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THE EFFECTS OF CONDENSED TANNINS IN SULLA (*HEDYSARUM CORONARIUM*) ON VALINE KINETICS IN THE OVINE MAMMARY GLAND

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SUMMARY

We tested the hypothesis that condensed tannins (CT) in Sulla (*Hedysarum coronarium*) increase the partitioning of essential amino acid (EAA) to the mammary gland in late lactating ewes. Lactating ewes (12) were surgically prepared with permanent indwelling arterio-venous catheters across the mammary gland, a cannula in the abomasum and a transonic flow probe fitted around the pudic artery at 1 week postpartum. All ewes were fed on fresh Sulla until the completion of the experiment. Two weeks after surgery (week 3 of lactation), half the ewes were orally drenched (4 times per day) with polyethylene glycol to remove the effects of the CT (PEG), whilst the remaining ewes (Control) received a drench of water according to a block design. At week 6 of lactation, a primed continuous infusion of [1-^{13}C]-valine into the abomasum was used to quantify the kinetics of valine across the mammary gland, using a 2- or 3-compartment model. Blood was obtained by consecutive 2-h continuous blood sampling (6-12 h from the start of infusion) from the mesenteric artery and caudal superficial epigastric vein, to measure the isotopic enrichment and concentration of valine in plasma. Mammary blood flow tended to decrease ($P<0.10$) with PEG, and this resulted in a reduction in the flow of valine to the mammary gland. The net flux of valine across the mammary gland was not affected ($P<0.10$). Transmembrane valine transport into and out of the mammary intracellular pool was higher ($P<0.05$) in the control group compared with the PEG group. Irreversible loss rate (ILR) of valine, calculated using a 2-compartment kinetic model, tended to be lower ($P=0.10$) in the PEG group while the ILR estimated from a 3-compartment model was not affected ($P>0.10$). The rate of valine released from protein degradation was not affected ($P>0.10$) by treatments. These results suggest that the CT in Sulla increased mammary blood flow, supplying more valine (and other EAA), for mammary protein synthesis and/or oxidation.

Keywords: mammary valine kinetics, compartment model, lactation, condensed tannins, sheep

INTRODUCTION

Moderate concentration of condensed tannins (CT) in the diet can reduce the degradation of dietary protein in the rumen (McNabb *et al.* 1996) and can increase the apparent absorption of essential amino acids (EAA), especially branched-chained AA such as valine from the small intestine (Waghorn *et al.* 1987; Bermingham *et al.* 2001). This may be responsible for the increased lactational performance observed in ruminants fed fresh CT-containing forages (Wang *et al.* 1996; Woodward *et al.* 1999). These results suggest that these legumes may have potential as improved forages for dairy ruminants.

Our hypothesis was that CT in Sulla increase the partitioning of EAA to the mammary gland in late lactating ewes. Polyethylene glycol preferentially binds and inactivates CT, and the effects of CT were elucidated by comparing animals fed Sulla and orally supplemented with polyethylene glycol (PEG; CT inactive) with animals that had not received PEG (Control; CT active).

MATERIALS AND METHODS

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

Experimental animals, design and diets

Three days post-lambing, 12 ewes were fed twice daily with Lucerne (*Medicago sativa*) pellets and chaff (600 g : 400 g) for a week. Water was available *ad libitum*. The ewes were milked twice daily. A week later, the ewes were prepared with an indwelling catheter in the mesenteric artery (Huntington and Reynolds 1987) and a permanent cannula in the abomasum under isofluorane anaesthesia. A transonic flow probe was also fitted around the pudic artery to measure mammary blood flow. Three

days prior to the start of the sampling periods, a temporary catheter was inserted into the caudal superficial epigastric (mammary) vein for blood sampling.

All ewes were then offered fresh Sulla (1500 g DM/d; 80 g CT/d) from 25 days after the start of lactation. Half the ewes were orally drenched (4 times per d) with polyethylene glycol (PEG; 160 g/d in water) to remove the effects of the CT, and the remaining ewes (Control) received a drench of water (day 0 of the experimental period). The treatments were applied according to a completely randomised block design, with the block representing the week the ewes underwent surgery (2 ewes of each treatment).

Infusion and blood sampling

On day 21 (day 46 of lactation), a 12 h continuous infusion of [1-^{13}C]-valine into the abomasum was used to quantify the kinetics of valine across the mammary gland. Blood was obtained by 3 consecutive 2-h continuous blood samples (from 6-12 h) from the mesenteric artery and mammary vein. Mammary blood flow was measured continuously during this period using a transonic flow probe surgically fitted around the pudic artery. Blood samples were centrifuged and the plasma removed and stored at -85ºC until assayed for valine concentration and isotopic enrichment.

Analytical measurements

To determine the concentration of valine in plasma, 0.5 mL of plasma was treated with 80 mM dithiothreitol and 3 mM norleucine (in 0.1% phenol), stored at -85°C, and the samples processed and analysed using HPLC separation (Bermingham *et al.* 2003). The plasma and mammary intracellular pool samples were processed and their isotopic enrichments determined by negative chemical ionisation gas chromatography mass spectrometry as described by Kulik *et al.* (1995).

Calculations

Plasma flow was calculated as {(100 - Hematocrit (%))/100} × blood flow. Isotopic enrichment (IE) of valine in the plasma and mammary intracellular pool (M) was calculated as described by Campbell (1974). Net flux of valine across the mammary gland was calculated by multiplying the plasma flow by the arterio-venous valine concentration difference. Inflow (Fin) and outflow (Fout) of valine was calculated by multiplying plasma valine concentration in the mesenteric artery (A) or mammary vein (V) by plasma flow. Mammary valine irreversible loss rate (ILR) was calculated according to 2-compartment (Equations 1 and 2; Harris *et al.* 1992) and 3-compartment (Equations 3, 4, 5 and 6; Biolo *et al.* 1994) models.

(1) $$^{13}\text{C-valine extraction} = \frac{[\text{VAL}]_A \times \text{VAL IE}_A - [\text{VAL}]_V \times \text{VAL IE}_V}{[\text{VAL}]_A \times \text{VAL IE}_A}$$

(2) Valine ILR (μmol/min) = ^{13}C-valine extraction × valine inflow (μmol/min)

The inward (Fma) and outward (Fvm) transmembrane transport of valine was calculated as follows:

(3) Fma (mmol/min) = Mammary plasma flow × ({(VAL IE$_M$ – VAL IE$_V$)/ (VAL IE$_A$-VAL IE$_M$) × [VAL]$_V$} + [VAL]$_A$)

(4) Fvm (mmol/min) = Mammary plasma flow × ({(VAL IE$_M$ – VAL IE$_V$)/ (VAL IE$_A$- VAL IE$_M$) × [VAL]$_V$} + [VAL]$_V$)

Bypass valine flow (Fva) was calculated by difference between valine arterial inflow (Fao) and inward transport (Fma). The use of valine to protein synthesis and oxidation (Fom) was calculated as follows:

(5) $$\text{Fom} = \frac{([\text{VAL}]_A \times \text{VAL IE}_A - [\text{VAL}]_V \times \text{VAL IE}_V) \times \text{mammary plasma flow}}{\text{VAL IE}_M}$$

The rate of intracellular valine appearance from endogenous sources (protein degradation; Fmo) in the intracellular pool was calculated as follows:

(6) Fmo = Inward transport × {(VAL IE$_A$/ VAL IE$_M$) – 1}.

Statistical analysis

Data were analysed using the GLM procedure of SAS using a block design. Significant statistical differences between treatments were declared at $P<0.05$, with a trend being $P<0.10$.

RESULTS

The CT in the diet did not affect feed intake (Control - 1365 *v.* PEG - 1347 (s.e.d. 55) g DM/d), but tended to increase plasma flow to the mammary gland (Control - 362 *v.* PEG - 260 (s.e.d. 40) mL/min). The concentration of valine in the mammary vein was higher in the control ewes (Table 1). Valine inflow to (Fao) and outflow from (Fov), the mammary gland were higher in the control ewes compared with the PEG ewes (Figure 1) resulting in similar net uptake of valine (Table 1).

Table 1. Least squared means and standard error of the difference (s.e.d.) of net valine flux and valine kinetics across the mammary gland of lactating ewes fed fresh Sulla either orally supplemented with polyethylene glycol (PEG; CT inactive; n= 6), or not supplemented with PEG (Control; CT active; n =6).

Parameters	Control	PEG	s.e.d.	P-value
Concentration and flux				
Arterial concentration (µmol/L)	180.8	155.8	15.2	0.24
Venous concentration (µmol/L)	114.8	86.1	11.8	0.10
Net flux (µmol/min)[A]	24.0	18.0	3.3	0.17
Isotopic enrichment (APE%)				
Mesenteric artery	3.3	7.1	1.2	0.03
Mammary vein	3.0	6.8	1.1	0.03
Mammary intracellular pool	2.0	3.8	0.7	0.07
Kinetics using a two compartment model				
^{13}C-valine arterial extraction (%)	0.43	0.47	0.04	0.57
Irreversible loss rate (µmol/min)	28.2	19.0	4.0	0.10

[A] Positive and negative values are indicative of net uptake and net release of the valine by mammary gland, respectively.

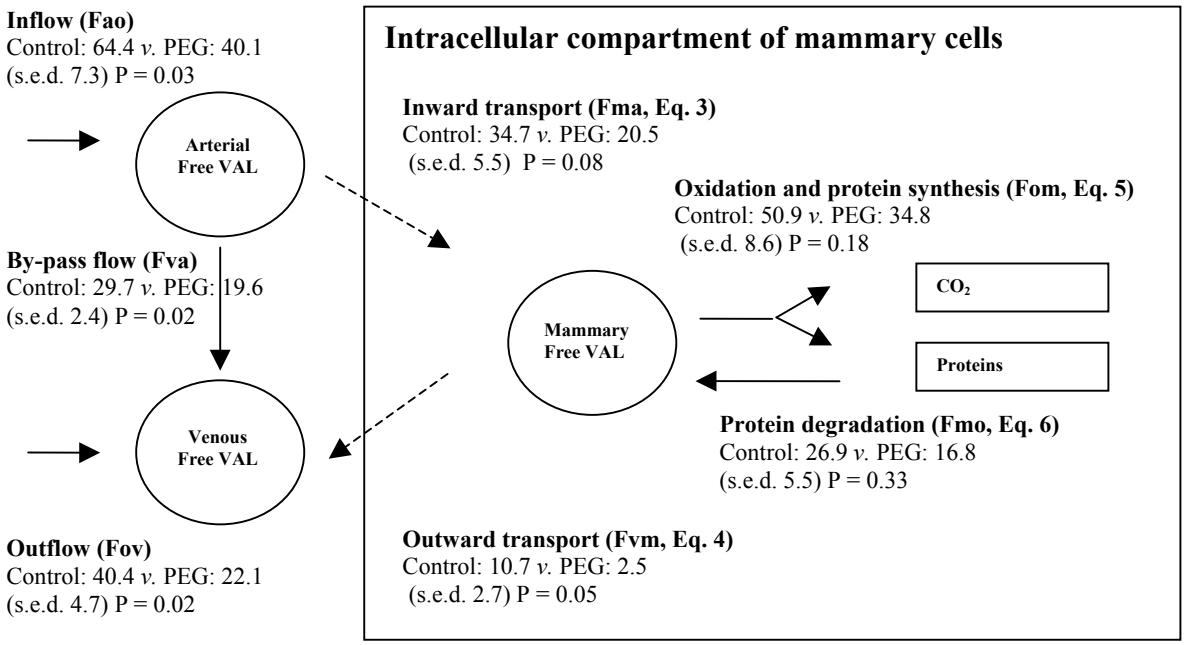

Figure 1. Least squared means and standard error of the difference (s.e.d.) of valine kinetics (µmol/min) across the mammary gland of lactating ewes fed fresh Sulla either orally supplemented with polyethylene glycol (PEG; CT inactive), or not supplemented with PEG (Control; CT active).

The isotopic enrichment of valine in arterial and venous plasma, and in the mammary intracellular pool (trend only), was lower in the control ewes (Table 1). The CT in Sulla did not affect the ^{13}C-valine extraction from the artery by the mammary gland (Table 1). Because the valine inflow (Fao) was higher in the control ewes, there was a tendency for the ILR of valine to increase (2-compartment model).

Inward transport of valine (Fma) to the mammary intracellular pool tended to be higher in control ewes (Figure 1). Similarly, the outward transport (Fvm) from this pool was increased. An increase of the by-pass flow of valine, i.e. from the artery to the mammary vein without entering the intracellular pool, was also observed (Figure 1). The CT did not affect the estimates of valine ILR (Fom) using a 3-compartment model. The CT in Sulla did not affect valine released from protein degradation (Fmo).

DISCUSSION

This study reports for the first time that CT in Sulla tended to increase mammary plasma flow. This could be the result of increased cardiac output, or shifts in the distribution of blood between tissues. Consequently, the inflow of valine (Fao) to the mammary gland increased in the control group. The CT in Sulla did not alter the percentage of valine extraction by the mammary gland. The ILR of valine across the mammary gland was higher in the control group, suggesting that protein synthesis (and/or

oxidation) was increased in this tissue. Our estimates of valine ILR are similar to those reported for the goat mammary gland (Bequette *et al*. 2002).

Increased arterial supply of valine (Fao) to the mammary gland in the CT group was also accompanied by increases in both the rate of inward and outward transport of valine. This indicates that the transport system for valine, mainly system L (Baumrucker 1985), was not saturated when the arterial supply of valine was increased to 181 μmol/L in the CT group and, therefore, the transport of valine was unlikely to limit mammary protein synthesis. This result is in accordance with *in vitro* measurements of the saturation of valine transport in the lactating porcine mammary gland that occurs at 640 μmol/L (Jackson *et al.* 2000). Our transport rates were within the range reported for the goat mammary gland (Bequette *et al.* 2000).

In conclusion, the improved protein secretion in milk reported in ruminants fed forages that contain CT appear, at least partly, to be attributable to an up-regulation of the mammary blood flow, thus providing more valine, and probably other AA, to the mammary gland.

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