The effect of condensed tannins in sulla (*Hedysarum coronarium*) on 4-methylphenol metabolism in the ovine gastrointestinal tract during late lactation

N.C. ROY, K. FRASER, G.A. LANE, B.R. SINCLAIR and W.C. MCNABB

AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand

ABSTRACT

Twelve lactating ewes were surgically prepared with permanent arterio-venous catheters across the gastrointestinal tract at 1 week post partum. From week 3 to 6 of lactation, all sheep were fed fresh sulla (1500 g dry matter/day; condensed tannins (CT) 4.4 g per 100 g dry matter). Half the ewes were orally drenched (4 times/day) with polyethylene glycol (PEG) (160 g/day in water) to remove the effects of the CT whilst the remaining ewes received a water drench. At week 6, para-aminohippurate was infused for 7 h into the cranial mesenteric vein for measuring mesenteric and portal plasma flow and 4-methylphenol concentrations. The mesenteric and portal plasma flows were not affected ($P > 0.05$) by the treatments. The concentration of 4-methylphenol in the mesenteric artery (Control: 2.8 vs PEG: 9.0 ± 1.2 µg/ml) was lower ($P < 0.01$) in the control ewes. The net portal appearance of 4-methylphenol was higher ($P < 0.01$) in the PEG ewes (Control: -663 vs PEG: -1430 ± 159 µg/minute). These results establish that sulla CT reduced the portal flux and peripheral concentration of 4-methylphenol and it is likely that this was a consequence of reduced formation of that metabolite in the rumen due to lowered ruminal protein degradation in the CT-treated ewes.

Keywords: plasma net fluxes; gastrointestinal tract; 4-methylphenol; lactation; sheep.

INTRODUCTION

Meat and dairy products derived from animals raised on pasture have a characteristic pastoral flavour that is perceived undesirable for some Asian and European markets (Keen, 1998; Young *et al.,* 2003). The alkylphenol, 4-methylphenol, is part of a group of flavour compounds that have been implicated as contributing towards 'pastoral flavours' in ruminant dairy fat (Urbach *et al.*, 1972). The concentration of 4 methylphenol in the rumen has been positively correlated with crude protein intake (Fraser *et al.*, 2003). There is a scarcity of information in the literature on how these metabolites are metabolised by the gastrointestinal tissues in grazing ruminants and sequestrated into muscle and milk fat, although 4-methylphenol is known to be excreted in the milk and urine largely as conjugates (Lopez & Lindsay, 1993; Lane & Fraser, 1999)

We have tested the hypothesis that reduced ruminal protein degradation observed with feeding animals with fresh forages containing condensed tannin (CT; McNabb *et al.*, 1996) such as sulla can reduce the ruminal concentration of tyrosine, a likely precursor of 4-methylphenol, and hence reduce the supply of 4-methylphenol to the peripheral blood and fat depots. Our objective was to investigate the effect of CT on the net flux of 4-methylphenol across the small intestine (mesenteric-drained viscera (MDV)) and gastrointestinal tract (portal-drained viscera (PDV) = stomachs, small intestine, large intestine, spleen and pancreas), the liver and the mammary gland of lactating ewes. The MDV and PDV fluxes for 4-methylphenol are presented in this paper.

MATERIALS AND METHODS

Animals and surgical procedures

The Animal Ethics Committee of AgResearch Limited approved the experimental protocol. Surgery occurred about 8 days after lambing. Four days after lambing, twelve Romney ewes were transferred to metabolism crates, held indoors and fed twice daily with lucerne pellets (600 g/day) and chaff (400 g/day) for the week before surgery. Water was available *ad libitum*. The ewes were milked twice daily using a portable milking machine with milk letdown assisted with an intravenous injection of oxytocin (1 i.u.).

Twelve ewes were prepared with a permanent cannula in the abomasum under isofluorane anaesthesia. During surgery, permanent indwelling catheters were placed in the mesenteric (2 sites; cranial for infusion and distal for sampling), portal and hepatic veins, and in the mesenteric artery (Huntington *et al.,* 1989). A transonic flow probe was fitted around the pudic artery. Three days prior to the start of the sampling period, a temporary catheter was inserted into the mammary vein for blood sampling.

Treatments

Three weeks post partum, all ewes were offered fresh sulla (*Hedysarum coronarium*; 1500 g dry matter (DM) DM/day; 4.4 g CT/100 g DM) on a hourly basis for 28 days using an automated belt feeder. Half the ewes were orally drenched (4 times per day) with polyethylene glycol (PEG; 160 g/day in water) to remove the effects of the CT (CT inactive; PEG group; McNabb *et al.,* 1996) whilst the remaining ewes received a drench of water (CT active; Control group). The treatments were applied according to a completely randomised block design. Due to the time-consuming

nature of the surgical procedure, a maximum of 4 ewes were surgically prepared with catheters in any one week. Therefore, the ewes were blocked according to the week that they underwent surgery, referred to as the group effect in the statistical model. Each block included two ewes from each treatment for a total of four animals.

Infusion and sampling

Six weeks post partum, para-aminohippurate was infused for 7 hours into the cranial mesenteric vein for measuring mesenteric and portal plasma flows. Blood was continuously sampled for three consecutive 2-hour periods from the mesenteric vein, portal vein and mesenteric artery. These blood samples were centrifuged at 3270 *g* for 15 minutes (4ºC) and the plasma stored at –20ºC until analysed for 4-methylphenol and para-aminohippurate concentration.

Analytical methodologies

The plasma concentration of 4-methylphenol (both conjugated and free) was measured using a gas chromatograph with a flame ionization detector after enzyme hydrolysis, as previously described (Fraser *et al.*, 2004). Plasma was deproteinised and the acetyl para-aminohippurate in the supernatant deacylated with HCl. The para-aminohippurate was then derivatised with N-1-napthyl-ethylene-diamino-dihydrochloride and measured against a standard curve using a spectrophotometer at 540 nm to determine its concentration (Lobley *et al.,* 1995).

Calculation

The MDV and PDV plasma flows were calculated as described by Lobley *et al.* (1995). Arterial inflow of 4-methylphenol was calculated by multiplying the plasma flow (ml/minute) by its arterial concentration (µg/ml). Net plasma flux of 4-methylphenol across the MDV and PDV was calculated as plasma flow (ml/minute) x (arterial concentration - venous concentration; µg/ml). A positive flux indicates a removal, whilst a negative flux indicates a net production by the gastrointestinal tract.

Statistical analysis

Data were subjected to the GLM procedure of SAS (SAS, 1999-2001) according to a completely randomised block design. Paired *t*-tests were used to verify whether the arterio-venous concentration differences for 4-methylphenol were different from zero. Least squares means (± overall standard deviation of the means) are presented. Significant differences between treatments were declared at a probability less than 0.05 whilst a probability below 0.1 but above 0.05 was considered to represent a trend.

RESULTS

The concentration of 4-methylphenol in the mesenteric artery, mesenteric vein and portal vein was lower ($P < 0.01$) in the control than the PEG ewes (Table 1). Results from the paired *t*-tests for the arterio-venous concentration differences of 4 methylphenol across the MDV indicate that they were not different from zero (data not shown) and therefore the parameters calculated using these data must be interpreted with care. The arterio-mesenteric vein concentration difference of that metabolite across the MDV was not affected ($P > 0.10$) by the PEG treatment (Table 1). The arterio-venous concentration difference of 4-methylphenol across the PDV was different from zero (data not shown) and was higher in the PEG ewes compared to the control ewes (Table 1).

TABLE 1: Effect of polyethylene glycol (PEG) on plasma concentration of 4-methylphenol in mesenteric artery (A), mesenteric vein (M) and portal vein (P) and arterio-venous concentration differences of 4-methylphenol across the mesenteric-drained viscera (A-M) and portal-drained viscera (A-P) in lactating ewes.

Parameter	Treatments		SEM	P value
	PEG (n = 6)	control (n = 5)		
Plasma concentration (µg/ml)				
A	9.03	2.82	1.22	0.007
M	9.10	2.78	1.19	0.006
P	9.65	3.13	1.28	0.007
Arterio-venous concentration difference (µg/ml)[1]				
A-M	-0.07	0.06	0.20	0.65
A-P	-0.63	-0.30	0.81	0.02

[1] Positive and negative values indicate net uptake and net production by the relevant organ

Plasma flows across the MDV and PDV were not affected by PEG and averaged respectively 689 and 2314 ml/minute (Table 2). The arterial inflow of 4-methylphenol to the MDV tended to be higher ($P < 0.10$) with the PEG treatment (Table 2). In the PDV, this increased inflow was highly significant ($P < 0.01$). Similar effects were found for the 4-methylphenol output from the MDV and PDV (Table 2). Net appearance of 4-methylphenol in the mesenteric drainage was similar ($P > 0.10$) between treatments whilst across the portal drainage this net flux was higher ($P < 0.01$) in the PEG group compared to the control ewes. The net mesenteric appearance of 4 methylphenol was less than 10% of its appearance in the portal vein.

DISCUSSION

This study shows that the oral addition of PEG to lactating ewes fed a CT-containing plant, sulla, increased the net appearance of 4-methylphenol in the portal vein. The concentration of 4-methylphenol in the rumen has been positively correlated with crude protein intake (Fraser *et al.*, 2003). A moderate concentration of CT in the diet can reduce the degradation of dietary protein in the rumen (McNabb *et al.*, 1996) and it would

be expected that with feeding sulla, the concentration of 4-methylphenol in the rumen would be reduced. The rumen concentration was not measured but the decrease in the net portal appearance of 4-methylphenol in the control ewes is consistent with the observations of McNabb *et al*. (1996) and Fraser *et al.* (2003). However, our results contrast with the lack of effect of *Lotus corniculatus* on the rumen concentration of 4-methylphenol observed in Fraser *et al.* (2003). *Lotus corniculatus* contains lower levels of CT than sulla.

TABLE 2: Effect of polyethylene glycol (PEG) on plasma flow and flux of 4-methylphenol across the mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) in lactating ewes.

Parameter	Treatments		SEM	P value
	PEG (n = 6)	control (n = 5)		
Plasma flow (ml/minute)				
MDV	560	817	139	0.24
PDV	2271	2357	92	0.51
Arterial inflow to (mg/minute)				
MDV	5.3	2.2	1.2	0.10
PDV	20.2	6.1	2.3	0.002
Venous outflow from (mg/minute)				
MDV	5.5	2.2	1.3	0.11
PDV	21.6	6.8	2.3	0.02
Net flux across (µg/minute)[1]				
MDV	-130	-22	171	0.65
PDV	-1430	-663	159	0.009

[1] Positive and negative values indicate net uptake and net production by the relevant organ

The net appearance of 4-methylphenol was substantially higher in the portal drainage than the mesenteric drainage. This suggests that most of that metabolite is absorbed through the rumen epithelium as the PDV estimate includes the rumen contribution. Previous studies have also shown that the absorption of the bulk of the plant oestrogens (phenolic compounds) probably occurs in the rumen (Cox & Braden, 1974).

Previous findings have shown that phenolic compounds are metabolized in the body via conjugation mainly in the gastrointestinal epithelium (Powell *et al.,* 1974; Lundh, 1990). The rumen is probably the most important tissue in the gastrointestinal tract as regards detoxification of plant phenolic compounds (Lundh, 1990). The ingested food stays in the rumen for a relatively long period and these metabolites are catabolized mainly by the microorganisms in the rumen via demethylation and reduction. Other tissues such as the reticulum, omasum and small intestine also have a significant role in the detoxification process (Lundh, 1990). This combined conjugation activity of the various tissues of the gastrointestinal tract acts as a first line of defence before the 4-methylphenol enters the blood circulation. The conjugation reactions occurring in the liver have been shown to be very slow and account for only a very minor part of the overall metabolism of 4-methylphenol confirming the main detoxification role of the gastrointestinal tract (Lundh *et al.*, 1988). Thus higher fluxes of conjugated 4-methylphenol in the portal drainage of the PEG ewes resulted in an augmentation of the peripheral blood concentration of the metabolite.

Overall, these results establish that sulla CT reduced the net portal appearance and peripheral concentration of 4-methylphenol and it is likely that this was a consequence of reduced formation of that metabolite in the rumen due to lowered ruminal protein degradation in the CT-treated ewes.

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