Glycosyl ureides in ruminant nutrition

3. In vivo studies on the metabolism of glycosyl ureides and corresponding mixtures of their free component molecules

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1. Steers and sheep were given basal diets of barley and straw (1:1, w/w), usually containing urea, which for certain experimental periods were supplemented with pure glucosyl urea (GU), pure lactosyl urea (LU) or a product prepared from whey concentrate (EW) which contained 65–80% of the lactose in the form of LU.

2. On the morning of an experiment ureide (or EW) was omitted from the feed and a dose of either ureide (or EW) or equivalent amounts of free lactose and urea (L+U) was added to the rumen within 30 min of feeding, together in some experiments with polyethylene glycol (PEG) as a fluid-phase marker. Samples of rumen contents, and in some experiments abomasal contents, were taken at intervals for up to 8 h.

3. For both steers and sheep given GU and LU for the first time (unadapted animals) there was little or no accumulation of ammonia in the rumen or cleavage of the sugar-urea bond. Galactose was, however, slowly liberated from LU.

4. For steers and sheep which had been given GU, LU or EW for approximately 7–10 d or more (adapted animals) some accumulation of ammonia occurred after adding GU or LU to the rumen, but for LU it occurred less rapidly and to a lower peak concentration than when L+U was added. In adapted animals cleavage of the sugar-urea bond in LU was virtually complete in 2–4 h. Degradation of the components of L+U was virtually complete within 1 h.

5. Recovery at the abomasum of ureide (present either as GU or LU) estimated from ureide:PEG values, appeared to be complete in experiments with unadapted sheep given a dose of EW. In adapted sheep only very small amounts of ureide in an EW dose (on average 6%) entered the abomasum undegraded. Amounts lost in this way appeared to be positively correlated with the rate of fluid turnover in the rumen.

Many attempts have been made to improve on urea as a source of dietary nitrogen for ruminants (Fonnesbeck *et al.* 1975; National Academy of Sciences, 1976). These have generally taken the form of attempting to reduce the rate at which ammonia is produced, or ensuring the presence of a suitable energy source, or both (Bartley & Deyoe, 1977). Reduction of ammonia concentration, which can be achieved by these expedients means less risk of overt or marginal toxicity (Lewis & Buttery, 1973; Buttery, 1977; Barej & Harmeyer, 1979). Although it is generally believed that matching the rate of energy and N release also favours efficient microbial growth, it may be argued that moderately-high ammonia concentrations are unlikely to depress greatly the efficiency of utilization of N from a non-protein-N (NPN) source, unless rumen pH is exceptionally high (Smith, 1979). There is, however, limited evidence that microbial protein synthesis is more efficient when the energy source is starch, and particularly cooked starch (Borgida *et al.* 1976; Durand *et al.* 1976), rather than the more-rapidly-metabolized soluble sugars (Al Attar *et al.* 1976; Oldham *et al.* 1977).

In vitro studies showed that lactosyl urea (LU), formed by heating lactose and urea at low pH and high temperature (Merry *et al.* 1982*a*) was metabolized more slowly in rumen contents from sheep and steers than either of its free component molecules (Merry *et al.* 1982*b*), even after the donor animal had been adapted by protracted feeding of LU. It appears probable therefore that the use of this compound rather than its components in ruminant diets would not only reduce the risk of ammonia toxicity but may also lead to energy becoming available at a more suitable rate; indeed at a rate similar to that observed

Table 1. *Daily intake and composition (kg dry matter) of major components of diets for steers and sheep, together with nitrogen (g) and metabolizable energy (MJ) contents*

	Daily amounts given in the diets								
	Steers				Sheep				
Diet...	A	A+GU	B	B+EW1*	C	C+LU	C+EW2*	D	D+EW3*
Barley–straw (1:1, w/w) cubes	3·41	3·18	—	—	0·81	0·61	0·56	—	—
Alkali-treated straw cubes	—	—	1·72	1·72	—	—	—	0·49	0·49
Rolled malting barley	—	—	1·72	1·17	—	—	—	0·49	0·31
Urea	—	—	0·025	0·041	0·006	—	—	0·007	—
Glucosyl urea (pure)	—	0·20	—	—	—	—	—	—	—
Lactosyl urea (pure)	—	—	—	—	—	0·160	—	—	—
Ewoplus	—	—	—	0·492	—	—	0·175	—	0·164
Supplying:									
Lactosyl urea	—	—	—	0·154	—	—	0·058	—	0·073
Lactose	—	—	—	0·068	—	—	0·029	—	0·087
Urea	—	—	—	0·017	—	—	0·008	—	0·016
Metabolizable energy	34·4	34·7	34·7	30·9	8·2	8·3	8·2	9·8	9·9
N	51·2	57·7	49·8	48·5	16·7	22·1	21·6	14·1	17·7

* For details of EW1, EW2 and EW3, see Table 2.

Table 2. *Composition (g/kg dry matter) of Ewoplus (EW) preparations**

	Preparation no.		
	EW1	EW2	EW3
Nitrogen	57·7	62·8	59·8
Lactosyl urea	313·4	333·9	446·3
Glucosyl urea	4·8	6·6	7·2
Galactosyl urea	3·2	13·3	11·3
Free lactose	137·7	164·0	119·1
Free urea	34·3	34·2	20·6

* See below.

with cooked starch as the main energy component (Merry *et al.* 1982*b*). Thus it seems possible that mixtures containing LU may have a potential for improving the efficiency of use of NPN. However, it must be borne in mind that under in vivo conditions a reduction in NPN degradation rate may lead to appreciable loss of the compound by flow out of the rumen before it has been metabolized. This has been shown to occur for other slowly-degraded NPN sources, e.g. isobutylidene diurea (Kaufmann & Hagemeister, 1973) and Biuret (Tiwari *et al.* 1973), but in vitro studies (Merry *et al.* 1982*b*) did not suggest that resistance of ureides to degradation was such that extensive loss in this way would occur. The work reported here was carried out to study the potential nutritional value of glycosyl ureides, and particularly LU, when they were given to sheep and young steers. Part of this work has been briefly reported elsewhere (Merry *et al.* 1979; Merry *et al.* 1981).

EXPERIMENTAL

Animals and diets

Four Friesian steers (C1-4) weighing 140–160 kg were equipped with rumen cannulas (Smith & McAllan, 1970) and housed in individual pens with expanded-metal floors. Four Suffolk × Scottish Blackface mature wether sheep (S1-4) weighing 50–70 kg were similarly housed and provided with rumen cannulas (Smith & McAllan, 1970). Sheep S2-4 were also given 'T'-piece cannulas (Williams & Smith, 1974) in the fundic region of the abomasum. Periods of at least 3 weeks after the operation were allowed before experiments were begun. Composition and amounts of ingredients in diets for all animals are shown in Table 1. At the start of an experiment steers were given diets of barley and straw, with or without urea (diets A and B) providing energy intake for a growth rate of 0·5 kg/d, and sheep were given similar diets (C and D) with energy intakes for maintenance. On days of sample collections during experimental periods, parts of the diets were sometimes replaced in terms of energy value by supplements containing glycosyl ureides, either in the pure form or in mixtures prepared from whey given directly into the rumen. Preparations from whey containing LU were produced commercially by Ewos AB, Södertälje, Sweden, and will subsequently be referred to as Ewoplus (EW). Different samples were employed (EW1, EW2, EW3) with the compositions shown in Table 2.

Feeds were offered in equal amounts at 09.00 and 17.00 hours and water generally allowed *ad lib.* Water was withheld during the period of sampling when experiments were done. Any refusals of the basal diet during experimental periods were added directly to the rumen via the rumen cannula.

Sampling and treatment of digesta

Rumen content samples were obtained by suction using a perforated tube (i.d. 20 mm) connected to a vacuum pump. Digesta samples from the abomasum were obtained by unstoppering the cannulas and waiting for gushes of digesta. Approximately 100 ml of digesta were generally obtained in less than 5 min.

Samples collected in experiments were treated as follows. Rumen digesta (25 g) for ammonia analysis were mixed with 5 ml 2 M-hydrochloric acid strained through four layers of surgical gauze and then centrifuged at 35000 ***g*** for 15 min. In some experiments a further sample of rumen digesta (50 g) was strained and the strained fluid divided into two portions to one of which mercuric chloride solution (50 g/l) was added to give a final concentration of 5 mg/ml. Supernatant fractions (35000 ***g***) were also prepared from these samples, that treated with $HgCl_2$ were used for analysis of ureides, urea or lactose and the untreated one for polyethylene glycol (PEG). Supernatant fractions of untreated abomasal samples were also prepared by centrifuging at 35000 ***g***. All samples were stored at -20 °.

Treatments

Expt 1. A steer (C1) was given diet A and then transferred to a diet containing glucosyl urea (GU) for a period of 30 d. This diet is referred to in Table 1 as A + GU. On different days during this period samples of rumen contents were taken before, and at intervals after, the morning feed for measurements of ammonia concentration.

Expt 2. A sheep (S1) was given diet C (Table 1) for a period of 21 d followed by a further period of 35 d in which diet C + LU (Table 1) was given. On different days during the feeding of these diets prefeed samples of rumen contents were taken followed by further samples at intervals after feeding. Two similar experiments were carried out on days 33 and 35 of adaptation to LU but in which equivalent amounts of lactose and urea (L + U) were substituted for LU as the supplement.

Expt 3. Three steers (C2-4) were adapted to diet B (Table 1) for a period of 21 d followed by a further period of 26 d in which diet B + EW1 (Table 1) was given. On particular days during this period experiments were done as follows. For the morning feed on the day of an experiment diet B was given. When this had been consumed (usually in less than 30 min) a supplement of either EW1 (Table 2) or L + U, together with PEG (22·5 g) in 250 ml water were added rapidly to the rumen. Samples of rumen contents were collected at intervals after the time of addition of the supplement without further feeding.

Expt 4. A sheep (S2) received diet C (Table 1) for 21 d, and then an experimental diet (C + EW2, Table 1) for 42 d. On appropriate days during this period, experiments were performed as follows. On the morning of the day of experiments samples of rumen and abomasal contents were taken and diet C offered. When this had been consumed (usually approximately 30 min), a dose of EW2 together with PEG (10 g) in 250 ml water were added to the rumen. Rumen and abomasal contents were collected at intervals after addition of the dose.

Expt 5. Two sheep (S3 and 4) received diet D (Table 1) for 21 d and then diet D + EW3 (Table 1) for a further period of 26 d. Finally, one sheep was returned to diet D for a further 7 d. On the mornings of particular days during these periods, experiments were done in which diet D was given followed by supplements of EW3 or L + U accompanied by PEG (20 g). Procedures were the same as for Expt 4.

Analytical

The method of Smith (1959) was followed to estimate PEG in digesta samples, except that 20 min instead of 1 h were allowed for turbidity to develop. Disappearance curves of PEG

Table 3. *Expt 1. Changes in rumen ammonia concentration (mmol/l) after giving a diet of barley, straw and 100 g pure glucosyl urea to a steer (S1) during a period of adaptation to glucosyl urea*

Period after feeding (h)	Period of feeding glucosyl urea (d)									
	0	1	3	4	9	10	12	17	26	29
0·0	1·01	0·72	0·72	0·98	1·06	3·00	0·58	6·38	2·60	3·32
0·5	1·29	1·42	1·73	1·22	4·39	5·04	8·88	5·90	7·10	10·56
1·0	0·86	0·72	0·60	0·41	8·83	10·62	12·92	9·46	9·60	14·30
2·0	0·42	0·55	0·60	0·24	12·50	11·26	17·38	11·90	10·96	11·16
4·0	0·18	0·19	0·19	0·14	0·53	9·26	0·48	3·88	0·40	1·20

from the rumen were used to calculate fluid turnover rate (Maeng & Baldwin, 1976). GU and LU were determined by a chromatographic procedure described by Merry *et al.* (1982*a*) and lactose by the method of Smith & McAllan (1971). Ammonia ($NH_3 + NH_4^+$) was determined by the procedure of Merry (1980) and urea as described by Merry *et al.* (1982*a*).

Statistical analysis

Statistical significance was determined by paired *t* tests by the method described by Snedecor & Cochran (1972).

RESULTS

Ammonia concentration

Expt 1. Adaptation to glucosyl urea. On several days before and during the period in which diet A + GU (Table 1) supplying 100 g GU/feed was given, ammonia concentration in the rumen was examined before and at various times after a morning feed. Before addition of GU to the feed, ammonia concentration in rumen fluid remained below 2 mM over the period examined (Table 3). Similar results were obtained for samples taken up to 4 d after starting to add GU. After 9 d of feeding GU, however, there was appreciable accumulation of ammonia in the rumen, indicating that degradation of GU (i.e. adaptation) had occurred. Maximum values of approximately 12 mM were observed 2 h after feeding. Patterns and levels similar to the 9 d observations were observed over the rest of the period examined.

Expt 2. Adaptation to lactosyl urea. At intervals during a period when a sheep was being offered a diet containing LU (80 g/feed) ammonia accumulation in the rumen was examined before and at intervals after a feed containing LU, or sometimes an equivalent amount of L + U was given. Results for these experiments are shown in Fig. 1. After the diet had included LU for 1 d, ammonia concentrations of not more than approximately 10 mmol/l were attained on the following day. After 5 d on the same diet including LU a maximum ammonia concentration after 6 h of 18 mmol/l was observed. After 8 d, corresponding ammonia concentrations were even greater; the establishment of a fairly similar pattern and extent of ammonia accumulation for collections made after this time indicated that complete adaptation to LU had occurred after 8 d. Corresponding values for ammonia concentrations after addition of L + U to the rumen of the LU-adapted animal showed a completely different trend (Fig. 1). Ammonia was produced fairly rapidly with a peak value of nearly 60 mmol/l at 2 h after feeding and decreased markedly after this time.

Expt 3. Metabolism of lactosyl urea and lactose and urea supplied in a whey preparation: steers. Rumen ammonia concentrations were examined after doses of EW1 (Table 2)

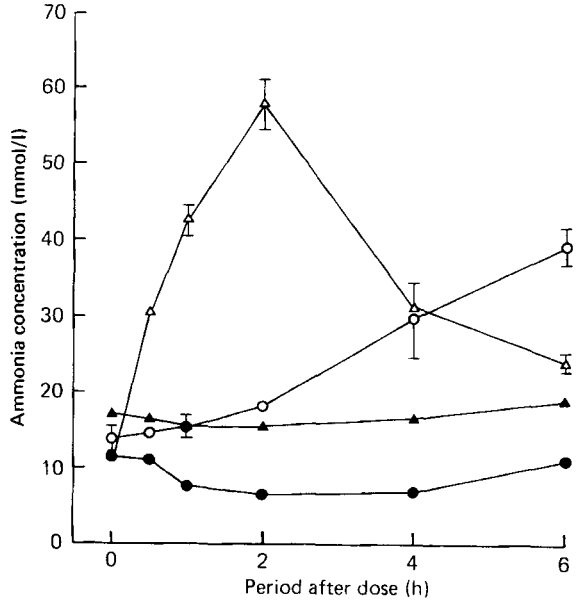

Fig. 1. Expt 2. Changes in rumen ammonia concentrations (mmol/l) in sheep S1 after giving doses of 80 g lactosyl urea (LU) into the rumen. Results are shown for experiments done on days 1 (●) and 5 (▲), and as mean values with their standard errors represented by vertical bars (when these exceeded 1 mmol/l) for days 13, 19, 26, 28, 32 and 34 (○) after changing from diet C to diet C+LU (for details of diets, see Table 1). Corresponding values are also given for times when doses of L+U replaced LU on days 33 and 35 (△).

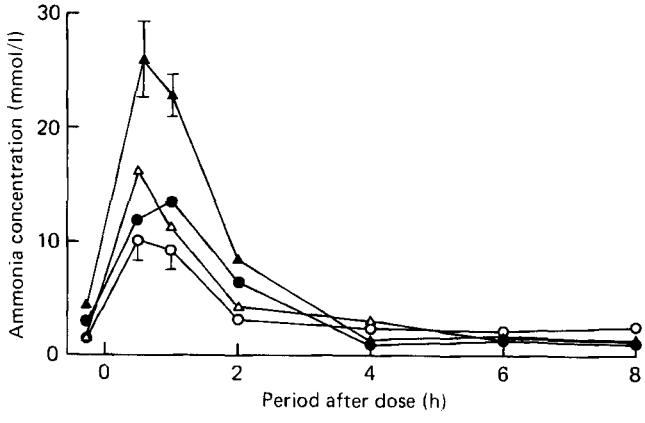

Fig. 2. Expt 3. Changes in rumen ammonia concentrations (mmol/l) in steers C2-4 after giving doses of 246 g Ewoplus (EW1; see Table 2) containing 77 g lactosyl urea (LU) into the rumen. Results are mean values for three observations with their standard errors represented by vertical bars (when these exceeded 1 mmol/l) for experiments done on days 1 (○) and 21 (●) after changing from diet B to B+EW1 (for details of diets, see Table 1). Corresponding values are also given for times when doses of L+U replaced EW1 in experiments done 7 d before (△) and 26 d after (▲) changing diets.

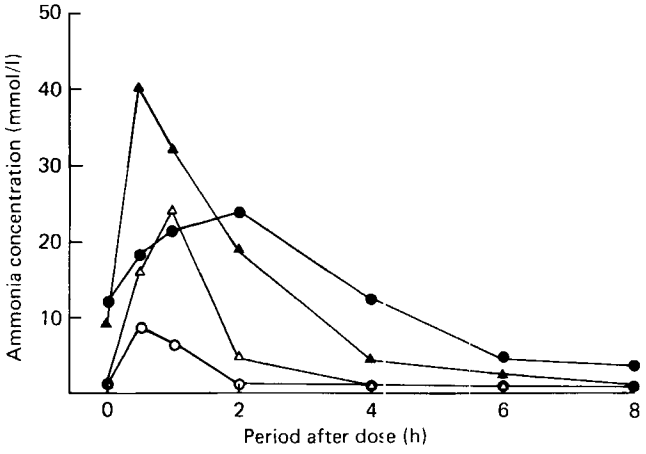

Fig. 3. Expt 5. Changes in rumen ammonia concentrations (mmol/l) in sheep S3 after giving doses of 82 g Ewoplus (EW3; see Table 2) containing 36·5 g lactosyl urea (LU) into the rumen. Results are shown for experiments done on days 1 (○) and 21 (●) (one observation on each day) after changing from diet C to C+EW3 (for details of diets, see Table 1). Corresponding values are also given for times when doses of L+U replaced EW3 in experiments done 7 d before (△) and 26 d after (▲) changing diets.

(supplying 77 g LU/feed together with small amounts of free lactose and urea), or amounts of L+U equivalent to the totals in the EW preparation (103 g lactose and 20.5 g urea) had been added to the rumens of three steers. The animals were either unadapted (day 1) or had been adapted for at least 3 weeks to the diet containing EW.

In unadapted animals given EW1 (1st day of feeding LU) it might be expected, according to earlier in vitro findings (Merry *et al.* 1982*b*), that as no bound urea was likely to be degraded, ammonia concentrations in the rumen would be fairly low. However, because the EW samples contained free urea and whey proteins as well as LU, accumulation of ammonia occurred, even before adaptation to LU was evident (Fig. 2). The amount of unreacted urea was fairly high in preparation EW1 (Table 2) with a bound urea: free urea value of 1·4. After 21 d, when adaptation had occurred, amounts of ammonia accumulating in the rumen after addition of the EW1 supplement increased, but were considerably lower and more evenly spread than when equivalent amounts of L+U were given (Fig. 2). Peak concentrations (approximately 13 mmol/l) 1 h after EW1 was given was later and lower than when L+U was given (26 mmol/l after 30 min).

Expt 5. Metabolism of lactosyl urea and lactose and urea supplied in a whey preparation: sheep. Patterns of ammonia accumulation in the rumens of adapted and unadapted sheep given doses of LU (36·5 g) in the form of EW3 (Table 2) or equivalent amounts of L+U (43·5 g lactose and 8 g urea) were similar to those obtained in Expt 3 with steers. An example of results for a single animal are shown in Fig. 3. Closely similar results were obtained with another animal. For both unadapted sheep with L+U, peak rumen ammonia concentrations (approximately 20 mmol/l) were attained between 30 min and 1 h after feeding, and there was then a rapid decline. After adaptation ammonia accumulated more rapidly when L+U was given, with a sharp peak at 40 mmol/l after 30 min. The presence of unreacted urea and whey proteins in the EW3 supplement again caused elevation of rumen ammonia concentration even in the unadapted sheep although not to such a marked extent as in Expt 3. The sample of Ewoplus (EW3) used in this series of experiments was one in which urea conversion into LU was greater, with consequently less free urea (bound urea: free urea value

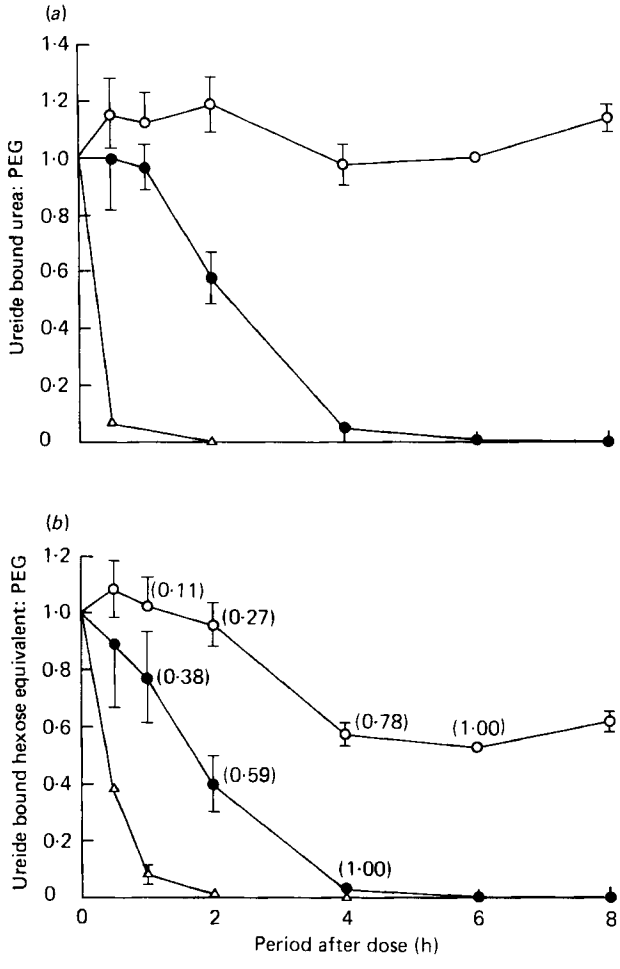

Fig. 4. Expt 3. Changes in (*a*) ureide-bound urea:polyethylene glycol (PEG), (*b*) ureide-bound hexose equivalent:PEG (mean values for three observations with their standard errors represented by vertical bars, when these exceeded 0·02) in steers C2-4 after giving doses of 246 g Ewoplus (EW1; for details, see Table 2) containing 77 g lactosyl urea (LU) and 22·5 g PEG into the rumen. Results are shown for experiments done on days 1 (○) and 21 (●) after changing from diet B to B+EW1 (Table 1). Corresponding values are also given for times when doses of L+U replaced EW1 on day 26 (△). Ureide-bound urea and hexose equivalent:PEG value in the dose was 1·0. Values in parentheses are proportions of ureide-bound hexose equivalent present as glucose.

of 3·4 in the product). When this was given ammonia concentrations were much lower and more evenly spread than when equivalent amounts of L+U were given. Similar values were observed for ammonia concentrations after addition of LU to the rumen on days 7 and 14 of adaptation to LU, indicating that adaptation was complete after 7d.

Disappearance of ureide and free sugar and urea in the reticulo-rumen

Expt 3. During the experiments described previously where ammonia concentrations were being examined (see p. 309), concurrent studies were made of the metabolism of LU in EW1 compared to the metabolism of amounts of pure L+U equivalent to those in the product.

Analyses of rumen contents showed that amounts of urea and hexose equivalents (free

or bound) changed relative to those of PEG as shown in Fig. 4(*a*,*b*). Bound urea and hexose equivalents were calculated from values for concentrations of LU or GU or both in the rumen and were expressed as proportions of the dose given. In unadapted animals (first day of feeding EW) a slow liberation of galactose occurred and GU accumulated in the rumen contents, until after 6 h all bound hexose equivalents (approximately 50% of total bound hexose equivalents added) were in the form of GU (Fig. 4(*b*)). As glucose represents 50% of the hexose equivalents in LU and only GU was present in the rumen contents after 6 h it appeared that GU was not further degraded. This was also indicated by estimates of bound urea remaining in the rumens of unadapted animals (Fig. 4(*a*)). After 23 d of adaptation liberation of galactose occurred at a faster rate and there was degradation of GU; bound urea and hexose equivalents both had virtually disappeared from the rumen after 4 h. Mean differences between unadapted and adapted animals in the proportions of bound urea and hexose equivalents remaining in the rumen 4–8 h after addition of EW1 were significant ($P \leqslant 0{\cdot}01$). Degradation rate was, however, slower than that seen when free urea and lactose were added after adaptation. In this instance urea had almost completely disappeared after 30 min and only 8% of the lactose remained after 1 h (Fig. 4(*a*,*b*)). Amounts of urea and hexose equivalents that remained in the rumens of adapted animals 4 h after feeding were significantly greater ($P \leqslant 0{\cdot}05$) when EW1 was given than when the corresponding free components were given.

Apart from adaptation to LU there was also an adaptive response to the feeding of free L+U. The mean ($\pm$SE) amount of free urea remaining in the rumens of unadapted animals 1 h after addition of L+U was $0{\cdot}05 \pm 0{\cdot}03$, whilst complete disappearance of urea had occurred in this time with 26 d adapted animals. Corresponding values for lactose remaining were $0{\cdot}69 \pm 0{\cdot}11$ and $0{\cdot}08 \pm 0{\cdot}03$ for unadapted and adapted animals respectively. The mean difference between unadapted and adapted animals in amounts of free lactose remaining in the rumen 1 h after feeding L+U was significant ($P \leqslant 0{\cdot}05$) although no significant differences in rates of degradation of urea were apparent.

Expt 4. Disappearance of LU was followed up to 8 h after the addition of doses of EW2 supplement (containing 29 g LU) into the rumen of a sheep. Degradation of LU after the animal had been adapted to the compound followed a closely similar pattern to that seen with the adapted steers in Expt 3 (Fig. 4). After 7 d adaptation 95% of bound urea and 86% of bound sugar had disappeared by 4 h after feeding. Corresponding disappearance values after 14, 21 and 42 d adaptation were 40, 89 and 80% of bound urea respectively, and 63, 94 and 96% of bound sugar respectively.

Expt 5. Results in Fig. 5 are for mean amounts of bound urea remaining in the rumens of two sheep after the addition of EW3. Observations were made at intervals during a period of adaptation to LU (see p. 308). Also included in Fig. 5 are corresponding mean values for steers obtained in Expt 3. These results indicate that adaptation to LU was virtually complete after approximately 7 d in both sheep and steers. Withdrawal of EW3 from the ration of one of the adapted sheep led to a rapid loss of LU-degradative activity. Analysis of samples taken from this animal after 2 and 5 d of deadaptation indicated that very little degradation of bound urea had occurred (Fig. 5).

Residual flow of ureide at the abomasum

Expt 4. This was a preliminary experiment in which a sheep was given EW2 for a period of 42 d. Details of experimental procedure have been described previously (p. 308). On particular days during the period of adaptation to EW2, a dose of the supplement was added directly to the rumen, followed by collection of abomasal digesta samples for the next 8 h. Analyses of samples collected on the first day of feeding EW2 showed bound urea:PEG values at all times which were closely similar to those of these compounds in the feeds. Bound

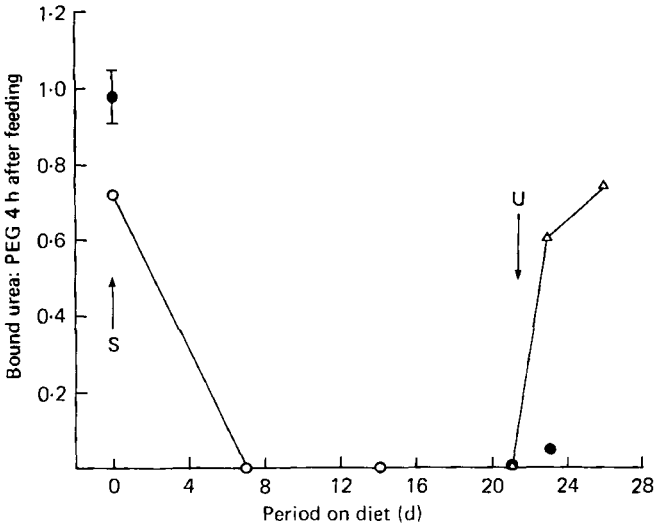

Fig. 5. Expts 3 and 5. Ureide-bound urea:polyethylene glycol (PEG) values in rumen samples taken 4 h after adding doses of Ewoplus (EW; for details, see Table 2), containing approximately 5 g lactosyl urea (LU)/kg rumen contents, to the rumens of steers and sheep (mean values for three and two observations for steers and sheep respectively with their standard errors represented by vertical bars, when these exceeded 0·02). Times of changing from diet B to B+EW1 (steers C2-4, Table 1) (●) and from C to C+EW3 (sheep S3 and 4, Table 1) (○) are shown (S). Supplemented diets continued for the remainder of the experiment except that for sheep S4 (△), the diet was changed from C+EW3 to C at time (U).

Table 4. *Expt 5. Cumulative recoveries of ureide (mol/mol dose) at the abomasum and proportions as glucosyl urea (in parentheses) at different times after giving 36·5 g lactosyl urea at Ewoplus (EW3)* into the rumens of adapted and unadapted sheep (S3 and 4)*

(Values for adapted sheep were means for samples collected 7, 14 and 21 d after starting to give EW3 with the feed)

	Period after adding dose (h)	Sheep S3		Sheep S4	
Unadapted	1	0·079	(0·16)	0·057	(0·18)
	3	0·216	(0·54)	0·161	(0·46)
	6	0·366	(0·81)	0·286	(0·79)
		Sheep S3		Sheep S4	
		Mean	SE	Mean	SE
Adapted	1	0·028	0·003	0·037	0·008
	3	0·045	0·002	0·067	0·015
	6	0·047	0·004	0·074	0·015

* For details, see Table 2.

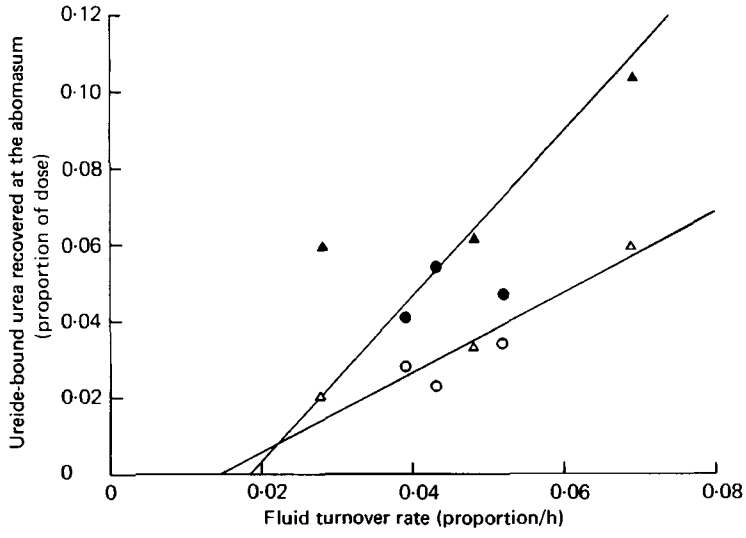

Fig. 6. Expt 5. Relationship between rumen fluid turnover rates and proportions of ureide recovered at the abomasa of sheep S3 (△, ▲) and S4 (○, ●) at 1 h (△, ○) and 6 h (▲, ●) after adding a dose of Ewoplus (EW3; for details, see Table 2) containing 36·5 g lactosyl urea (LU) to the rumen. Animals were fully-adapted to LU and values were estimated as described below.

urea:PEG values in similar samples taken from the same sheep after 7, 14 and 21 d of adaptation were lower than in the diet even at 1 h after addition of the EW supplement, and very little flow of bound urea occurred at all between 3 and 6 h.

Expt 5. A similar experiment was carried out in which two sheep were adapted to EW3 supplement. For details, see p. 308. In Expt 4 no bound urea was detected at the abomasum of the adapted animal after 6 h, therefore, sample collections in this experiment were made only for periods up to this time.

Cumulative flows of ureide to the abomasum were calculated for both unadapted and adapted sheep and the estimated mean values are shown in Table 4. Values at particular times were calculated in the following way. The proportion of the dose of PEG leaving the rumen up to that time was estimated from the change in concentration of PEG in the rumen. The ureide:PEG value in digesta which had entered the abomasum up to that time was then used to estimate the amount of ureide that had entered the abomasum.

Results for adapted sheep are means for collections made on days 7, 14 and 21 after starting to add EW3 to the feed assuming that adaptation was complete after 7 d (see Fig. 5). In unadapted sheep ureide showed a fairly linear flow to the abomasum; approximately 30% of the amount consumed had passed to the abomasum after 6 h, of which nearly 80% was in the form of GU. After adaptation only approximately 6% of the ureide flowed out of the rumen and most of this was lost in the first 1–3 h after addition of EW to the rumen. In the previous experiments fluid turnover rates in the rumen were estimated from rates of decrease of PEG concentration in the rumen. A plot was made of values obtained for fluid turnover rate and estimates of total flow of ureide recovered at the abomasum after 1 and 6 h; the results are shown in Fig. 6. There appeared to be a linear relationship between the two measurements, suggesting that losses of undegraded material from the rumen may be positively linked with fluid turnover rate.

DISCUSSION

Findings of the present experiments were in accord with the in vivo observations of Milligan *et al.* (1972) for GU, and confirmed the in vitro findings of Merry *et al.* (1982*b*) in showing that the sugar-urea bonds in GU and LU were virtually unattacked in the rumens of unadapted sheep or steers. The observations that rates of breakdown of both the energy and N fractions increased during adaptation to LU and appeared to be maximal after approximately 7 d, were in broad agreement with previous conclusions from in vitro observations (Merry *et al.* 1982*b*). Breakdown rates of LU even in the adapted animals remained, however, markedly slower than those of a mixture of free L+U.

Previous reports (Milligan *et al.* 1972; Merry *et al.* 1982*b*) and the present in vivo studies demonstrate that ammonia concentrations are considerably lower when LU (or GU) is given as a supplement, than when equivalent amounts of lactose and urea (or glucose and urea) are given, and imply that the ureides would be potentially less toxic than the free compounds. This has also been shown in practice (Widell, 1979). Even the whey products (EW; which contain whey proteins and some unreacted urea) showed lowered and more-evenly-spread ammonia concentrations than comparable amounts of free lactose and urea when added to the rumens of adapted animals (Figs. 2 and 3). EW preparations also have low toxicity (Widell, 1979). Clear-cut clinical signs of toxicity can be avoided with urea feeding provided that care is taken to avoid excessive intakes, and the main value of a product such as LU is in reducing the chance of accidental overdoses. It should, however, be remembered that subclinical toxicity due to moderately-high rumen ammonia concentrations can also occur and may have deleterious effects on animal production. For example, such concentrations may interfere with rumen motility (Juhász & Szegedi, 1980) and absorption of an excessive amount of ammonia has been shown to lead to interference with carbohydrate metabolism (Buttery, 1977; Barej & Harmeyer, 1979). Such effects, which may be partly responsible for the poor use of urea which has often been attributed to inadequate amino acid supply (Chalupa *et al.* 1970), are less likely to occur with LU than with free urea in the diet.

Some workers have put forward the view that a high concentration of ammonia in the rumen after NPN feeding is wasteful because bacterial requirements are met by an ammonia concentration as low as 3·5 mM, and excess ammonia can be lost by absorption through the rumen wall (Satter & Roffler, 1975). A recent report by Meggison *et al.* (1979) provides some support for this idea. However, other studies have indicated that maximum organic matter digestion occurs with much higher ammonia concentrations of up to 14 mM (Mehrez *et al.* 1977). There is also abundant evidence that provided rumen pH is not above approximately 7, fairly high concentrations of ammonia may remain in the rumen for many hours and provide a reserve of ammonia for subsequent bacterial growth, and are not necessarily wasted (Smith, 1979). Potential improvement in the value of an NPN source by reducing the rate at which it liberates ammonia may therefore be limited. On the other hand encouragement of steady, vigorous bacterial growth by supplying energy in a controlled manner seems likely to lead to an improvement in the efficiency of microbial protein synthesis (Smith, 1979). Such an approach has been made in the present work by chemically combining lactose and urea and thereby modifying the rate of fermentation of lactose. Indeed, it was shown by Merry *et al.* (1982*b*) that despite the fact that galactose is released from LU and fermented relatively rapidly, the overall rate of sugar fermentation was markedly slower than that of free lactose, and also supported a similar pattern of bacterial growth to that supported by fermentation of cooked starch.

With some NPN sources over-protection from degradation reduces the value of the

compound and it was shown for example that too slow degradation led to excessive amounts of isobutylidenediurea and Biuret leaving the rumen (Kaufmann & Hagemeister, 1973; Tiwari *et al.* 1973) in adapted cows and sheep. This was undoubtedly so in the unadapted animals in our experiments as ureide was lost quantitatively with the digesta flow to the abomasum. As GU and LU are stable to acid at body temperature (R. J. Merry, unpublished results) they are unlikely to be digested in the abomasum and small gut, so that loss of part, at least, of the sugar and all the urea entering the duodenum up to the ileum is likely. It is not clear what would happen to the compound in the large gut.

Under the conditions examined in the present studies, losses of undegraded ureide in digesta flowing out of the rumen in adapted sheep were small (generally 6%). However, as rumen fluid turnover rates were on average approximately 0·05/h (at the lower end of the published physiological range (Harrison & McAllan, 1980)) and appeared to be positively correlated with amounts of ureide lost, a change in the basal diet to one that induced a higher turnover rate could lead to more extensive losses. In experiments with three adapted steers where fluid turnover rates were greater (range 0·09–0·17/h in eight experiments), calculations based on comparison of the degradation rate of bound urea with fluid turnover rate indicated that losses of bound urea would have been slightly higher than those obtained in sheep. Calculations were based on measured disappearance rates of PEG (fluid turnover rate) and bound urea from rumen contents. Mean ($\pm$SE) rate-constants (proportions/h) for the former (K_o) and latter ($K_o + K_d$), where K_d = degradation rate of bound urea were, $0{\cdot}11 \pm 0{\cdot}01$ and $1{\cdot}02 \pm 0{\cdot}09$ respectively. Thus losses of bound urea with the digesta flow from the rumen ($K_o/[K_o + K_d]$) would have been $10 \pm 1\%$. It seems possible that high producing animals such as cows on high intakes of diets which induce faster flow rates (up to 0·2/h; Hungate, 1966) might suffer more extensive losses of bound urea and sugar with the digesta flow, although LU degradation rate in the rumen may vary markedly under different conditions of feeding.

The finding that deadaptation occurred within a matter of days in one experiment in the present studies confirmed earlier in vitro findings (Merry *et al.* 1982*b*) and underlines the importance of maintaining a continuous dietary supply of NPN supplements such as LU. No information was obtained in the present work on the effect of altering LU level or other components of the diet on LU utilization, but by analogy with studies on Biuret it seems likely that such differences would have an effect (Gilchrist *et al.* 1968; Schröder & Gilchrist, 1969; Wyatt *et al.* 1975). Studies are needed to investigate these possibilities with ureides. For the conditions examined it appears that 7–10 d would be adequate for complete adaptation to occur to the metabolism of a ureide. Nevertheless, a longer period may be more suitable for adjustment of the microbial population to addition to the diet of EW, as metabolism of lactose and urea as well as ureide should be considered after a diet change. Indeed, the significant response in terms of increased rate of lactose degradation in the rumens of steers during adaptation to lactose, supports observations made in in vitro experiments using rumen contents from the same animals (Merry *et al.* 1982*b*). An increase was shown in urea degradation rate in Expt 3 after the urea level in the diet was increased although this was not significant. Increase in urea degradation rate with urea supplementation was not consistently shown in the experiments of Borhami *et al.* (1981) who followed rates of ureolysis and ammonia production in the rumens of different species of ruminants during adaptation to urea. In these experiments , however, the authors did not look at changes in the factors during the first month of adaptation and thus may have missed the changes indicated in our experiments.

It may be concluded that LU and preparations rich in this compound have certain properties likely to be advantageous in ruminant feeding. Possible advantages include low

rates of ammonia production, a steady, controlled release of energy, and high palatability (Merry, 1980). Nevertheless, care must be taken (as with other slow-release NPN sources) that excessive loss of undegraded N with the flow of digesta out of the rumen does not occur.

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