Effects of diet, level of intake, sodium bicarbonate and monensin on urinary allantoin excretion in sheep

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The present experiment was designed to study the effects of factors likely to alter microbial purine yield from the rumen on urinary excretion of allantoin-nitrogen (UAN). Sixteen mature Clun Forest–Welsh crossbred wethers were used in a $2 \times 2 \times 2 \times 2$ factorial design to investigate the effects of (1) level of intake, (2) wheat:nutritionally improved straw (NIS) ratio, (3) sodium bicarbonate inclusion and (4) monensin inclusion on diet digestibilities, fractional outflow rates of solids and liquids from the rumen and urinary allantoin excretion. Each treatment occurred in each of two experimental periods. The treatments were designed to influence microbial purine yield via changes in rumen outflow rate and microbial maintenance coefficient. Increasing the proportion of NIS and increasing feeding level decreased digestibility and increased the fractional outflow rate of solids. Increasing the level of intake increased the fractional outflow rate of liquids. Urinary allantoin excretion (/kg live weight$^{0.75}$ per d) was significantly increased by an increased proportion of wheat in the diet and increased level of intake, and significantly reduced by $NaHCO_3$. There was a significant interaction effect such that increasing level of intake did not increase UAN with the high-NIS diet, despite an increased fractional outflow of solids from the rumen, in contrast to the increase observed with the high-wheat diet. Taken together with other observations it is suggested that high sodium concentrations in the diet reduce the efficiency of microbial synthesis, probably by increasing the energy cost of maintaining osmolarity. Monensin had no overall effect on UAN but there were significant interactions between monensin and dietary Na; the inhibitory effect of monensin on UAN was eliminated or reversed in the presence of added $NaHCO_3$. This is consistent with theories that monensin increases the net influx of hydrogen ions into microbial cells and that this influx can be reduced by increased extracellular sodium ions.

Urinary allantoin: Rumen: Microbial maintenance coefficient: Sheep

Nucleic acids have been used widely to indicate the microbial content of digesta taken from the abomasum or duodenum (McAllan & Smith, 1969). This approach was improved by the measurement of their component purine bases (Schelling *et al.* 1982), which are more robust and readily measured (Zinn & Owens, 1986). To estimate the yield of microbial material from the rumen there was still a need for invasive techniques to assess the quantity of digesta flowing through the lower gut. Rys *et al.* (1975) proposed the use of urinary purine derivatives as indices of microbial yield from the rumen. Chen *et al.* (1990), using animals sustained by intragastric infusion, demonstrated a direct relationship between the supply of exogenous purine to the lower gut of sheep and their recovery as purine derivatives (hypoxanthine, xanthine, allantoin and uric acid) in urine. When purine derivative excretion exceeded 0·6 mmol/kg live weight $(W)^{0.75}$ per d there was no net

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endogenous contribution, i.e. endogenous losses were entirely replaced by utilization of exogenous purines. Above this level there was a linear relationship between the supply of exogenous purines and urinary excretion of purine derivatives with an apparent molar recovery of 0·84. Below this level an increasingly large net endogenous contribution was evident, making the relationship non-linear, though nonetheless positive.

In the absence of other limitations, microbial energetic efficiency (E), the relationship between fermented energy supply and microbial yield from the rumen, is influenced by (1) the ratio microbial outflow (yield):total synthesis and (2) the maintenance energy costs of the population. Microbial maintenance energy costs include those of motility, lysis, uncoupling, inefficient phosphorylation, secretion and active transport (Harmeyer, 1986). Estimates of the microbial maintenance coefficient range from 0·75 to 3·50 mmol ATP/g microbial dry matter (DM) per h (Harrison & McAllan, 1980).

Dewhurst *et al.* (1987) demonstrated changes in urinary allantoin excretion, relative to energy supply, with changes in the fractional outflow rate of solids from the rumen (K_s). This was interpreted as changes in E caused by increases in the ratio microbial yield:total synthesis. The present paper investigates further the effects on urinary allantoin excretion of factors designed to influence both fractional outflow rates from the rumen and the maintenance energy cost of the population. Level of intake, diet digestibility, proportion of nutritionally improved straw (NIS) and sodium bicarbonate treatments were imposed to manipulate fractional outflow rates from the rumen, whilst monensin was expected to influence the microbial maintenance coefficient.

MATERIALS AND METHODS

Animals and their management

Sixteen mature Clun Forest–Welsh crossbred wethers, weighing from 44 to 94 kg (mean 67 (SD 16) kg) were used. The sheep were housed in digestibility crates for 14 d preliminary periods and for 10 d periods of total collection of faeces into canvas bags and urine over 100 ml 2 M-sulphuric acid. Faeces and urine were removed daily and stored at 4° before bulking and storage at −20° at the end of the collection period. Sheep were weighed and given the appropriate dose of anthelmintic at the start of each experimental period. Diets were fed in two equal portions at 09.00 and 16.00 hours and sheep had continual access to drinking water.

Treatments and design

Sixteen sheep were used in a $2 \times 2 \times 2 \times 2$ factorially-designed experiment to investigate four sources of variation in apparent digestibilities, fractional outflow rates and urinary allantoin excretion. The sixteen treatments were replicated in two experimental periods and consisted of all combinations of the following factors (a) 8·2 or 15·6 g feed DM/kg W per d, (b) wheat: NIS values of 0·3:0·7 and 0·7:0·3, (c) 0 or 50 g $NaHCO_3$/kg and (d) 0 or 22 mg monensin/kg. During the 6 weeks between periods the sheep were grazed at pasture. The level of intake treatment was imposed according to the W of the sheep at the start of each experimental period. $NaHCO_3$ was weighed separately and sprinkled onto each day's feed allowance. The other treatments were imposed by formulating four diets (Table 1) which were passed through a 6 mm screen and fed in a loose-meal form. The mineral–vitamin premix supplied (mg/kg air-dry feed) vitamin A 4·05, cholecalciferol 0·068, vitamin E 6·7, manganese 120, zinc 220, iron 20, cobalt 0·5, iodine 5, selenium 0·2, and molybdenum 1. Since the level of intake, monensin and $NaHCO_3$ treatments took account of sheep W, the sheep were paired by W and randomly allocated to either the high-NIS or high-wheat diets; within these groups sheep were randomly allocated to the eight treatments.

Table 1. *Diet formulations (g/kg air dry feed)*

Diet no...	1	2	3	4
Wheat	283	663	283	663
Nutritionally improved straw*	659	284	659	284
Dicalcium phosphate	30	30	30	30
Urea	20	15	20	15
Mineral–vitamin premix	8	8	8	8
Monensin premix (100 g/kg)	0	0	0·22	0·22

* 32 Viton-NIS; Unitrition International Ltd, Basingstoke, Hants.

Fractional outflow rates from the rumen

Fractional outflow rates from the rumen for the solid- and liquid-phases of rumen digesta (K_s and K_l respectively) were determined using chromium-mordanted straw neutral-detergent fibre (NDF) and CoEDTA respectively (Uden *et al.* 1980). Sheep were given a pulse oral dose of 12 g labelled fibre and 8 g CoEDTA in approximately 250 ml water immediately before their morning feed. A daily subsample of the total collection of faeces was taken before the morning feeding on each of the following 10 d and dried at 100° in preparation for marker analysis.

Analytical methods

Samples were analysed, as appropriate, for proximate constituents (Association of Official Agricultural Chemists, 1965), NDF (Robertson & Van Soest, 1977), acid-detergent fibre and acid-detergent lignin (Van Soest & Wine, 1967). Cr and Co were determined by atomic absorption spectrophotometry and Na by flame photometry, both following a wet-digestion procedure (Milner & Whiteside, 1981). Acid-detergent-insoluble nitrogen (ADIN) was determined by performing a Kjeldahl N determination on an acid-detergent fibre residue. Allantoin in urine was determined using an autoanalyser method based on the colorimetric reaction of Borchers (1977). The autoanalyser was found to be extremely advantageous for this method since the manual method is highly sensitive to the timing of manipulations. Calibration curves were non-linear but could be linearized ($r > 0{\cdot}99$) by performing a double-reciprocal plot. The addition of allantoin to a sample of urine with a low (0·184 (SE 0·005) g/l) allantoin content showed recoveries of 0·98–1·04. Earlier work demonstrated the stability of allantoin in urine preserved with the level of sulphuric acid used in the present trial and kept at 4° or −20° (Dewhurst, 1989).

Statistics

Results were analysed using the residual maximum likelihood (REML) directive of GENSTAT 5 (Lawes Agricultural Trust, 1987). The model looked at period effects and the main and two-factor interaction effects of level of intake, diet, $NaHCO_3$ and monensin. Sheep were treated as a random variable. The most conservative estimates of residual degrees of freedom were used in arriving at levels of significance. Linear regression was undertaken using the MINITAB statistical package (Minitab Inc., 1980). Fitting of fractional outflow rates from the rumen involved selection, by eye, of points on the log descending phase of the marker excretion curve, followed by linear regression *v.* time. The slope of this line was taken to be the fractional outflow rate from the rumen. The number of daily samples included in these regressions varied with fractional outflow rate, though usually samples from days 2 to 6 were included.

Table 2. *Chemical analysis of the wheat, nutritionally improved straw (NIS) and diets used in the experiment (g/kg dry matter)*

			Diets			
	Wheat	NIS	1	2	3	4
Ash	32	115	100	68	99	80
CP	134	44	139	144	119	146
EE	10	14	12	12	12	10
CF	27	371	244	134	248	113
NFE	797	460	505	642	522	651
NDF	108	623	444	272	456	273
ADF	31	480	315	174	323	134
ADL	18	74	54	37	51	33
ADIN	0·6	2·5	1·9	1·4	2·0	1·2
Sodium			20	13	23	10

CP, crude protein (nitrogen × 6·25); EE, diethyl ether extract; CF, crude fibre; NFE, N-free extractives; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ADL, acid-detergent lignin; ADIN, acid-detergent-insoluble N.

RESULTS

The chemical analyses of the diets used are given in Table 2. The diets were fed at DM contents ranging from 869 to 898 g/kg. A few day's urine collections were lost owing to carboys running over or being knocked over and results were corrected for this. In the second period two sheep refused to eat a considerable portion of their allocated feed; their values were treated as missing values in subsequent analyses since level of feeding was a major source of variation being tested. Other feed refusals were negligible. Urine volumes tended to be very large (mean 3·3 (SD 1·5) l/d, range 1·3–7·1 including approximately 0·5 l washings/d), probably owing to a combination of warm weather and high intakes of Na. Urinary allantoin-N (UAN) excretion ranged from 120 to 1140 (mean 466 (SD 242)) mg/animal per d. This represented a range of 4·7–42·0 (mean 19·8 (SD 9·0)) mg/kg $W^{0·75}$ per d and 0·27 to 1·37 (mean 0·79 (SD 0·24)) g/kg digestible organic matter (DOM).

Table 3 summarizes the main effects of diet, level of intake and the inclusion of $NaHCO_3$ and monensin on digestibility of DM, NDF and neutral-detergent solubles (NDS), K_s and K_l, and daily UAN excretion.

Increasing the ratio wheat:NIS from 0·3:0·7 to 0·7:0·3 had the expected effects on digestibility, significantly increasing the digestibility of DM and NDS but decreasing the digestibility of NDF. The 0·7 wheat:0·3 NIS diet also produced a significantly greater excretion of UAN whether expressed as mg/kg $W^{0·75}$ per d or g/kg DOM.

Increasing the level of intake significantly reduced the apparent digestibility of DM and NDF, but not NDS which is more rapidly fermented, and increased both K_s and K_l. UAN increased significantly when expressed as mg/kg $W^{0·75}$ per d, but remained unchanged when expressed as g/kg DOM. Table 4 shows the significant interaction between diet type and level of intake on UAN. UAN was increased with increasing level of intake when feeding with the high-wheat diet, whilst there was no significant level of intake effect with the high-NIS diet.

Addition of $NaHCO_3$ significantly increased the digestibility of DM, NDF and NDS; K_s and K_l were unaffected but UAN was significantly reduced. Effects of monensin on all variables were small and, considered in isolation, reveal no consistent pattern. There were, however, significant interactions between monensin and $NaHCO_3$ (Table 4). Monensin

Table 3. *Effects of diet*† (*d*), *level of intake* (*l*), *$NaHCO_3$* (*b*) *and monensin* (*m*) *on the apparent digestibility of dry matter* (*DMD*), *neutral-detergent fibre* (*NDFD*) *and solubles* (*NDSD*), *fractional outflow rates from the rumen of solids* (K_s) *and liquid* (K_l) *and urinary allantoin N excretion* (*UAN*)‡

(Values are means with their standard error of difference for each pair of values given in parentheses)

	Wheat:NIS		Intake level		$NaHCO_3$ (g/kg)		Monensin (mg/kg)		Statistical significance of effects			
	0·3:0·7	0·7:0·3	Low	High	0	50	0	22	d	l	b	m
DMD	0·67	0·74	0·73	0·68	0·69	0·71	0·71	0·70	***	***	*	NS
	(0·005)		(0·005)		(0·005)		(0·004)					
NDFD	0·63	0·57	0·65	0·56	0·57	0·63	0·60	0·61	*	**	*	NS
	(0·019)		(0·019)		(0·019)		(0·018)					
NDSD	0·69	0·81	0·75	0·74	0·74	0·76	0·76	0·74	***	NS	*	*
	(0·005)		(0·005)		(0·006)		(0·004)					
K_s (%/h)	3·27	1·79	1·92	3·14	2·15	2·91	2·95	2·11	*	*	NS	NS
	(0·392)		(0·422)		(0·422)		(0·372)					
K_l (%/h)	3·90	3·54	2·77	4·66	3·62	3·82	4·32	3·12	NS	**	NS	NS
	(0·521)		(0·521)		(0·521)		(0·521)					
UAN: mg/kg $W^{0·75}$ per d	15·6	23·7	14·4	24·9	22·4	16·9	20·6	18·6	***	***	**	NS
	(1·295)		(1·310)		(1·355)		(1·216)					
g/kg DOM	0·68	0·87	0·77	0·77	0·89	0·66	0·79	0·76	*	NS	**	NS
	(0·059)		(0·060)		(0·062)		(0·055)					

NIS, nutritionally improved straw; NS, not significant; W, live weight; DOM, digestible organic matter.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Tables 1 and 2.
‡ For details of procedures, see pp. 346–347

Table 4. *Interaction effects on the apparent digestibility of neutral-detergent fibre* (*NDFD*) *and urinary allantoin N excretion* (*UAN; mg/kg digestible organic matter* (*DOM*) *and mg/kg $W^{0·75}$ per d*)*

(1) Diet × level of intake interaction						Statistical significance of interaction
Wheat:NIS...	0·3:0·7		0·7:0·3			
Level of intake...	Low	High	Low	High	SED	*P*
UAN: mg/kg $W^{0·75}$ per d	13·3[a]	17·8[a]	15·4[a]	32·0[b]	2·16	< 0·001
UAN g/kg DOM	0·76[a,b]	0·60[a]	0·78[a,b]	0·95[b]	0·099	< 0·01
(2) Monensin × sodium interaction						Statistical significance of interaction
Sodium bicarbonate (g/kg)...	0		50			
Monensin (mg/kg)...	0	22	0	22	SED	*P*
NDFD	0·55[a]	0·60[a,b]	0·65[b]	0·61[b]	0·027	< 0·05
UAN: mg/kg $W^{0·75}$ per d	25·1[a]	19·6[b]	16·2[b]	17·6[b]	1·89	< 0·05
g/kg DOM	0·97[a]	0·82[a,b]	0·61[b]	0·70[b]	0·086	< 0·05

[a,b] Values in the same row with different superscript letters were significantly different at the level indicated.
NIS, nutritionally improved straw; SED, standard error of difference.
* For details of procedures, see pp. 346–347

increased the digestibility of NDF and decreased UAN (g/kg DOM and mg/kg $W^{0.75}$ per d) in the absence of $NaHCO_3$, whilst these effects were negligible or reversed in the presence of $NaHCO_3$.

DISCUSSION

Apparent digestibilities

Treatment effects on apparent digestibilities were broadly as expected. Wheat NDF (bran) was significantly ($P < 0.05$) less digestible than NDF from NIS despite the significantly ($P < 0.05$) lower K_s acting on the high-wheat diet (0·018 *v.* 0·033 /h). There was no overall effect of monensin on apparent digestibility. There was, however, a significant interaction effect with $NaHCO_3$ on the apparent digestibility of NDF. In the absence of $NaHCO_3$ monensin increased the apparent digestibility of NDF whilst the effect was reversed in the presence of $NaHCO_3$. The effect of monensin on fibre digestion (in the absence of $NaHCO_3$) is the opposite of that noted in the review of Demeyer *et al.* (1986). It is, however, consistent with the findings of Allen & Harrison (1979) and may be explained by the same mechanism of increased rumen retention times (a non-significant trend in the current work).

Fractional outflow rates from the rumen

Significant increases in K_s were associated with increases in the level of intake and reductions in digestibility at a given level of intake. This is in agreement with the regression analysis of literature values undertaken by Evans (1981*b*). Increasing level of intake increased K_l, in agreement with Evans (1981*a*), though in the present experiment there was no evidence of a depression in K_l with increasing energy density of the diet. K_l was on average 0·5 greater than K_s (0·036 *v.* 0·024 /h). The reduction in K_l with monensin was consistent with the majority of experiments reviewed by Bergen & Bates (1984), though the absence of an effect of $NaHCO_3$ was more surprising, this being an initial objective of this treatment.

Urinary allantoin excretion

For several dietary treatments the level of urinary allantoin excretion was below 0·6 mmol/kg $W^{0.75}$ per d. In these circumstances the findings of Chen *et al.* (1990) would predict a significant net endogenous contribution to allantoin excretion. Thus, the reduction in microbial yield caused by some of the treatments is likely to be greater than is apparent from considering UAN.

UAN increased with increasing proportion of wheat in the diet and with increasing level of intake, and was reduced by the inclusion of $NaHCO_3$ in the diet. The effect of level of intake was proportional to the increase in DOM intake, so that increasing level of intake had no effect on urinary allantoin excretion/kg DOM. Since it is likely (Chen *et al.* 1990) that there was a net endogenous allantoin excretion at the low level of intake this observation is not inconsistent with an increasing E with increasing level of intake. There was no significant relationship between urinary allantoin excretion (expressed per kg DOM) and fractional outflow rates from the rumen, despite K_s ranging from 0·76 to 6·77/h and K_l from 1·66 to 6·79/h. This may have resulted from a net endogenous allantoin contribution at low outflow rates or the greater effect of other sources of variation in microbial yields. $NaHCO_3$ did not increase fractional outflow rates from the rumen as had been expected so that effects on UAN must have been mediated by some other means. The effects of Na supplementation in the present experiment are confounded somewhat by the high concentration of Na in the NIS used. It is, however, possible to examine the effect of Na in both NIS and $NaHCO_3$ by comparing effects of diets with and without $NaHCO_3$ supplementation. Table 5 presents results from the present study and an earlier experiment

Table 5. *Comparison of the effects of changing from 0·3:0·7 to 0·7:0·3 nutritionally improved straw (NIS):wheat, with different sodium concentrations in the straw samples**

(Values are means and standard deviations for observations based on the results of the present experiment and that presented by Dewhurst *et al.* (1987))

		Urinary allantoin-N (g/kg DOM)						K_s (%/h)					
		0·3:0·7			0·7:0·3			0·3:0·7			0·7:0·3		
Na content of NIS (g/kg DM)	$NaHCO_3$ (g/kg)	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
11·0	0	3	1·04	0·48	3	1·79	0·08	3	2·25	0·69	3	2·83	0·32
23·4	0	3	1·41	0·34	3	1·46	0·32	3	1·54	0·51	3	2·28	0·42
29·5	0	4	0·71	0·15	4	0·65	0·29	4	1·99	0·96	4	2·51	1·67
29·5	50	4	1·03	0·26	3	0·78	0·15	4	2·81	0·27	3	4·01	0·92

DM, dry matter; DOM, digestible organic matter; K_s, fractional outflow rate from the rumen of solids.
* For details of procedure, see pp. 346 and 351.

(Dewhurst *et al.* 1987) which used identical procedures to those used in the present experiment and a single level of feed intake, irrespective of W, which averaged 12·6 g feed DM/kg W per d. This comparison shows that increasing the NIS proportion of NIS–wheat diets from 0·3 to 0·7 increased UAN/kg DOM when Na concentration in NIS was 11·0 g/kg DM, presumably via the increase in K_s. As Na concentration in NIS increased, this increase first disappeared and then reversed despite an increase in K_s in all cases. This strongly suggests that increasing Na supply at high levels reduces E; increased energy costs of osmoregulation are a likely mechanism (Mackie & Therion, 1984). This effect also explains the level of intake × diet interaction effect on UAN (Table 4), with the effect of increasing level of intake suppressed by the effect of a very large supply of Na with the high-NIS diet.

Although monensin had no overall effect on urinary allantoin excretion, there was a significant interaction with Na (Table 4). The reduction in UAN noted when monensin was added in the absence of $NaHCO_3$ was reversed in the presence of Na. Both recent models of monensin action (Bergen & Bates, 1984; Russell, 1987) envisage monensin increasing the net influx of hydrogen ions into microbial cells, i.e. reducing the transmembrane proton motive force, and thereby reducing microbial yield and efficiency. The interaction between monensin and Na noted here can be explained by both models; in the former model increased extracellular sodium ions reduces the influx of H^+ whilst in the latter it increases the efflux of H^+ without expenditure of ATP.

An interaction between Na and monensin in their effects on a number of measures of the growth and efficiency of both host animals and rumen microbes in vitro has been demonstrated frequently (Perski *et al.* 1982; Rogers & Davis, 1982; Rumpler *et al.* 1986; Schwingel *et al.* 1989). The effect of urinary allantoin excretion (E) noted in the present experiment is consistent with the in vitro results of Mackie & Therion (1984) in which the negative effect of monensin on bacterial yield (per unit glucose used) was reduced by increasing extracellular Na.

Reduced E on the addition of monensin (without high levels of Na) have been observed by Poos *et al.* (1979) and Isichei & Bergen (1980) in vivo and by Van Nevel & Demeyer (1977) using a constant outflow rate in vitro system. Hoeller *et al.* (1985) used both ^{15}N and thiamin net synthesis to demonstrate reduced microbial yields in dairy cows given monensin at 33 mg/kg. E was reduced from 1·47 to 1·12 g microbial N (MN)/MJ

metabolizable energy (ME) (^{15}N values). For wether sheep, Allen & Harrison (1979) estimated a 0·14 decline in E with inclusion of monensin. Laurent & Vignon (1979) and Laurent *et al.* (1980) demonstrated a reduced urinary allantoin excretion when monensin was included in diets, though the effect may have partly resulted from reduced levels of intake.

The demonstration of this interaction effect in vivo is an illustration of the strength of the urinary allantoin excretion technique in permitting the identification of the effects of a number of the many interacting factors that influence microbial yield from the rumen. It is likely that a number of other important factors contribute to the large variation in E noted in the literature (Agricultural Research Council, 1984). Assuming that energy (ATP) is the limiting factor for microbial yield, these will operate by one of the two routes demonstrated in the present paper and the paper of Dewhurst *et al.* (1987), these being fractional outflow rates from the rumen and the microbial maintenance coefficient.

The Agricultural Research Council (1984) preferred value of 1·34 g MN/MJ ME is clearly only applicable to ruminants fed in a similar way to the majority of animals used to derive this mean value, i.e. standard diets given at close to the maintenance level of intake. The model of Webster (1987) attempted to take account of plane of nutrition and diet digestibility effects on E, but ignored factors influencing the microbial maintenance coefficient. These experiments reveal that the non-invasive method for estimation of microbial protein yield from urinary purine derivative excretion offers a powerful approach to the analysis of the complex factors affecting outflow and maintenance energy costs of the microbial population in the rumen which can, after further study, be incorporated into improved models.

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