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Feeding frequency of fresh white clover (*Trifolium repens*) and ammonia metabolism in the ovine liver*

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ABSTRACT

The splanchnic net fluxes of ammonia, urea and glutamine were measured during and after feeding. Hepatic glutamine synthetase activity was also estimated. Ammonia load to and extraction by the liver increased following feeding but returned to baseline within seven hours. During feeding, hepatic glutamine production increased and was complemented by an increase in glutamine extraction when the ammonia load had returned to baseline. Hepatic glutamine synthetase activity followed an inverse trend.

KEY WORDS: sheep, white clover, ammonia, urea, glutamine metabolism, liver

INTRODUCTION

Fresh forages such as white clover contain a high proportion of highly soluble proteins that are rapidly degraded in the rumen resulting in large quantities of ammonia produced. The rumen microbes can use this ammonia directly (Parker et al., 1995) but ammonia production is usually much higher than microbial utilization. Excess ammonia is passively absorbed across the rumen wall into the gastric vein and transported to the liver to be converted to urea. Ureagenesis typically exceeds the rate of hepatic ammonia-nitrogen (N) extraction and the shortfall in N for ureagenesis is contributed by amino acid. This can reduce productive gain (Lobley et al., 1995).This study tested the hypothesis that the elevated hepatic ammonia extraction required when ruminants are fed fresh white

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clover over two 2 h periods would require a concomitant increase in hepatic glutamine metabolism to supply the additional N required for ureagenesis.

MATERIAL AND METHODS

Animals, surgical procedures and dietary treatments

Six Romney-cross wether lambs (BW 33.4 (SE 0.6) kg) fed fresh white clover were prepared with catheters in the caudal aorta and in the mesenteric, portal and hepatic veins (Greaney, 2001). Ten days prior to sampling, rapid consumption of 800 g DM/d of fresh white clover was achieved by restricting the access to feed to two 2 h periods per day (08.00-10.00 and 16.00-18.00).

Infusion protocol, blood sampling and analytical measurement

On day 11, the sheep were continuously infused with sodium para-aminohippurate (pAH) into the cranial mesenteric vein for 11 h (06.00-17.00) to measure splanchnic blood flow (Lobley et al., 1995). Heparinised blood was withdrawn from the portal, hepatic and arterial catheters for the first 30 min of each sampling period (0, 1, 3, 4, 6, 9 and 11 h) to quantify the net flux of ammonia, urea and glutamine across the splanchnic tissues. Two weeks later, a similar pAH infusion protocol was repeated and the animals were euthanased at the times mentioned above to measure the hepatic glutamine synthetase activity. Ammonia and urea concentrations were measured using commercial kits and amino acid concentrations were determined using reverse phase high performance liquid chromatography (Cohen et al., 1986).

Calculations and statistics

Calculated blood flows and net metabolite flux across the splanchnic tissues (Lobley et al., 1995) were subjected to analysis of variance using GLM procedure of SAS with animals treated as blocks for the effect of time relative to feeding and the significance of the difference between means was assessed by t-test and declared at probability < 0.05 (probability < 0.10 indicates a trend).

RESULTS

There was no difference in blood flow across the splanchnic tissues, or in ammonia, urea and glutamine concentrations in the portal and hepatic vein, and the posterior aorta at any of the nine time points (Greaney, 2001 and Table 1).

The net production of ammonia across the portal-drained viscera and the net uptake of ammonia by the liver were affected by the time after feeding. No changes in the net flux of urea across these tissues were observed. Net production of glutamine by the

Table 1. Net flux of ammonia, urea and glutamine across the splanchnic tissue in sheep fed fresh white clover in two 2 hour periods per day[1]

	Average	Sampling period, h									SED	P
		1	2	3	4	5	6	7	9	11		
Blood flow, ml/min												
portal-drained viscera	1807	1648	1288	1759	1973	1976	1871	2050	2157	1536	290	NS
splanchnic tissue	2120	2118	1568	1788	2642	2219	2313	2561	2163	1709	389	NS
hepatic artery	309	470	280	29	669	92	442	616	6	173	339	NS
Net flux of ammonia, µmol/min[2]												
portal-drained viscera	700	^{da}529	^{dc}421	^{a}733	^{b}663	^{b}830	^{b}1019	^{b}904	^{a}690	^{ac}511	199	0.03
liver	-821	^{da}-557	^{dc}-446	^{a}-780	^{a}-818	^{c}-836	^{a}-1035	^{a}-1113	^{a}-1125	^{a}-682	179	0.009
Net flux of urea, mmol/min[2]												
portal-drained viscera	-0.01	-0.16	-0.05	0.40	-0.25	0.26	-0.33	-0.15	-0.29	0.51	0.42	NS
liver	0.71	0.99	0.54	0.34	0.92	0.89	1.09	0.75	0.85	0.02	0.58	NS
Plasma concentration of glutamine, µmol/L												
arterial	214.0	185.2	192.1	203.0	218.4	234.3	235.5	221.7	226.3	218.1	25.9	NS
portal	193.8	155.8	145.5	187.1	200.0	229.6	211.3	205.7	232.2	204.8	32.0	NS
hepatic	196.8	160.3	177.8	190.8	220.8	242.2	229.1	206.3	189.0	186.3	44.9	NS
Net flux of glutamine, µmol/min[2]												
portal-drained viscera	-27.0	-43.5	-26.3	-29.0	-37.1	-12.4	-51.0	-32.5	15.1	-26.0	32.57	NS
liver	-2.1	1.9	5.2	4.9	42.8	35.7	66.7	-4.6	-128.0	-43.4	46.83	NS

[1] mean value and standard error of the difference between means (SED) for six sheep; feeding between sampling hours 3 and 4, beginning at 08.00; NS means not significant at 0.05; means with no superscript or the same superscript are not significantly different at 0.05

[2] positive and negative values indicate net production and net extraction of the metabolite by the relevant organ

liver tended to be higher at the start of feeding followed by an increase in glutamine extraction when ammonia returned to baseline (Table 1). Hepatic glutamine synthetase activity followed an inverse trend (from 30 to 95 units/100 mg liver; Greaney, 2001).

DISCUSSION

The major findings of the current study were; a significant increase in both portal ammonia production and hepatic ammonia extraction following feeding without a corresponding increase in hepatic urea production. The increase in portal ammonia production had receded within seven h following the end of feeding. This was also demonstrated in cows fed fresh ryegrass during two 2 h periods, with a peak in rumen ammonia concentration recorded two h after the start of feeding, returning to pre-feeding concentration within two h following the end of feeding (Waghorn et al., 1989).

Our data indicate that there might be another mechanism temporarily used for disposing of the increased ammonia load that could not be removed immediately by hepatic ureagenesis. Indeed, there was a trend for increased hepatic glutamine production following the onset of feeding that mirrored the increased in ammonia load to the liver and this changed to net glutamine extraction by the liver once the ammonia load returned to pre-feeding levels.

CONCLUSIONS

Our results suggest that hepatic glutamine synthesis might be another mechanism used to remove excess the hepatic ammonia in support of ureagenesis during a short-term period of elevated ammonia load.

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