THE EFFECT OF DIETARY UREA ON INSULIN REGULATION OF GLUCOSE METABOLISM IN SHEEP

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Abstract – High levels of muscle glycogen prior to slaughter are essential for avoiding dark cutting meat. This study assessed the impact of diets high in rumen degradable nitrogen on glucose metabolism in sheep. Variation in nitrogen content was achieved by using rations containing 0%, 3% or 4% urea. A significant increase in plasma urea and plasma ammonia was evident in the 3% and 4% diets when compared to 0%. After being challenged with two levels of insulin, there was no difference in the glucose response between the high and low insulin doses when 3% or 4% urea was fed, suggesting diets high in rumen degradable nitrogen cause resistance to exogenous insulin and therefore likely to increase the risk of dark cutting.

Key Words – dietary nitrogen, dark cutting, hormone.

I. INTRODUCTION

A key quality problem facing the red meat industry globally is dark cutting. This is characterised by meat with a darker colour, shorter shelf life, poor palatability and reduced tenderness [1,2]. Dark cutting is caused by low levels of muscle glycogen at slaughter, which is the result of the initial muscle glycogen 'on-farm' minus the amount lost as a result of stress during the pre-slaughter period.

The initial muscle glycogen concentration 'on-farm' is strongly influenced by nutrition. Previous work in sheep [3] and cattle [4] has demonstrated that increasing levels of metabolisable energy increases basal muscle glycogen concentration. In addition to metabolisable energy, the ruminant ration must contain enough rumen degradable protein to optimise the growth of rumen micro-organisms. When the ration is incorrectly balanced with high level of readily degradable nitrogen and low levels of fermentable carbohydrates, excessive production and absorption of ammonia from the rumen can occur [5].

A common source of non-protein nitrogen fed to ruminants is dietary urea. This supplement increases feed intake and digestibility of low quality roughages. Under normal physiological circumstances the dietary urea is converted to ammonia in the rumen which can then be converted to microbial protein with excess ammonia synthesized back into the less toxic urea by the liver. When the rate of ammonia absorption exceeds the liver's detoxification capacity, systemic ammonia concentration increases.

Previous work has demonstrated that high levels of systemic ammonia reduce glycogen concentration in the brain, liver and muscle tissue of rats [6] and glycogen concentration in the muscle of cattle [7]. This is likely due to the under-utilisation of glucose, as demonstrated in both cattle [8] and sheep [9,10] by elevated blood glucose concentrations during urea toxicosis.

Therefore, we hypothesise that increasing levels of dietary urea will increase plasma urea and plasma ammonia concentration and induce resistance to exogenous insulin.

II. MATERIALS AND METHOD

Animals and Diet

Twenty-four merino wether lambs were selected from the Meat and Livestock Australia Katanning resource flock. Lambs were the progeny of 12 different sires. Each sire was represented by 2 progeny.

The sheep were fed a complete pelleted diet consisting of 10.5 MJ/kg dry matter of metabolisable energy, 15% crude protein and 0%,

3% or 4% urea. Diets were randomly allocated using a Latin square design, to ensure that at least one progeny of each sire was allocated to each urea level. Lambs were acclimated to the diet for 14 days prior to the hormone challenge week. During challenge week sheep were fed a quarter of their feed at 7.30am, another quarter at 11.30am and the remaining half at 4pm.

Experimental Design

On day 9 sheep were relocated to individual pens measuring 900mm by 1500mm. On day 14 the sheep were weighed and this weight was used to calculate the amount of hormone required for each challenge on a per kg live weight basis.

On day 17 an indwelling cannulae was inserted into the jugular vein of each sheep. To prevent clotting, the catheters were flushed with an EDTA saline solution during blood sampling. Catheters remained in the animal for 5 days before being removed.

Between days 18 to 21, each sheep was subjected to 5 hormone challenges; 2 adrenaline – 0.6 µ/kg live weight and 3 µg/kg live weight; 2 insulin – 0.5 IU/kg live weight (low) and 2 IU/kg live weight (high); and one dextrose (50% glucose) – 0.35 g/kg live weight. Each challenge was randomly allocated throughout the five days for each sheep. On day 1, 2 and 4 of the challenge week, 12 sheep were challenged in the morning and the remaining 12 in the afternoon. On day 3 of challenge week, 24 sheep were challenged in the morning and 24 sheep in the afternoon.

Blood samples were taken at time points -30, -15, -10, -5, 0, 2.5, 5, 10, 15, 20, 30, 45, 60, 90, 120, 125 and 130 minutes relative to the administration of the challenge. Blood was collected using a S-Monovette Vacutainer® tubes (Sarstedt Australia Pty. Ltd, SA, Australia, Cat No. 02.1066.001).

Immediately after collection the S-Monovette Vacutainer® tube was placed on ice. Within 30 minutes of collection, samples were centrifuged in the S-Monovette Vacutainer® tubes at 3000rpm for 15 minutes. 1.5ml of plasma was transferred to microtubes and stored at -80°C. The -30 sample for each animal had an additional 1ml of plasma transferred to a separate microtube, containing 0.5ml of 5% perchloric acid for the determination of ammonia. .

Laboratory analyses of plasma urea, glucose, lactate and NEFA concentration was carried out using enzymatic methods [11,12]. Analyses were automated using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, New York) and the Olympus kits for urea and glucose (Olympus Cat No. OSR6193).

Ammonia samples were thawed and centrifuged at 3500rpm for 5 minutes. To neutralize the sample, 0.6ml was removed and placed in a microtube with 0.03ml of KOH and 0.03ml KOH3. Samples were then centrifuged and immediately analysed for ammonia using the automated analyser, Olympus AU400 (Olympus Optical Co. Ltd, Melville, New York).

Statistical Analysis

A derived function with multiple exponential components (Equation 1) was fitted to each animal's plasma glucose response to the insulin challenges.

Equation 1:

$$y(t)=Int+(e^{[-\beta t]*[-\gamma/(\beta-\alpha)+\gamma/(\beta-\alpha-\Delta)-\varepsilon/\beta+\gamma/(\beta-\alpha)*e((\beta-\alpha)-\gamma/(\beta-\alpha-\Delta)*e((\beta-\alpha-\Delta)+\varepsilon/\beta*e(\beta t)])}$$

Where:

y(t)	substrate concentration (mM) at any given point in time
t	time (minutes)
Int	basal substrate concentration (mM) prior to challenge
γ,β,α,Δ	exponential constraints
ε	the adjustment from basal substrate concentrations

The use of this function allowed the plasma concentration response curve to be modelled at different time points, pre and post challenge. This enabled the quantification of the substrate response and out-putting components for analysis, including area under curve (AUC) up to any time point specified. It was noted that the peak response was being reached at around 10 minutes, as such we chose to analyse the AUC between 0 and 10 minutes. Thus AUC analysis reflects the initial substrate response phase following the administration of insulin.

The plasma glucose AUC, plasma urea at time -30 and plasma ammonia at time -30 were analysed using a linear mixed effects model in SAS (SAS Version 9.2, SAS Institute, Cary, NC, USA). The base model for plasma glucose AUC included fixed effects for diet (0%, 3%, 4% urea inclusion), challenge (high, low), birth-type rear-type and age of dam. Animal identification within sire was included as a random term. The base model for plasma urea and plasma ammonia was the same except that it also included day as a random term. All relevant first order interactions between fixed effects were tested and non-significant (P>0.05) terms were removed in a stepwise manner.

III. RESULTS AND DISCUSSION

Plasma urea and plasma ammonia concentrations were markedly different (P<0.01) between dietary treatments (Fig 1. and Fig. 2). A 99% increase in plasma urea was evident between the 0% and 4% diets whilst a 47% increase in plasma ammonia was evident for the dietary urea treatments, thereby confirming our initial hypothesis. This result aligns well with previous work which demonstrated a 50% increase in plasma ammonia concentration in cattle fed 3% urea compared to those fed 1% [7] as well as increased ammonia absorption from the rumen in lambs fed a wheat straw diet and 2.5% urea [13].

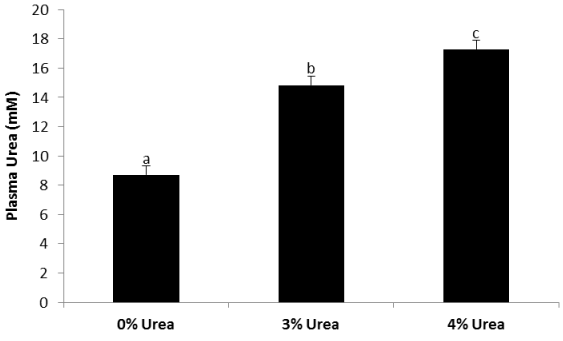

Figure 1. The effect of diet on plasma urea concentration (mM). Values are lsmeans ±S.E.

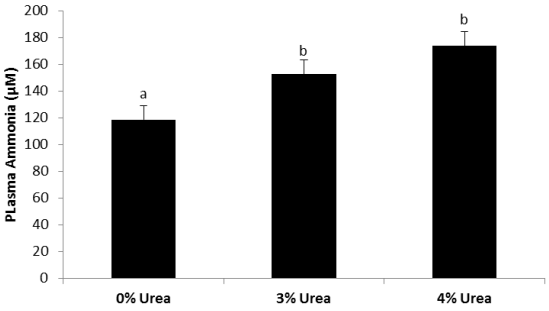

Figure 2. The effect of diet on plasma ammonia concentration (uM). Values are lsmeans ±S.E.

As shown in Fig. 3, the response to the high insulin dose was almost 27% higher than the low insulin dose in the group fed 0% urea (p<0.05). However when comparing the high and low insulin doses in the groups fed 3% and 4% urea, there was no difference in the plasma glucose response. The lack of a greater response under the high insulin challenge suggests that hyper ammonaemia causes a loss of insulin responsiveness leading to an under-utilisation of glucose by insulin sensitive tissues, supporting out initial hypothesis. This aligns with previous work by Emmanuel et al [12] who found plasma glucose remained elevated following the intra-ruminal administration of urea and subsequent insulin infusion. Furthermore, given the depressed response is only evident in the high insulin dose, it is plausible that the most marked effect would be evident in the immediate post-prandial period, during which time the absorption rate of glucose would be at its peak and plasma insulin at its highest concentration. This aligns with Emmanuel et al [10] who concluded ammonia mechanistically caused the development of hyperglycaemia due to a resistance of plasma glucose to exogenous insulin.

In practice this finding would likely result in a reduced uptake of glucose by skeletal muscle post feeding and so increase the likelihood of reduced glycogen levels leading to an increased risk of the dark cutting syndrome. Such an outcome is probable when ruminants graze lush high protein pastures and deserves further studies.

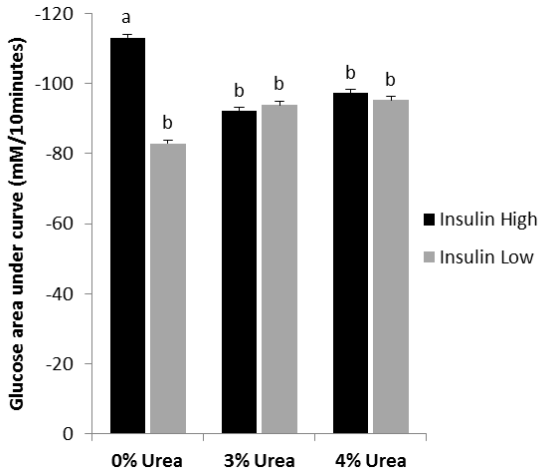

Figure 3. Effect of diet and insulin dose (high and low) on glucose response represented by area under curve. Values are lsmeans ±S.E.

IV. CONCLUSION

This paper demonstrates that increasing dietary urea results in elevated plasma urea and ammonia concentrations and a reduced whole body insulin response. We propose that this response is likely to reduce glucose uptake by insulin responsive tissues, thereby limiting muscle glycogen storage. Therefore rations containing high levels of urea, or other dietary ingredients leading to increased blood ammonia, could be associated with an increased incidence of dark cutting.

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REFERENCES

1. Ferguson, D.M., Bruce, H.L., Thompson, J.M., Egan, A.F., Perry, D. & Shorthose, W.R. (2001). Factors affecting beef palatability – farmgate to chilled carcass. Australian Journal of Experimental Agriculture 41(7): 879-891.
2. Purchas, R.W. & Aungsupakorn, R. (1993). Further investigations into the relationship between ultimate pH and tenderness for beef samples from bull steers. Meat Science 34: 163-178.
3. Pethick, D.W. & 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.
4. 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(7): 1306-1314.
5. Johnson, R.R. (1976). Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. Journal of Animal Science 43(1): 184-191.
6. Visek, W.J. (1984). Ammonia: its effects on biological systems, metabolic hormones and reproduction. Journal of Dairy Science 67(3): 481-498.
7. Gardner, G.E., McIntryre B.L., Tudor, G.D. & Pethick, D.W. (2013). Diets high in rumen degradable nitrogen reduce bovine muscle glycogen concentration at slaughter. International Congress of Meat Science and Technology. Izmir. Turkey.
8. Fernandez. J.M., Croom, W.J., Johnson, A.D., Jaquette, R.D. & Edens, F.W. (1988). Subclinical ammonia toxicity in steers: effects on blood metabolite and regulatory hormone concentrations. Journal of Animal Science 66(12): 3259-3266.
9. Edjthehadi, M., Szabuniewicz, M. & Emmanuel, B. (1978). Acute urea toxicity in sheep. Canadian journal of comparative medicine. 42: 63-68
10. Emmanuel, B., Thompson, J., Christopherson, R., Milligan, L. & Berzins, R. (1982). Interrelationships between urea, ammonia, glucose, insulin and adrenaline during ammonia-urea toxicosis in sheep (ovis aries). Comparative biochemistry and physiology part a: physiology 72(4): 697-702.
11. Trinder, P. (1989). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry: An international journal of biochemistry in medicine 6(1): 24-27.
12. Braham D. & Trinder P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 97(1151): 142-145.
13. Sudana, I. & Leng, R. (1986). Effects of supplementing a wheat straw diet with urea or a urea-molasses block and/or cottonseed meal on intake and live weight change of lambs. Animal feed science technology 16(1): 25-35.