The metabolic cost of hepatic ammonia detoxification

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ABSTRACT

The quantity of ammonia removed from portal blood by the ovine liver was measured using four sheep prepared with in-dwelling catheters in the hepatic region and fed either lucerne pellets (L) or white clover (WC) at 900 gDM/day. Following blood sampling, plasma ammonia and oxygen concentrations were determined. The portal ammonia concentration and flow were significantly ($P<0.01$) affected by the diet (304 and 646 µM; 641 and 1480 µM/min respectively). The ammonia flow across the liver was significantly ($P<0.01$) affected by the diet (-578 and -1346 (µM/min respectively). Hepatic oxygen consumption for the L diet was significantly ($P<0.05$) less than that for WC (1.6 and 2.8 mM/min respectively), of which 24 and 32% respectively can be directly attributed to ammonia detoxication to urea.

This work confirms earlier reports (Lobley *et al.*, 1995) that the ovine liver can detoxify at least 1400 µM/min of ammonia from the portal blood, albeit at an elevated metabolic cost.

Keywords: Ammonia; protein digestion; liver; sheep.

INTRODUCTION

Ruminant metabolism of nitrogen (N) is relatively inefficient, for example, only 13% of dietary N was converted into body tissue N by wethers fed twice maintenance levels of pelleted grass (Lobley, 1992). Microbial degradation of ingested plant protein to ammonia in the rumen and the subsequent detoxification of this absorbed ammonia to urea by the liver accounts for a large portion of this inefficiency. Hepatic detoxification of ammonia to urea is an energetically expensive process (Waghorn and Wolff, 1984), which has recently been shown to require an additional N cost in the form of amino acid catabolism (Lobley *et al.*, 1995). The study of Lobley *et al.* (1995) involved sheep fed concentrates and hepatic ammonia loadings elevated exogenously using mesenteric infusions of NH_4Cl. However, Egan and Ulyatt (1980) report elevated blood urea concentrations for sheep fed fresh pasture compared to dried feeds, which suggests that the ammonia detoxification costs associated with sheep fed fresh feeds could be naturally even higher than those attained through exogenous manipulation.

This paper describes studies using sheep surgically prepared with trans-hepatic catheters and fed fresh white clover in order to naturally elevate the hepatic ammonia load relative to a pelleted lucerne diet. The ammonia removed by the liver from the portal blood was quantified and the metabolic cost of ammonia detoxification in terms of both lost substrate (protein nitrogen) and the energy consumed was estimated.

MATERIALS AND METHODS

Four Romney cross wether lambs (35-40 kg live weight, 6-9 months old) were prepared with in-dwelling silicone based catheters in the aorta (A: via the femoral artery) and mesenteric (M), portal (P), and hepatic (H) veins, utilising the technique of Katz and Bergman (1969), as modified by Lobley *et al.* (1995). All animals returned to pre-surgery feed intake levels within 48 hours. The animals were kept in metabolism crates and fed 900 gDM/d in hourly portions of either lucerne pellets (L; 27 gN/kgDM) or fresh white clover (WC; 44 gN/kgDM), in a cross over design.

Each animal was placed on a nitrogen balance for ten days prior to blood sampling. After a minimum of three weeks on each diet, four 10 ml blood samples from each catheter were collected over ice into polypropylene tubes containing 0.1 ml heparinised (1000 IU/ml) saline using a peristaltic pump at a rate of 0.46 ml/min, over a four hour period at half hourly intervals. Para-amino hippurate (PAH) was infused into the mesenteric vein for the measurement of blood flow by dye dilution using the procedures of Lobley *et al.* (1995). The blood samples were centrifuged (4300G for 15 minutes) immediately following collection, with plasma stored at 4°C until the completion of each sampling period when the ammonia concentrations were determined using an enzymatic assay (Sigma #171-B). Blood oxygen concentrations were determined using a galvanic oxygen cell (Grubb and Mills, 1981).

The ammonia flow from the mesenteric and portal drained viscera were calculated as follows;

Mesenteric drained viscera = (mesenteric ammonia concentration - arterial ammonia concentration) X mesenteric blood flow.

Portal drained viscera = (portal ammonia concentration - arterial ammonia concentration) X portal blood flow.

The data were analysed by analysis of variance.

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RESULTS AND DISCUSSION

All catheters remained patent for the duration of the trial (5 mo), with the exception of one mesenteric sampling catheter, which failed within one week of surgery.

For the ten days preceding blood collection, the nitrogen (N) intake for the respective L and WC diets was significantly different (P<0.05) 22.9±1.4 and 37.9±1.4 (gN/d; mean±SE), whilst net retention of N was not significantly affected by the diet (1.7±0.6 and -1.8±1.5; gN/d).

The blood flow for each of the four sampling positions (M, P, H, A) was not significantly different for the two diets, with an average portal flow of 2094±234 and 2306±213 (g/min), and an average hepatic flow of 2244±376 and 2337±229, for the L and WC diets respectively. The lack of statistical significance between these and some other results presented in this section is predominantly because of the low number of animals per treatment group (four) together with between-animal variation. Further studies with greater animal numbers are currently being undertaken to alleviate this problem.

The portal ammonia concentration was significantly (P<0.01) affected by the diet, being 304±37 and 646±127 ((M) for L and WC respectively (Table 1). The hepatic values were 38±38 and 72±35 µM (NS) respectively. Lobley *et al.* (1995) recorded comparable portal and hepatic ammonia concentration values following the infusion of either 25 or 150 µM/min of NH_4Cl into the mesenteric vein of sheep fed L (P 379 and 506; H 93 and 110 µM). Severe signs of intoxication can be expected when arterial ammonia concentrations exceed 800 µM (Symonds *et al.*, 1981) but were not observed in this trial. This indicates that the liver effectively removed the additional ammonia load imposed by the high protein WC diet.

The portal ammonia flow was significantly (P<0.01) different for the two diets, being 641±110 and 1480±334 (µM/min) for L and WC respectively (Table 1). The low hepatic values (68±64 and 167±66 respectively) again confirm that the liver effectively removed the additional ammonia load.

Assuming that the liver mass was 2.5% of body live weight (Orzechowski *et al.*, 1987) the measured rates of hepatic ammonia clearance were equivalent to 0.57 to 1.31 µM/min/g wet liver tissue, which is below the quoted metabolic maximum 1.5 µM/min/g wet liver tissue (Symonds *et al.*, 1981; Orzechowski *et al.*, 1987). However, Lobley *et al.* (1994) assumes a lower percentage of live weight for the liver (1.6%) which generates values of hepatic ammonia clearance equivalent to 0.9 to 2.05 µM/min/g wet liver tissue, which suggests that these pasture fed sheep are approaching the maximum hepatic ammonia load.

The ammonia flows (µM/min) from the mesenteric drained viscera (MDV) and portal drained viscera (PDV) were not significantly affected by the diet (MDV 376±103 and 574±238; PDV 597±110 and 1168±294 respectively). The ammonia flow from the rumen (by subtraction; PDV - MDV) was also not significantly affected by diet (due to between-animal variation) being 197±48 and 725±178 µM/min respectively. The hepatic ammonia flow (H-P-A) for the L diet was significantly (P<0.01) less than that for WC (-578±140 and -1346±332 µM/min respectively), which confirms the findings presented above for the portal ammonia flow. Lobley *et al.* (1995) recorded similar PDV and hepatic ammonia flows for 25 or 150 µM/min NH_4Cl infusions (PDV 452 and 659; hepatic -450 and -664 µM/min).

Due to between-animal variation, there was no significant effect of diet on blood oxygen flow for the four sampling positions (Table 1). However, liver oxygen consumption (H-P-A) for the L diet was significantly (P<0.05) less than that for WC, 1.6±0.6 and 2.8±0.6 (mM/min) respectively. Assuming four high energy phosphate bonds per urea molecule synthesised and six of these bonds per molecule of oxygen consumed, Parker *et al.* (1995) calculated that only 13% of liver oxygen usage can be attributed to urea synthesis. Using these assumptions in the current study it was estimated that of the total hepatic oxygen consumption, 24 and 32% was directly associated with the ammonia detoxification to urea, for L and WC, respectively. This does not make allowance for the elevated amino acid catabolisation, sodium pump activity and oxidative phosphorylation which are also likely to occur as a result of the increased ammonia loading. The current work indicates larger percentages of oxygen consumption attributable to ammonia detoxification and urea synthesis than previously reported (13.3%, Reynolds *et al.*, 1991; 11-16%, Lobley *et al.*, 1995), which is due to the elevated ammonia loading associated with fresh feeds, especially white clover.

TABLE 1: Average plasma ammonia concentration (NH_3 conc.; µM) and flow (µM/min) and blood oxygen flow (O_2; mM/min) in the mesenteric (M), portal (P), hepatic (H) venous blood and the aorta (A) of sheep fed lucerne pellets (L) or fresh white clover (WC). SEM = standard error of the mean (four animals per treatment).

Variable	Diet	M	P	H	A (Hepatic)
NH_3 Conc.	L	326±40	304±37	38±38	20±7
NH_3 Conc.	WC	467±102	646±127	72±35	132±52
Sig.		NS	**	NS	NS
NH_3 Flow	L	391±99	641±110	68±64	5±5
NH_3 Flow	WC	673±260	1480±334	167±66	33±26
Sig.		NS	**	NS	NS
O_2 Flow	L	4.19±0.1	6.65±0.8	5.63±0.5	0.53±0.6
O_2 Flow	WC	4.66±0.6	7.53±0.5	4.87±0.5	0.14±0.6
Sig.		NS	NS	NS	NS

CONCLUSION

The hepatic ammonia flow for L was less than half that of the WC diet (-578 and -1346 (µ/min respectively). In practise, grazing lambs normally consume more than the 900 gDM/d used for this work, hence severely challenging the liver's ability to detoxify the resultant higher quantities of ammonia produced. Similarly, the feeding system which is used in most dairy farm situations in this country (two main periods of release onto pasture) may also result in very large ammonia surges to the liver.

Although the hepatic ammonia load for the WC diet was more than double that recorded by Lobley *et al.* (1995), the present data confirm that the ovine liver can successfully remove and detoxify at least 1.4 mM ammonia/min from the portal vein without exhibiting any metabolic signs of ammonia toxicity. Nevertheless such detoxification comes at increased metabolic cost as indicated by the elevated oxygen requirements of the liver.

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REFERENCES

Egan, A.R. and Ulyatt, M.J. 1980: Quantitative digestion of fresh herbage by sheep. VI. Utilisation of nitrogen in five herbages. *Journal of Agricultural Science*. Cambridge, **94:** 47-56.

Grubb, B.R. and Mills, L.P. 1981: Blood oxygen content in microlitre samples using an easy to build galvanic oxygen cell. *Journal of Applied Physiology*, **50:** 456-464.

Katz, M.L. and Bergman, E.N. 1969: A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. *American Journal of Veterinary Research*, **30:** 655-661.

Lobley, G.E. 1992: Control of the metabolic fate of amino acids in ruminants: *A review. Journal of Animal Science*, **70:** 3264-3275.

Lobley, G.E.; Connell, A.; Milne, E.; Newman, A.M. and Ewing, T.A. 1994: Protein synthesis in splanchnic tissues of sheep offered two levels of intake. *British Journal of Nutrition*, **71:** 3-12.

Lobley, G.E.; Connell, A.; Lomax, M.A.; Brown, D.S.; Milne, E.; Calder, A.G. and Farningham, D.A.H. 1995: Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *British Journal of Nutrition*, **73:** 667-685.

Orzechowski, A.; Motyl, T.; Pierzynowski, G. And Barej, W. 1987: Hepatic capacity for ammonia removal in sheep. *Journal of Veterinary Medicine A*, **34:** 108-112.

Parker, D.S.; Lomax, M.A.; Seal. C.J. and Wilton, J.C. 1995: Metabolic implications of ammonia production in the ruminant. *Proceedings of the Nutrition Society*, **54:** 549-563.

Reynolds, C.K.; Tyrrell, H.F. and Reynolds, P.J. 1991: Effect of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: Net nutrient metabolism by visceral tissues. *The Journal of Nutrition*, **121:** 1004-1014.

Symonds, H.W.; Mather, D.L. and Collis, K.A. 1981: The maximum capacity of the bovine liver to metabolise ammonia. *The Proceedings of the Nutrition Society*, **40:** 63A.

Waghorn, G.C. and Wolff, J.E. 1984: Theoretical considerations for partitioning nutrients between muscle and adipose tissue. *Proceedings of the New Zealand Society of Animal Production*, **44:** 193-200.