Anim. Prod. Aust. 2002 Vol. 24: 133-136

THE EFFECTS OF PARASITE INFECTION ON METHIONINE METABOLISM IN SHEEP

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SUMMARY
The effect of *Trichostrongylus colubriformis* infection on methionine (Met) metabolism in Dorset-Romney lambs was examined. The parasitic larvae were dosed orally for 6 days. Met metabolism was determined six weeks later with an infusion of DL-[3,3,4,4-deuterium$_4$]Met. The infection reduced the Met : d_4-Met ratio in the mesenteric vein (P = 0.09), and increased Met uptake from the artery relative to Met sourced from tissue protein breakdown (P= 0.07) in the duodenum. The results demonstrated that a considerable reduction in Met absorption incurred with *T. colubriformis* infection.

Key words: methionine, parasites, sheep

INTRODUCTION
Internal parasite infection damages the mucosal epithelium of the gut and affects nutrient digestion and absorption. The costs of infection include the repair of damaged gastrointestinal tissue the mounting of immune responses (Sykes 2000). Methionine (Met) is the first limiting amino acid for wool production in sheep. It is involved in the initiation of all protein synthesis and through its contributions to cysteine and polyamine synthesis, plays an essential role in supporting high protein synthesis rate in the gut and immune responses. This experiment was designed to examine the effect of a parasite infection on Met absorption and metabolism.

MATERIAL AND METHODS
Animals and experimental procedures
Ten Dorset-Romney castrated male lambs (initial weight of 36.9 kg, SEM 0.16) were hand-reared indoors from weaning to minimise prior exposure to *Trichostrongylus colubriformis* (immunologically naive). The sheep were housed indoors and fed with lucerne pellets (800 g DM per d) and lucerne chaff (200 DM per d) prior to surgery and during the recovery period following surgery. After recovering from surgery, these sheep were fed with fresh lucerne (800 g of DM per d). On the day of the measurement of Met metabolism, sheep were fed hourly from overhead feeders.

All catheters were made according to the procedures described by Huntington *et al.* (1989). Catheters were placed in the mesenteric, portal and hepatic veins, and in the mesenteric artery according to the procedure described by Huntington *et al.* (1989) whilst the catheters for the arterio-venous hind limb preparation were inserted in the abdominal aorta and vena cava according to the procedures of Ortigues *et al.* (1994).

One week after surgery, 5 lambs (parasite group) received third stage *T. colubriformis* larvae by a stomach tube daily for 6 days (6000 larvae/d). The remaining 5 lambs (control group) were drenched with water. Infection was monitored by measuring faecal egg counts on day 21 and every second day thereafter.

On day 45 from start of the infection, DL-[3,3,4,4-deuterium$_4$)Met (>99%, CDN Isotopes, Pointe-Claire, Quebec, Canada) was infused over a period of 8 h via a jugular catheter. The infusion rate was constantly at 0.042 g d_4-Met per h in a volume of 22 mL of sterilised saline. Blood from the aorta, vena cava and mesenteric, portal and hepatic veins was constantly drawn into a syringe (buried in ice) by a peristaltic pump, and samples were collected every 2 h. Plasma was collected and stored at -80 C.

Immediately after the infusion period of 8 h, the lambs were killed. Samples of the duodenum, liver, muscle and skin were collected immediately, frozen in liquid nitrogen and stored at -80 C. Sample preparations and analysis for the enrichment of d_4-Met in plasma and tissue homogenate by gas chromatography-mass spectrometry were performed according to Calder and Smith (1988).

Calculations

Met irreversible loss rate (ILR). Total irreversible loss rate of Met was calculated from

$$ILR = \frac{0.99}{Ea} \times I \quad (1)$$

where 0.99 is the isotope enrichment of Met in the infusate, *Ea* represents the enrichment of Met in arterial blood collected during hour 6 to 8 of infusion (Lee *et al.* 1995), and *I* is the infusion rate of d_4-Met (g/d). Because ILR includes the infusate, the infusion rate of d_4-Met was then subtracted from ILR and referred to as unlabelled Met which should cover Met absorbed from the gut and from breakdown of body protein.

The ratio of Met uptake from the artery to Met from tissue protein breakdown. Assuming breakdown of tissue protein contributes to the tracee but not to the tracer, and that the tracer enrichment reaches plateau after 8 h infusion, the enrichment of the tracer in tissue homogenate, for example in the skin, should be determined by uptake of both the tracer and tracee from the artery and the tracee from breakdown of tissue protein, ie

$$\frac{Ua \times Ea}{Ua + Ds} = Es$$

where *Ua* represents uptake of Met from the artery (including d_4-Met plus Met, g), *Ea* is d_4-Met enrichment in the artery sampled from hour 6 to 8 of the infusion, *Ds* stands for Met from protein breakdown in the skin (g) and *Es* is d_4-Met enrichment in skin homogenate at 8 h. After a rearrangement, the ratio of uptake of Met from the artery by the skin to Met from protein breakdown in the skin can be calculated from

$$\frac{Ua}{Ds} = \frac{Es}{Ea - Es}. \quad (2)$$

Similarly, the uptake : breakdown ratio in the muscle can be calculated using formula 2 by substituting *Es* with *Em* (d_4-Met enrichment in muscle homogenate) and *Dm* (Met from protein breakdown in the muscle, g).

In the liver the contribution of the tracer (d_4-Met) is from both the hepatic artery (*Ua*) and the portal vein (*Up*), therefore d_4-Met enrichment in liver homogenate (*El*) is determined from

$$\frac{Ua \times Ea + Up \times Ep}{Ua + Up + Dl} = El$$

where *Ep* and *El* are d_4-Met enrichment in the portal vein and the liver homogenate, and *Dl* represents Met from protein breakdown in the liver (g). Since the difference between *Ea* and *Ep* was small, they could be substituted with *Eap*, the mean of *Ea* and *Ep*, to simplify the formula to

$$\frac{Ua + Up}{Dl} = \frac{El}{Eap - El}. \quad (3)$$

As for the gut, d_4-Met enrichment (*Eg*) in gut homogenate can be calculated from

$$\frac{Ua \times Ea}{Ua + Dg + A} = Eg$$

where *A* represents Met absorption from the gut (g), and *Dg* is Met from protein breakdown in the gut (g). Again, with a rearrangement

$$\frac{Ua}{Dg + A} = \frac{Eg}{Ea - Eg}. \quad (4)$$

Statistical analysis

The effect of parasite infection was examined by comparing group means using unpaired T-Test (one-sided). Since a limited number of animals were used in this experiment, the significance level of 0.1 was used to assess the parasite effect. The analysis was performed using GenStat 5. Release 4.2 (VSN International Ltd).

RESULTS

Met flux

Total Met ILR was 2.37 g/d in the infected group and 2.59 g/d (SE 0.19) in the control sheep. After subtracting the infused d_4-Met, the ILR was 1.37 and 1.61 g/d (SE 0.20) for the infected and control sheep. Parasite infection did not significantly reduce Met ILR (P>0.10).

Met : d_4-Met ratios in the artery and various veins

Since d_4-Met was systemically administrated, the Met : d_4-Met ratio is used to indicate relative contribution of Met from tissue protein breakdown and/or absorption from the gut, depending on the tissues. As shown in Table 1, the ratio was consistently lower in all the blood vessels in the infected group, but differences were not statistically significant (P>0.1). Amongst the blood vessels, there was a trend that the lowest ratio was found in the mesenteric vein (P=0.09).

Table 1. The effects of parasitic infection on Met : d_4-Met ratio in blood vessels of Dorset-Romney lambs

	Artery	Mesenteric vein	Portal vein	Hepatic vein	Vena cava
Control	1.64	2.70	2.07	1.88	1.86
Parasite	1.40	1.78	1.68	1.53	1.53
SE	0.24	0.45	0.25	0.27	0.21
P vales	0.25	0.09	0.15	0.19	0.15
Parasite/control	0.85	0.66	0.81	0.81	0.82

Ratio of uptake of Met from the artery to Met from protein breakdown in the tissues. The uptake of Met from the artery by the duodenum and the absorption from the lumen accounted for only a fraction of the Met from protein breakdown in the gut tissue, and parasite infection tended to increase the ratio (P = 0.07). A similar low uptake (from the artery and portal vein): breakdown ratio was found in the liver. In the skin the uptake provided the major source of Met compared to that from protein breakdown. The infection did not influence these ratios in the liver, skin and muscle.

Table 2. The ratio of Met uptake from the artery to Met from protein breakdown in the tissue

	Muscle	Skin	Liver	Duodenum
Control	0.549	2.407	0.122	0.144
Parasite	0.419	2.401	0.128	0.209
SE	0.185	0.457	0.014	0.028
P vales	0.32	0.50	0.39	0.07

DISCUSSION

Subclinical infection in the small intestine of the lambs was successfully created by oral dosing with *T. colubriformis*, described previously by Bermingham *et al.* (2000). Met available from the gut tended to decline with the infection, as indicated by a reduction in the Met : d_4-Met ratio in the mesenteric vein. The decline in the ratio suggests less Met (tracee) available relative to the labeled Met. Unlabelled Met in the gut tissue is sourced from both absorption from gut lumen, and protein breakdown in the tissue, and a reduction of either could contribute to the decline. Protein synthesis in the gut is elevated with parasite infection (reviewed by Sykes 2000), and protein degradation is unlikely to be suppressed as both processes are usually accompanying each other though at different magnitudes. Therefore, it is likely that the absorption of Met was impaired with *T. colubriformis* infection.

The direct result of parasite infection is mucosal damage of the gastrointestinal tract. Repairing the damaged tissue is coupled with an increase in protein synthesis. The demand of amino acids for protein synthesis is from both absorption, which tended to be reduced by the infection, and from artery uptake. To match the demand and compensate for the decline in the absorption, the uptake of Met from the artery by the duodenum tended to increase, as shown in Table 2. This is supported by the observation that leucine sequestration by the gastrointestinal tract was significantly increased in response to a parasite infection (Yu *et al.* 2000).

The *T. colubriformis* infection reduced Met ILR by 15%, and cysteine ILR by a similar level (Bermingham *et al.* 2000). However, the reductions were not statistically significant, possibly due to the limited number of animals used in the experiment. As parasite infection affects directly the gut tissue, whole-body ILR appeared not to be a sensitive index to study the effects of the infection. This is supported by the little differences in ILR of leucine (Yu *et al.* 2000) and valine (Bermingham *et al.* 2000). In combination of data of the flux and the isotopic ratios in the various blood vessels this paper suggests that parasite infection created Met repartitioning in the body, and a more sensitive method is required to define this repartition.

REFERENCES

BERMINGHAM, E.N., MCNABB, W.C., REYONLDS, G.W., WAGHORN, G.C., SUTHERLAND, I.A., REVELL, D.K. and ROY, N.C. (2000). *Asian-Aust. J. Anim. Sci.* **13 Vol. C**, 196-9.

CALDER, AG and SMITH A. (1988). *Rapid Comm. Mass Spec.* **2**, 14-6.

HUNTINGTON, G.B., REYNOLDS, C.K. and STROUD, B.H. (1989). *J. Dairy Sci.* **72**, 1583-95.

LEE, J., HARRIS, P.M., SINCLAIR, B.R. and TRELOAR, B.P. (1995). *Aust. J. Agric. Res.* **46**, 1587-1600.

ORTIGUES, I., DURAND, D. and LEFAIVRE, J. (1994*). J. Agric. Sci. Camb.* **122**, 299-308.

ROY, N.C., ZUUR, G., DENNISON, N. and LOBLEY, G.E. (1999). *Brit. J. of Nutr*. Submitted.

SYKES, A.R. (2000). *Asian-Aust. J. Anim. Sci.* **13 Vol. A,** 343-50.

YU, F., BRUCE, L.A., CALDER, A.G., MILNE, E., COOP, R.L., JACKSON, F., HORGAN, G.W. and MACRAE, J.C. (2000). *J. Anim. Sci.* **78**, 380-90.

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