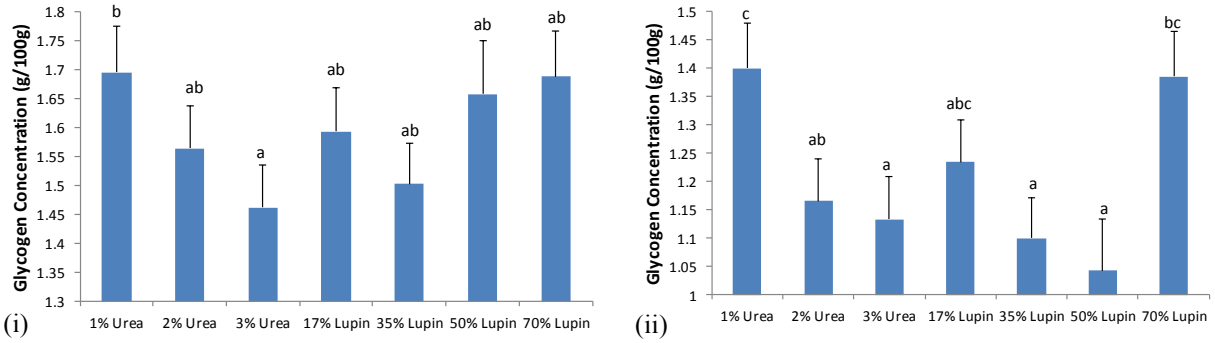

Figure 1. Differences in glycogen concentration (g/100g) at slaughter between dietary treatments within the (i) *M. semimembranosus* and (ii) *M. semitendinosus*. Values are lsmeans ±S.E.

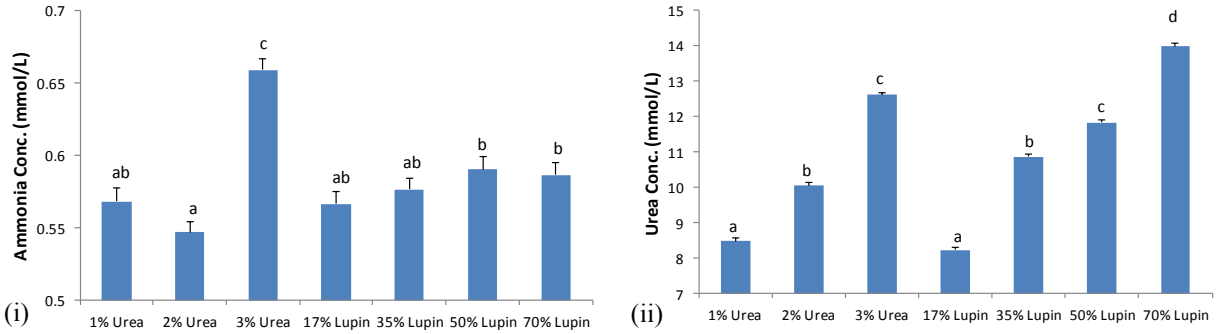

Figure 2. Differences in plasma (i) ammonia concentration (mmol/L) and (ii) urea concentration (mmol/L) between dietary treatments. Values are lsmeans ±S.E.

Lastly, there were no differences in muscle glycogen concentration between any of the dietary treatments groups during the post exercise sampling periods. This implies that differing levels of dietary rumen degradable nitrogen do not impact on subsequent rates of muscle glycogen repletion. This lends further support to the notion that hyper-ammonaemia has not impacted sufficiently to reduce the molar insulin:glucagon ratio leading to an under-utilisation of glucose by insulin sensitive tissues. However, some care must be taken with this interpretation as the average level of glycogen depletion, as a percentage of the basal muscle glycogen, across all treatment groups was only 23.9 ± 2.13% and 12.4 ± 1.80% for the SM and ST. This is only a fraction of the 50% depletion level suggested by Gardner et al [10], to provide enough scope for measuring differences in

either the amount of glycogen depleted, or subsequent rates of glycogen repletion.

IV. CONCLUSION

This paper demonstrates the potential for beef cattle rations containing 3% urea to decrease muscle glycogen at slaughter. Furthermore, it has highlighted the rate of nitrogen absorption from the rumen as being the likely factor causing this effect, as opposed to the total dietary nitrogen load. Furthermore it has highlighted that this response is linked to a greater sensitivity to stress, as opposed to reduced glucose uptake by insulin responsive tissues. Therefore rations containing high levels of urea should be modified in the weeks preceding slaughter to minimize the incidence of dark cutting beef.

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