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Ammonia and urea utilisation in lambs infected with *Trichostrongylus colubriformis* and fed fresh lucerne (*Medicago sativa*)

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ABSTRACT

The effect of a *Trichostrongylus colubriformis* infections on ammonia and urea fluxes across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), and liver was investigated using an arterio-venous preparation in lambs fed fresh lucerne (*Medicago sativa*). The lambs were infected with 6000 *T. colubriformis* L3 larvae per day for 6 days (n=5) or kept as parasite free controls (n=5). On day 48 of the experiment, the lambs were infused continuously for 8 hours with ρ -aminohippuric acid (688 mg/h) into the mesenteric vein in order to measure plasma flow across the MDV, PDV and liver. Blood was continuously collected from the mesenteric artery and vena cava for consecutive two hour periods throughout the infusion period and plasma was harvested. Plasma concentrations of ammonia and urea were unaffected by the presence of intestinal parasites. Ammonia fluxes across the MDV, PDV, and liver were similar between treatment groups. Urea fluxes were also similar between treatment groups with the exception of the PDV, which showed a net release of urea in the parasite group and a net uptake by the control group ($P = 0.03$). This suggests that the presence of a sub-clinical *T. colubriformis* infection does not increase the demand on the lamb for amino acids for ureagenesis.

Keywords: ammonia; urea; *Trichostrongylus colubriformis*; sheep.

INTRODUCTION

Intestinal parasite infections are characteristic of pastoral feeding systems and many parasite species are show resistant to anthelmintic drenches. The immune response created by a parasite infection places additional demands on the animal for amino acids (AA; Nieto & Lobley, 1999). These additional requirements may be met by diverting AA from storage protein (e.g., muscle; MacRae, 1993) to the immune system and to the repair of damaged tissue. Transamination of AA occurs in the muscle and the gastrointestinal tract (GIT). This results in the formation of amine groups, which are mainly used for ureagenesis in the liver. Ammonia produced from either dietary sources or by AA breakdown, needs to be removed from the blood circulation, as it is toxic to the animal when the concentration in the peripheral circulation exceeds 50 μmol . This is achieved primarily by the production of urea, which aside from being an energetically expensive process in terms of ATP and oxygen consumption, requires the input of an additional N from an AA (Lobley *et al.*, 1995). Therefore, in the parasite-infected animal, it is possible that there will be an increase in urea production due to the increase in AA oxidation in the muscle and GIT.

The aim of this study was to quantify the effects of a parasite infection on ammonia and urea production in the small intestine and liver. This will enable us to determine if there is any further burden on the parasitised lamb arising from increased ammonia and urea production. These preliminary results are part of a larger study investigating the effects of a *T. colubriformis* infection on the partitioning of AA between skeletal muscle, the GIT and liver using arterio venous preparations across the mesenteric drained viscera (MDV: small intestine), the portal-drained viscera (PDV; small and large intestine, rumen, spleen, and pancreas), the liver and the hind limbs,

of lambs fed fresh lucerne or sulla.

MATERIALS AND METHODS

The experimental procedures for this trial were reviewed and approved by the Crown Research Institute, Animal Ethics Committee in Palmerston North, New Zealand according to the Animals Protection Act (1960) and Animals Protection Regulations (1987) and amendments.

Ten castrated male Dorset-Romney cross lambs aged approximately six months, with initial live weight of 38 ± 1.4 kg were kept on feed pads for approximately three weeks prior to surgery. The lambs were fed lucerne pellets (800 g DM/day) and chaffed lucerne hay (200 g DM/day) before, and for three days after surgery. One week before surgery, the lambs were housed indoors in individual metabolism crates.

The lambs were prepared with permanent indwelling catheters in the mesenteric artery, and the mesenteric, portal and hepatic veins and vena cava for blood sampling as described previously in Bermingham *et al.* (2000).

For four days after surgery, the lambs received an intramuscular injection of procaine penicillin (3 mL; Bomacillin, Bomac Laboratories Ltd. Auckland, New Zealand). Three days after surgery the lambs were offered fresh lucerne (*Medicago sativa*; NEAA 123.2; EAA 91.4; Total AA 214.5 mg g/DM; 800 g DM/day). The lucerne was harvested with a sickle bar mower every two days by 10.00 am and stored at 4°C. The lambs were fed at hourly intervals from overhead feeders, and water was offered *ad libitum*. The lambs were weighed weekly to monitor liveweight changes during the experimental period.

One week after surgery (day 1 of the experiment) five lambs were given 6000 *T. colubriformis* L3 larvae per day by mouth for six days while the remaining five lambs were drenched with water to serve as controls

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(Bermingham *et al.*, 2000).

Parasitology

To monitor parasite burdens in the lambs, faecal egg counts on individual lambs were determined every second day from day 20 to day 45 of infection. Egg counts were determined using the modified McMaster method (Whitlock 1948) with the presence of one egg representing 50 eggs per gram of wet faeces. Total intestinal worm burdens were determined after slaughter, when the proximal 10 m of the small intestine was detached from the abomasum, ligated and refrigerated. The contents of the intestines were washed, and a 10% subsample of the washings was passed through a 38 mm sieve to collect worms for counting (Sutherland *et al.* 1999).

Splanchnic Fluxes

On day 48, ρ -aminohippuric acid (PAH) (688 mg/h; 0.15 mmol/L NaPAH, dissolved in water) was continuously infused into the mesenteric vein for 8 hours. Thirty mL of blood was withdrawn continuously every two hours from the mesenteric artery, the mesenteric, portal, and hepatic veins and the vena cava over the 8-hour infusion period using a peristaltic pump (302F, Watson-Marlow Limited, Cornwall, England). To prevent blood coagulation, 6000 iu/h of ovine heparin was infused into the jugular vein during the continuous sampling. In addition, the sampling lines and syringes were kept in an ice-water bath to reduce metabolic breakdown of blood constituents. Immediately after each two-hour collection period, 25 mL of whole blood was centrifuged at 4°C for 15 minutes (3270 g) to obtain plasma. The plasma was then either processed as described below or stored at -85°C for the analysis of urea and PAH.

Analytical methods

Ammonia and urea concentration

The concentration of ammonia in plasma was determined by mixing 1.5 mL of plasma with 30% TCA and centrifuging at 3270 g for 15 minutes at 4°C. The supernatant was filtered and stored at -85°C until it was injected onto a Tecator FIAstar Flow Injection 5010 Analyser (590 nm wavelength), to determine the concentration of ammonia.

Urea concentrations in plasma were determined using a commercial assay (Roche Diagnostics Ltd, Basel, Switzerland) that utilises the enzymes urease and glutamate dehydrogenase and follows the production of NADH at 340 nm. This assay measures urea in plasma by first converting it to ammonia. Therefore, the urea concentrations were corrected for endogenous ammonia, using a second assay (Sigma Diagnostics Ltd, St Louis, Missouri, USA) that lacked urease. The urea and ammonia assays were performed on a Cobas Fara II analyser (Hoffmann la Roche, Basel Switzerland).

Plasma flow

Plasma (0.5 mL) was analysed for PAH concentration according to the method outlined by Katz & Bergman (1969) and with a deacylation step as described by Lobley *et al.* (1995) to determine plasma flow through the MDV,

PDV and total splanchnic tissues (TSP).

Calculations

Plasma flows across the tissue beds were calculated according to the dye dilution method outlined in Katz & Bergman (1969).

Net flux of ammonia and urea across the MDV, PDV, and liver were determined according to the equation described by Lapierre *et al.* (2000). Negative flux represents a net release of ammonia or urea across the tissue bed, whilst positive values represent a net uptake of the metabolite.

Statistical analysis

Probability (P) values lower than 0.05 were considered to indicate a significant difference and values between 0.05 and 0.10 to indicate a trend. A General Linear Model (SAS Institute Inc., 1996) was used to analyse the data. Results are presented as treatment means and their associated standard errors. One sheep was omitted from the statistical analysis as it stopped eating two days before the sampling period due to a blockage the reticulo-rumen orifice.

RESULTS

The dosing of *T. colubriformis* larvae was successful in creating a sub-clinical infection in the small intestine of lambs fed fresh lucerne with total intestinal worm counts of 353 ± 117 and 22050 ± 991 found in the control and parasite groups, respectively ($P=0.001$). The presence of *T. colubriformis* in the small intestine had no effect on feed intake (777 ± 8 g DM/day; $P>0.05$). There was also no effect of parasite infection on plasma flow through the MDV (647 ± 125 mL/min; $P>0.05$), PDV (1330 ± 8 mL/min; $P>0.05$) or liver (1397 ± 100 mL/min; $P>0.05$). As indicated in Table 1, there were no differences in ammonia concentrations in plasma from either the mesenteric, portal, and hepatic veins or the mesenteric artery.

The flux of ammonia and urea were not significantly different across the MDV, PDV, or liver (Table 1), with the exception of urea flux across the PDV. There was a net release of urea from the PDV in the lambs infected with *T. colubriformis* ($59 \mu\text{mol/min}$) while in the control lambs urea was taken up by the PDV ($74 \mu\text{mol/min}$; $P<0.05$).

DISCUSSION

To our knowledge there have been no studies that investigate the effect of parasite infections on ammonia and urea utilisation in the MDV, PDV, or liver. This study aimed to quantify these effects to have a better understanding of the metabolic cost associated with intestinal parasites in lambs fed fresh forages. The concentrations of ammonia in the mesenteric, portal, and hepatic veins and the mesenteric artery are consistent with values obtained with lambs fed fresh white clover (Greaney *et al.*, 1996), lucerne pellets (Lobley *et al.*, 1995) and grass pellets (Milano *et al.*, 2000). Plasma urea concentrations reported in the present study are higher than those reported by Lobley *et al.* (1995) and Milano *et al.*

TABLE 1: Ammonia and urea concentrations and flux across the mesenteric drained viscera (MDV) and portal drained viscera (PDV) and liver of lambs fed fresh lucerne (*Medicago sativa*) with or without *Trichostrongylus colubriformis* infection.

	Control n=5	Parasite n=4	P
Ammonia concentration (µmol/mL)			
Mesenteric vein	0.65 ± 0.185	0.81 ± 0.177	NS
Portal vein	0.87 ± 0.172	0.89 ± 0.123	NS
Hepatic vein	0.13 ± 0.029	0.09 ± 0.000	NS
Mesenteric artery	0.13 ± 0.034	0.09 ± 0.016	NS
Urea concentration (µmol/mL)			
Mesenteric vein	7.37 ± 0.650	8.67 ± 0.520	NS
Portal vein	7.23 ± 0.600	8.83 ± 0.658	NS
Hepatic vein	7.71 ± 0.634	8.84 ± 0.790	NS
Mesenteric artery	7.35 ± 0.603	8.83 ± 0.627	NS
Ammonia flux (µmol/min)			
MDV	-324 ± 125.1	-428 ± 42.7	NS
PDV	-999 ± 220.1	-1048 ± 188.1	NS
Liver	848 ± 204.1	996 ± 137.1	NS
Urea flux (µmol/min)			
MDV	21 ± 0.04	113 ± 0.1	NS
PDV	74 ± 0.02	-59 ± 0.1	*
Liver	-765 ± 0.3	-1203 ± 1.1	NS

Where negative values represent a net release from the tissue and positive values a net uptake by the tissue

NS = not significant

* P<0.05

al. (2000), but this is expected given that in this study fresh lucerne was fed rather than pelleted forages.

The release of ammonia across the MDV (574 µmol min⁻¹) and PDV (1168 µmol min⁻¹) presented in this study are consistent with white clover-fed lambs with the net release being similar to that reported by Greaney *et al.* (1996). Ammonia flux across the liver followed similar patterns of uptake as those reported by Lobley *et al.* (1995; 450 µmol/min) and Milano *et al.* (2000; 319 µmol/min). Urea was removed by the MDV for both the control and parasite lambs, however it was released by the liver, which is consistent with the release reported by Lobley *et al.* (1995; 314 µmol/min) and Milano *et al.* (2000; 710 µmol/min). The differences in PDV urea utilisation may be due higher production of urea in the large intestine which has been observed during parasite infection (Roseby, 1977). This increase is thought to be due to the increase in endogenous N during parasite infection that is secreted into the gastrointestinal tract and fermented in the large intestine to urea which is subsequently absorbed into the sheep (Roseby, 1977).

The similarities between ammonia and urea production or utilisation in the lambs with *T. colubriformis* infection, compared to the control lambs, suggests that intestinal parasites do not create an additional demand on the animal for both the catabolism of AA or energy for ureagenesis. This result also suggests that it is unlikely that there has been an increase in protein degradation in the muscle due to the presence of intestinal parasites. Any increase in protein degradation would result in an increase in AA flux to the liver and any AA surplus to the requirements by the liver or other tissues would be used

for urea production. However, the weight loss measured in these lambs together with the similar whole body utilisation of cysteine and valine (Bermingham *et al.*, 2000) suggests that there is some alteration in protein synthesis, degradation or AA metabolism within the different tissues of the parasitised lamb. These alterations have been observed during *T. colubriformis* infections where there was an increase in leucine sequestration in the GIT of infected lambs (Yu *et al.*, 2000). It is envisaged that the completion of the larger study involving isotopes of cysteine and valine will enable us to quantify the metabolic changes in the GIT, liver and hind limbs of lambs that is caused by intestinal parasites.

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