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AMINO ACID FLUX ACROSS THE HIND LIMBS IN LAMBS FED FRESH LUCERNE *(MEDICAGO SATIVA)* WITH OR WITHOUT *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION.

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SUMMARY

The effect of parasite infections on amino acid (AA) flux across the hind limbs in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh lucerne (*Medicago sativa*), was investigated using an arterio-venous preparation. 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 indocyanin green (14.7 mg h^{-1}) into the abdominal aorta in order to measure plasma flow across the hind limbs. Blood was continuously collected from the mesenteric artery and vena cava for two hour periods throughout the infusion period and plasma was harvested. The concentration of AA in plasma from the mesenteric artery and vena cava, and plasma flow across the hind limbs were determined and the corresponding net AA flux was calculated. Parasite infection had no effect on the feed intake (777 g DM d^{-1} (SE 7.69); P>0.05) or plasma flow across the hind limbs (498 mL min^{-1} (SE 46.7); P>0.05). In most cases, the plasma concentration and the net flux of AA were not affected by the presence of an intestinal parasite infection (P>0.05). Due to a decrease in average daily gain in these lambs it is likely that there may be an alteration in the utilisation of AA in other tissues (e.g. gut and liver) of lambs infected with intestinal parasites.

Keywords: *Trichostrongylus colubriformis*, sheep, amino acid, hind limb

INTRODUCTION

Intestinal parasites have been shown to decrease weight gain, milk and wool production (Steel and Symons, 1982; Sykes, 1982; Poppi *et al.*, 1990) and alter body composition (Parkins and Holmes, 1989). These decreases in animal production may be due to an alteration in the way that amino acids (AA) and other nutrients are partitioned within the body of the infected animal, as there is an increase in the amino acid (AA) requirement for the immune response and tissue repair (Parkins and Holmes, 1989). This increase in AA requirement is thought to be met by the breakdown of the body protein stores – mainly muscle (MacRae, 1993). By quantifying the pattern of AA flux across the hind limbs it is possible to get an understanding of the metabolic processes occurring in this tissue. The aim of this study was to investigate the effects of an intestinal parasite infection on the flux of AA across the hind limbs in order to determine whether such repartitioning does occur in lambs. These preliminary results are part of a larger study investigating the effects of a *T. colubriformis* infection on AA utilisation in skeletal muscle, the gastrointestinal tract and liver using arterio-venous preparation across the small intestine, the portal-drained viscera, the liver and the hind limbs of lambs fed fresh lucerne or sulla.

MATERIALS AND METHODS

Animals and feed

Ten castrated male Dorset-Romney cross lambs aged approximately 6 months old, with initial live weight of 38 kg (SE 1.4) were housed indoors in individual metabolism crates and offered fresh vegetative lucerne (*Medicago sativa*) at about maintenance intake (800 g DM d^{-1}). The lucerne was harvested every

two days by a sickle bar mower by 10.00 am and stored at 4°C. The sheep were fed at hourly intervals from overhead feeders.

Permanent indwelling catheters were placed in the mesenteric artery and the mesenteric, portal and hepatic veins (Huntington *et al*., 1989) and vena cava (Ortigues and Durand, 1995) for blood sampling. Additional permanent catheters were placed in the mesenteric vein and abdominal aorta for infusion of ρ-aminohippuric acid (PAH) and indocyanin green (ICG) to measure plasma flow through the splanchnic tissues and the hind limbs of the sheep, respectively. A permanent cannula was fitted in the abomasum for the infusion of [1-^{13}C]-valine and [^{35}S]-cysteine. A temporary catheter was inserted into the jugular vein two days before blood sampling for the infusion of [3,4-^{3}H]-valine.

One week after surgery (day 1 of the experimental period) five sheep were given 6000 *T. colubriformis* L3 larvae per day by mouth for 6 days while the remaining five sheep were drenched with water to serve as controls. Faecal egg counts on individual sheep were determined every second day from day 20 to day 45 using the modified McMaster method (Whitlock, 1948) where one egg counted equated to 50 eggs per gram wet faeces. Total intestinal worm burdens were determined after slaughter on day 48 as described in Bermingham *et al*. (2000).

Arterio-venous sampling

On day 48 of the experiment the lambs received a continuous infusion of ICG (14.7 mg h^{-1}) into the abdominal aorta for 8 hours to measure the plasma flow across the hind limbs. The ICG infusion was prepared according to the method outlined by Wester *et al.* (2000). To prevent blood clotting during the continuous sampling, 6000 iu ovine heparin h^{-1} was infused into the jugular vein of the lambs, and sampling lines and syringes were kept in an ice-water bath. As part of the larger study, 30 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 infusion period. This paper focuses on AA flux across the hind limbs and therefore only AA concentrations in the mesenteric artery and vena cava and related calculated parameters are presented. After each two-hour collection period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. The blood was centrifuged (4°C; 3270 g for 15 minutes), and the plasma harvested and either processed as described below for AA or ICG concentrations or stored at -85°C.

Amino acid concentrations in plasma (0.5mL) were determined as described in Bermingham *et al.* (2000). Plasma flow across the hind limbs was calculated using the ICG concentration measured in plasma (1.0 mL) which was centrifuged at 3270 g for 15 minutes at 4°C before measuring the absorbance of plasma at 790 nm using a spectrometer. Plasma concentrations of ICG were determined from a standard curve generated by known concentrations of ICG and their corresponding absorbance.

Calculations and statistical analysis

Plasma flow across the hind limbs was calculated according to the following calculation:

$$\text{Hind limb plasma flow } (\text{HLPf; mL min}^{-1}) = \frac{\text{ICG concentration in the infusate x Infusion rate}}{\text{ICG concentration}_{\text{Vena cava}} - \text{ICG concentration}_{\text{Arterial}}}$$

Amino acid flux across the hind limbs (mmol min^{-1}) was calculated by:

$$\text{Net AA Flux } (\text{mmol min}^{-1}) = (\text{concentration}_{\text{Arterial}} - \text{concentration}_{\text{Vena cava}}) \text{ x HLPf}$$

Negative flux indicates a net release of AA from the hind limbs, whilst positive values represent a net uptake of AA by the hind limbs.

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 means and associated standard error.

RESULTS

The control and parasite groups had total intestinal worm counts of 353 (SE 116.7) and 22050 (SE 991.1; P=0.001), respectively as determined after slaughter on day 48 of the experiment. However, the presence of intestinal parasites did not affect daily feed intake (777 g DM d^{-1} (SE 7.69); P>0.05) nor plasma flow across the hind limbs (498 mL min^{-1} (SE 46.7); P>0.05).

Amino acid concentrations in the arterial and venous blood and arterio-venous differences (data not presented) did not differ between the control and infected lambs. Amino acid fluxes (see Table 1) showed similar patterns of uptake (branched chain AA; BCAA) or release (Total, non-essential, and essential AA) across the hind limbs, despite the presence of parasite infections. Only the flux of proline was affected by the presence of parasite infections (P=0.07).

Table 1. Net amino acid flux (mol min^{-1}) across the hind limbs in lambs infected with or without *Trichostrongylus colubriformis* infection and fed fresh lucerne (*Medicago sativa*)

	Control n=5	SE	Parasite n=4	SE	P=
Arg	-8.9	2.72	-9.4	2.50	0.89
Cys	-19.7	4.58	-18.8	2.71	0.88
His	-4.9	4.04	-6.1	3.86	0.84
Ile	1.9	0.52	1.9	0.72	0.99
Leu	3.1	0.74	4.1	0.61	0.31
Lys	-1.3	3.55	-6.4	0.87	0.25
Met	-1.0	1.04	-1.0	0.44	0.99
Phe	-0.2	0.33	0.3	0.42	0.44
Thr	2.0	2.18	-0.4	1.31	0.41
Val	5.1	3.12	5.3	0.65	0.96
Ala	-1.2	1.47	-3.6	1.09	0.26
Asn	1.3	0.91	1.8	0.48	0.68
Asp	1.6	0.82	1.3	0.25	0.81
Gln	-31.2	7.47	-29.0	4.08	0.82
Glu	-11.5	10.28	9.3	3.18	0.13
Gly	-7.0	9.16	-10.5	3.47	0.73
Pro	-1.7	1.14	1.3	0.39	0.07
Ser	4.2	1.37	2.3	0.42	0.57
Tau	1.6	0.78	1.9	0.67	0.75
Tyr	0.04	0.96	-1.0	0.31	0.40
Total	-67.8	6.62	-56.6	10.06	0.37
NEAA[1]	-43.9	11.27	-25.1	10.54	0.27
EAA[2]	-33.9	15.81	-42.0	2.60	0.64
BCAA[3]	10.0	3.81	11.3	0.15	0.77

Negative values represent a net release from the tissue and positive values a net uptake by the tissue.
[1.] Non essential amino acids (Asp, Glu, Ser, Asn, Gly, Gln, Tau, Ala, Pro)
[2.] Essential amino acids (His, Thre, Arg, Tyr, Met, Phe, Lys, Cys; do not include the BCAA)
[3.] Branched-chain amino acids (Val, Ile, Leu)

DISCUSSION

As previously reported (Bermingham *et al.*, 2000) the dosing of *T. colubriformis* larvae was successful in causing a sub-clinical infection in the small intestine of lambs, however there was no effect on feed intake. There is very little data available on the flux of AA across the hind limbs of lambs fed fresh forages.

Moreover to our knowledge, there is no data available on the effects of parasite infections on the utilisation or release of AA by the hind limbs. Plasma flow across the hind limbs was similar to that found by Roy *et al.* (unpublished data) who used a similar arterio-venous preparation. The pattern of uptake or release of AA from the hind limbs in most cases, were similar to lambs fed pelleted rations (e.g. Hoskin *et al*. 2001; Roy *et al*. unpublished data), where the essential- and non-essential AA were released by the hind limbs, and the BCAA were taken up by the hind limbs.

There was no difference in the AA flux across the hind limbs between the control and parasite infected lambs. This result does not necessarily mean that there is no shift in the metabolic processes (protein synthesis and degradation or AA oxidation) with parasite infection. It is possible that there may be an alteration in the way that AA are utilised within the hind limbs, with changes in protein synthesis, degradation and/or AA oxidation resulting in a similar net flux. However, there are no differences in the traditional carriers of N-groups resulting from the oxidation of AA (e.g. alanine). Average daily gain over the N retention period was lower in the infected lambs (-100 versus 200 g d^{-1} ($P<0.10$) in the infected and control lambs, respectively), while the whole-body utilisation of cysteine and valine was similar between treatment groups (Bermingham *et al.*, 2000). This suggests that an alteration in the protein synthesis and/or degradation in other tissues may also have occurred. Such alterations in protein and AA metabolism have been described in the gut by Yu *et al.* (2000), where the presence of *T. colubriformis* resulted in increased sequestration of leucine in the gastrointestinal tract during *T. colubriformis* infection in lambs fed pelleted rations. An increase in protein synthesis in the liver has also been reported following a *T. colubriformis* infection (Jones, 1982). These alterations in tissue metabolism may become apparent when the analysis of net AA flux, protein synthesis, protein degradation and AA oxidation rates in these tissues from the larger study are completed.

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