Animal Production in Developing Countries
Occasional Publication No. 16 — British Society of Animal Production 1993
edited by M. Gill, E. Owen, G. E. Pollott and T. L. J. Lawrence

Urinary hippuric acid excretion as related to changes in cell wall digestibility due to variation in degradable protein supply

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Introduction

It has been established that dietary phenolic cinnamic acids are the main source of the hippuric acid excreted in ruminant urine. However, changes in hippuric acid output due to variation in the extent of cell wall digestion have so far received little attention.

Material and methods

To study the effect of rumen degradable protein (RDP) : metabolizable energy (ME) ratio on cell wall digestibility and its relationship to the urinary output of hippuric and benzoic acids three levels of ME (0·19 s.d. 0·003 (1), 0·35 s.d. 0·005 (2) and 0·49 s.d. 0·039 (3) MJ ME per kg live weight $(LW)^{0.75}$ per day) and five levels of RDP (1·69 s.d. 0·069 (A), 2·02 s.d. 0·026 (B), 2·80 s.d. 0·057 (C), 3·26 s.d. 0·056 (D) and 4·14 s.d. 0·048 (E) g RDP per kg $LW^{0.75}$ per day) were supplied to 45 mature Merino ewes in a 3 × 5 factorial design. RDP represents potential degradable protein determined by the *in sacco* procedure.

Forage : concentrate ratios in the experimental diets (dry matter basis) were 64·5 : 35·5 (1), 43·6 : 56·4 (2) and 29·5 : 70·5 (3). The forage was a mixture of barley straw and a low-quality meadow hay in proportions 59 : 41 (1), 50 : 50 (2) and 39 : 61 (3). Concentrates, offered as pellets, were formulated from sodium caseinate, gluten meal, tapioca meal and sunflower oil in different proportions to achieve the experimental range of RDP : ME ratios. Neutral-detergent fibre (NDF) intakes (g/kg $LW^{0.75}$ per day) were 12·6 s.e. 0·054, 13·93 s.e. 0·15 and 14·56 s.e. 0·17 to levels 1, 2 and 3 of ME intake, respectively. For acid-detergent fibre (ADF) the corresponding figures were 8·01 s.e. 0·04, 8·62 s.e. 0·07 and 8·92 s.e. 0·06. All diets included a mineral and vitamin supplement, equivalent to 0·5 g/kg LW per day, and were offered in two equal meals daily.

The experimental period lasted 23 days. During the first 10 days animals received diet 2-C and then the experimental diets for 13 days. Total collection of faeces and urine (by self-retaining urethral catheters) being carried out during the last 5 days.

Results and discussion

NDF and ADF digestibility and free benzoic and hippuric acid outputs are shown in Tables 1 and 2, respectively.

As was expected NDF and ADF digestibilities were influenced by RDP intake. This effect changed with level of ME intake. At level 1, RDP supply was negatively correlated with NDF ($r = -0{\cdot}67$, $P < 0{\cdot}01$) and ADF ($r = -0{\cdot}72$, $P < 0{\cdot}01$) digestibilities. However, at the highest level, these correlations were positive ($r = +0{\cdot}75$, $P < 0{\cdot}001$ and $r = +0{\cdot}72$, $P < 0{\cdot}001$, respectively).

When NDF digestibility (Y) data were analysed according to the following variance-covariance model: $\ln Yij = \mu + MEij + b \times \ln Xij + c \times Xij + eij$, derived from the gamma function, X being the RDP : ME ratio, it was observed that the effects of both covariables were statistically significant ($P < 0{\cdot}05$). Below a RDP : ME value of 7·5, a tendency ($b = +0{\cdot}3$) to increase NDF digestibility with RDP supply was observed but this tendency was negative ($c = -0{\cdot}04$) when RDP : ME ratios on the diet were above that value.

Although the variability between animals within groups in the amount of hippuric acid excreted in the

Table 1 *Digestibility data*

Rumen degradable protein ($g/kg\ LW^{0.75}$ per day)	NDF digestibility for ME inputs ($MJ/kg\ LW^{0.75}$ per day) of:				ADF digestibility for ME inputs ($MJ/kg\ LW^{0.75}$ per day) of:			
	0·19	0·35	0·49	Mean	0·19	0·35	0·49	Mean
1·69A	44·55	48·93	34·65	42·71[a]	46·19	47·98	27·84	40·67[a]
2·02B	48·05	46·80	39·00	44·62[a]	47·25	46·66	36·64	43·52[a]
2·80C	40·82	49·69	39·66	43·39[a]	42·16	49·92	37·50	43·19[a]
3·26D	39·62	43·61	41·00	41·41[a]	41·62	43·21	37·61	40·81[a]
4·14E	39·56	40·83	47·61	42·67[a]	40·44	38·07	44·95	42·15[a]
Mean	42·52[a]	45·97[b]	40·38[a]		43·54[ab]	45·17[b]	36·91[a]	
Residual s.d.		3·32				3·50		

[a,b] Means in the same row or column with different superscript letter were significantly different ($P < 0·05$).

Table 2 *Acid excretion data*

Rumen degradable protein ($g/kg\ LW^{0.75}$ per day)	Benzoic acid excretion ($mg/kg\ LW^{0.75}$ per day) for ME inputs ($MJ/kg\ W^{0.75}$ per day) of:				Hippuric acid excretion ($mg/kg\ LW^{0.75}$ per day) for ME inputs ($MJ/kg\ LW^{0.75}$ per day) of:			
	0·19	0·35	0·49	Mean	0·19	0·35	0·49	Mean
1·69A	5·08	2·23	5·95	4·42[a]	102·32	102·04	30·22	78·19[a]
2·02B	11·42	9·49	11·24	10·72[ab]	155·10	105·84	56·55	105·83[a]
2·80C	19·75	24·92	14·99	19·89[bc]	120·86	136·90	58·97	105·58[a]
3·26D	19·43	22·18	32·40	24·67[cd]	128·24	128·97	73·06	110·09[a]
4·14E	15·50	29·86	48·51	31·29[d]	81·03	91·84	115·23	96·04[a]
Mean	14·24[a]	17·74[a]	22·62[a]		117·51[b]	113·12[b]	66·81[a]	
Residual s.d.		11·82				27·22		

[a,b,c,d] Means in the same row or column with different superscript letters were significantly different ($P < 0·05$).

urine was very high (CV = 0·2752), hippuric output was correlated with NDF digestibility (r = +0·78, $P < 0·001$) and ADF digestibility (r = +0·78, $P < 0·001$), provided RDP supply was equal to or lower than the estimated nitrogen requirements of the rumen micro-organisms (Agricultural Research Council, 1980).

Urinary free benzoic acid was not affected by changes in NDF or ADF digestibilities but was correlated with RDP intake (r = +0·64, $P < 0·001$). This coefficient was higher (r = +0·74) when the results of treatments with excess RDP were not taken into account.

On the other hand, benzoic acid conjugation rate, which ranged from 90·04% ± 3·67 (level A) to 67·63% ± 4·13 (level E), also appears to be related to RDP supply (r = –0·59, $P < 0·001$).

Conclusions

When there is a deficit of RDP, cell wall digestibility is affected probably due to a limited ability of animals to recycle nitrogen. Surplus of RDP also appears to reduce cell wall digestibility and it could be related with the cellulolytic bacterial growth. The experimental results indicate that renal excretion of hippuric acid may reflect, to some extent, changes in cell wall digestibility due to deficiencies in RDP of the diet.

Acknowledgement

PROYECTO CICYT (GAN 88-0071).

Reference

Agricultural Research Council. 1980. *The nutrient requirements of farm livestock. No. 2. Ruminants.* Commonwealth Agricultural Bureaux, Slough.