Utilization of endogenous and dietary urea in the large intestine of the mature horse

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The dynamics of N metabolism in mature horses were investigated when they were fed on a low-N diet or the same diet supplemented with sufficient urea or soyabean meal to meet their theoretical N requirements. There were no differences in DM, organic matter or neutral-detergent-fibre digestibilities for the three diets. N digestibilities and digestible-N intakes were similar for the urea- and soyabean-supplemented diets and very low for the low-N diet. For all three diets plasma urea was degraded in the digestive tract to NH_3 which was utilized by the bacterial population as a N source. NH_3 was absorbed from the large intestine into the blood and converted to urea. NH_3 was also incorporated into plasma proteins. The horses fed on the low-N diet degraded a greater proportion of endogenous urea in the digestive tract than did horses fed on the urea- or soyabean-supplemented diets. However, the horses fed on the urea diet had the highest degradation rate of urea. The quantity of urea degraded in the digestive tract of horses fed on the urea-supplemented and the low-N diets could not compensate for a lack of dietary crude protein in these diets. The horses were in a negative N balance when fed on the low-N and urea-supplemented diets and a positive N balance when fed on the soyabean-supplemented diet. Dietary urea supplementation did not benefit the horses.

Urea: Nitrogen metabolism: Horse

There is some debate concerning the horse's ability to utilize urea. At present it is not known if dietary urea supplementation has a place in the horse industry, particularly in the case of mature horses grazing poor quality pastures. It has been shown that endogenous urea is degraded in the large intestine and the proportion of endogenous urea degraded increases with low-protein diets (Houpt & Houpt, 1971; Prior *et al.* 1974). These results indicate that horses utilize some endogenous urea and therefore it is possible that supplementary urea may be of nutritional benefit.

The aim of the present experiment was to investigate the utilization of urea in the large intestine of the mature horse and to provide further information on the dynamics of N metabolism in horses fed on low- and adequate-protein diets.

METHODS

Animals

Three adult geldings, two Standardbreds and one Arabian (average weight 400 kg), aged between 5 and 7 years were each surgically fitted with two polyvinyl chloride (PVC) cannulas which had an outside diameter of 15 mm and an internal diameter of 10 mm. One

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Table 1. *Composition of diets (g/kg DM) fed during each experimental period*

Ingredient	Diet		
	LN	LNU	LNS
Barley straw	762·7	747·6	672·8
Molasses	204·9	200·5	182·2
Urea	0	20·3	0
Soyabean meal	0	0	116·3
Vegetable oil	19·7	19·2	17·4
Iodized salt	4·2	4·1	3·8
Dicalcium phosphate	7·1	6·9	6·3
Vitamin–mineral mix*	1·4	1·4	1·2
Nitrogen	4·4	13·7	13·3

LN, low nitrogen; LNU, low nitrogen + urea; LNS, low nitrogen + soyabean meal.

* Each kg of mix contained (g): retinol acetate 4·5, cholecalciferol 0·8, riboflavin 4, cyanocobalamin 0·011, α-tocopherol 10, Ca 2, Cu 1·25; Fe 6, Mn 4, Zn 5, NaCl 30, Co 0·025, I_2 0·1, Mg 0·5.

cannula was placed in the right ventral colon (just past the caeco-colic junction) and the other in the right dorsal colon (approximately 100 mm before the small colon) using a method similar to that described by Simmons & Ford (1988).

Diets and experimental design

At 2 months after the surgery the horses were individually stabled and fed on one of three diets in a 3 × 3 Latin square design. Within each of the three experimental periods there were three 5 d collection periods. The diets consisted of a low-N diet (diet LN), a low-N diet supplemented with adequate urea to meet the theoretical N requirements (National Research Council (NRC), 1978) of each horse (diet LNU), and a low-N diet supplemented with adequate soyabean meal to meet the N requirements of each horse (diet LNS). The diets were designed to meet the theoretical digestible energy requirements (NRC, 1978) of each horse. The composition of the diets is listed in Table 1.

Each horse was fed on its respective diet for an adaptation period of 3 weeks before each of the three experimental periods. For 2 d before each of these periods and throughout the experimental periods the horses were given one sixth of their respective diet at intervals of 4 h. Feed intake and total output of faeces and urine of each horse were measured for each 5 d collection period. This resulted in three measurements of digestibility for each horse for each experimental period. Samples of the dietary ingredients and the feed refusals from each horse were collected daily for each 5 d collection period, weighed and stored at −20°.

The horses were restrained in individual metabolism stalls during the three 5 d collection periods of each experimental period, except for 0·5 h each day when they were given mild exercise on a treadmill. Urine-collecting harnesses were fitted to the horses. The urine produced each day was collected into 20-litre containers to which 200 ml 12 M-HCl had been added. Faeces were voided onto plastic sheets placed behind the horses. Each horse was weighed at the beginning and end of each 15 d experimental period.

Experimental procedures

During the 15 d collection period of each experimental feeding period there were three isotope programmes; each isotope programme contained a 5 d collection period. At 1 h before each period of isotope administration a 15-gauge teflon indwelling catheter was aseptically inserted into the jugular vein of each horse and sutured in place.

For the first isotope programme a 55 ml aqueous solution containing [$^{15}N_2$]urea (250 mg 99% enriched ^{15}N) and ^{51}CrEDTA (9·25 MBq) was administered via a nasogastric tube into the stomach. The intragastric dose of [^{15}N]urea and ^{51}CrEDTA was administered only to those horses fed on diet LNU.

In the second programme a single intravenous injection of [$^{15}N_2$]urea (250 mg 99% enriched ^{15}N) in 5 ml isotonic saline was administered to each horse (diets LN, LNU and LNS). Additionally, in the second and third experimental periods a 50 ml solution containing 9·25 MBq ^{51}CrEDTA was administered into the right dorsal colon of each horse.

In the third isotope programme an aqueous solution containing 4 ml $(^{15}NH_4)_2SO_4$ (200 mg 99·3% enriched ^{15}N) and 50 ml ^{51}CrEDTA (9·25 MBq) was administered through the PVC cannula into the right ventral colon of each horse (diets LN, LNU and LNS).

Sampling procedures and preparation of samples

Jugular blood samples (20 ml) were obtained from each horse before each isotope administration and then at 0·5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60 and 72 h after administration. The heparinized blood samples were centrifuged at 1510 *g* for 10 min and the plasma was stored at −20°.

Samples of ventral and dorsal colonic fluid (70 ml) were collected from each horse before each tracer administration and at 0·5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 24, 30, 36, 48, 60 and 72 h after administration. Each sample was obtained by suction through a plastic tube directed into the colon and then passed through a fine nylon gauze to remove large particulate matter. The pH of each sample was determined.

The colonic fluid samples were centrifuged at 1000 *g* for 2 min to separate plant material. The supernatant fraction was decanted and then centrifuged at 19000 *g* for 20 min. Duplicate samples of supernatant fraction (2 ml) were pipetted into isotope-counting tubes for ^{51}Cr analysis. A further 5 ml supernatant fraction was added to an equal volume of 0·2 M-HCl and stored at −20° for subsequent ^{15}N analysis along with the remaining supernatant fraction for subsequent volatile fatty acid (VFA) analysis. Bacterial samples were obtained by resuspending the precipitated solid material in isotonic saline and centrifuging again for 10 min at 19000 *g*. The supernatant fraction was discarded and the bacterial pellet was resuspended in 10 ml isotonic saline and stored at −20°. The bacterial samples obtained were examined microscopically and found to be free of plant material.

Faeces and urine produced by each horse were weighed daily during each 5 d isotope programme. Each day 0·1 of each horse's daily faecal output, together with 0·01 of the daily urinary output were collected and stored at −20°.

Analytical procedures

Plasma urea-N and urea-N concentrations in urine were determined spectrophotometrically using an enzymic method (Boehringer Mannheim, Castle Hill, NSW, Australia).

The dietary ingredients, feed refusals and faeces were analysed for DM and organic matter (OM) by the methods described by the Association of Official Agricultural Chemists (AOAC) (1965) and the gross energy content was determined by bomb calorimetry. Neutral-detergent fibre (NDF) was estimated using the method of Goering & Van Soest (1970). Total N was determined on the dietary ingredients, feed refusals, faeces and urine by the Kjeldahl procedure (AOAC, 1965).

Total VFA concentrations and the molar proportions were determined on colonic samples by GLC (Hewlett Packard 8530A Gas Chromatograph and Hewlett Packard

18850A Gas Chromatograph terminal, Palo Alto, CA, USA). Samples were pooled so that only one ventral and one dorsal colonic sample were analysed for each isotope programme for each horse fed on each diet. Colonic samples were prepared for VFA analysis using a similar method to that described by Cottyn & Boucque (1968).

The N in the colonic, microbial, faecal and urinary samples was converted to $(NH_4)_2SO_4$ as required for ^{15}N analysis by the method described by Nolan & Leng (1972).

NH_3 derived from plasma urea was obtained after precipitating the protein from the plasma with TCA (100 g/l). The supernatant fraction was then passed through an ion-exchange column which bound the amino acids (Mills *et al.* 1981). The eluate was collected and evaporated to dryness at 70°, then dissolved in distilled water, digested and distilled for NH_3 as described by Nolan & Leng (1972).

The plasma protein precipitates obtained by the above procedure from samples collected 36 h after $(^{15}NH_4)_2SO_4$ administration into the ventral colon (isotope programme 3) were digested and the distilled NH_3 quantified.

The enrichment of samples with ^{15}N was determined with a mass spectrometer (Model VG Micromass 622 and Isotope Ratio Mass Spectrometer upgrade, Europa Scientific Ltd, Urmston, Greater Manchester) after converting $(NH_4)_2SO_4$ to N_2 gas using sodium hypobromite (Nolan & Leng, 1972). Abundance of ^{15}N in the N in the samples was calculated from the ratio of mass 28 and 29 peaks after correcting for background peaks and air contamination. The total ^{15}N enrichment of the plasma protein precipitates was not quantified. These samples were analysed for the presence or absence of ^{15}N.

The specific radioactivity of ^{51}CrEDTA in the ventral and dorsal colonic supernatant fractions and urinary samples was determined using a gamma spectrometer (Packard Instrument Co., CT, USA).

Analysis of isotope dilution data

Right ventral colonic NH_3-N pool size, total flux rate (total N entering the colonic pool), irreversible loss rate (N irreversibly lost from the colonic pool) and recycling rate of NH_3-N (N leaving but returning to the colonic pool) following right ventral colonic administration of $(^{15}NH_4)_2SO_4$ were determined using the equations derived from exponential curves as described by White *et al.* (1969). When fitting the equation to the ^{15}N enrichment curve of plasma urea-N following intravenous administration of [^{15}N]urea the first sampling point was disregarded, as it appeared that [^{15}N]urea had not equilibrated in the body at 30 min. The rate of irreversible loss of urea-N from the plasma pool and body urea-N pool size were determined using equations derived from exponential curves. Urea-N degradation rate in the gastrointestinal tract was calculated as the difference between the irreversible loss of urea-N from plasma and urinary urea-N excretion.

The area under the ^{15}N enrichment-with-time curve (AUC) for the primary N pools for any time period was calculated by integrating equations obtained from fitted curves (Nolan & Leng, 1972). AUC for secondary N pools were determined by planimetry. The proportion of N in any secondary pool derived from a primary pool was calculated from the secondary:primary AUC ratio for the period from 0 to 24 h after isotope administration.

The rate of N transfer from plasma urea to ventral colonic NH_3 was calculated as the product of the proportion of ventral colonic NH_3-N derived from plasma urea-N and the irreversible loss rate of ventral colonic NH_3-N. The rate of N transfer from ventral colonic NH_3 to plasma urea was calculated as the product of the proportion of plasma urea-N derived from ventral colonic NH_3-N and the irreversible loss rate of urea-N from plasma (Nolan & Leng, 1972).

The proportion of intragastric [^{15}N]urea which was absorbed into the blood over the time period 0 to infinity was determined from the ratio of the AUC of plasma urea-N when the ^{15}N was administered by the intragastric route to the AUC of plasma urea-N when it was administered intravenously. The AUC were determined by integration.

Statistical analyses

The data were analysed with ANOVA considering the individual effects of diet, animal and experimental period and the interactions between these effects. Sequential repeated measures for each of the nine combinations of the Latin square were averaged for each horse for each isotope programme and then within each experimental period before ANOVA. Means were compared using *t* tests, using standard deviations based on the residual variance. All data obtained from horse 3, diet LNS during the third isotope programme (colonic $(^{15}NH_4)_2SO_4$), were excluded in calculating means of that group due to a negative N balance (twice that of the standard deviation of the mean).

Colonic NH_3, total VFA concentrations and colonic pH were analysed using ANOVA as described above but included another factor site (ventral or dorsal colon). As there was no diet × site interaction and site was significantly different, the sites were then analysed separately.

RESULTS

Digestibility of the diets

DM, OM, NDF and N digestibilities of the three diets are presented in Table 2. The horses, when fed on the LN and LNU diets, were in negative N balance whereas when they were fed on the LNS diet they were in positive N balance (Table 2). The horses lost on average 12·7 (range −25 to −3) kg in weight while fed on the LN diet, 13·3 (range −19 to −8) kg while fed on the LNU diet and 4·7 (range −11 to 0) kg while fed on the LNS diet.

Volatile fatty acid concentrations

Diet did not significantly influence the total VFA concentrations or the molar proportions of VFA in the right ventral or right dorsal colon. However, the total VFA concentration and the molar proportions of acetate, isobutyrate, isovalerate and n-valerate were significantly lower ($P < 0{\cdot}05$) in the ventral colon than in the dorsal colon. The molar proportions of propionate and n-butyrate were significantly higher ($P < 0{\cdot}01$) in the ventral colon than in the dorsal colon (Table 3).

Single intragastric dose of [^{15}N] urea

Following intragastric dosing the [^{15}N]urea was rapidly absorbed into the blood (Fig. 1) with peak enrichment reached within 2–3 h. The proportion of dietary urea-N entering plasma urea-N averaged 90 (SD 12·3, range 76–100)%.

In the first 24 h after the intragastric administration of [^{15}N]urea, 44 (SD 3·6)% of the dose was excreted in urinary N and 1 (SD 0·2)% was excreted in faecal N. Over the 5 d collection period, 64 (SD 3·9)% was excreted in urinary N and 10 (SD 1·0)% in faecal N. The proportion of dietary urea retained, determined after a single intragastric dose of [^{15}N]urea and 5 d collection of urine and faeces, was 26 (SD 3·4, range 23–30)%.

Single intravenous injection of [^{15}N]urea

An example of the enrichment of plasma urea-N with time following a single injection of [^{15}N]urea into the blood is presented in Fig. 2 for the diet LNU (horse 3). The [^{15}N]urea 'enrichment with time' curves in plasma were best described by equations with a single

Table 2. *Mean digestibility of nutrients (g/g) and energy content (MJ/kg DM) of each of the three diets and daily nitrogen intake, faecal nitrogen and urinary nitrogen excretion and nitrogen balance (g/d) of the three horses**

Item	Diet			SD
	LN	LNU	LNS	
DM digestibility	0·52	0·52	0·53	0·020
OM digestibility	0·51	0·51	0·52	0·021
NDF digestibility	0·41	0·40	0·37	0·025
N digestibility	0·06[a]	0·72[b]	0·71[b]	0·018
DE	8·0	7·7	8·4	0·35
N intake	28·2[a]	95·1[b]	98·2[b]	8·11
N intake (%)†	33	111	115	—
Faecal N excretion	26·2	26·4	28·7	2·38
Urinary N excretion	17·6[a]	74·4[b]	56·2[c]	2·46
Digestible N intake	2·0[a]	68·6[b]	69·5[b]	5·75
N balance	−15·6[a]	−5·7[a]	13·3[b]	5·86

OM, organic matter; NDF, neutral-detergent fibre; DE, digestible energy; LN, low nitrogen; LNU, low nitrogen + urea; LNS, low nitrogen + soyabean meal.

[a,b,c] Mean values within a row with different superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 374–375.

† Expressed as a percentage of the recommended amount (National Research Council, 1989).

Table 3. *Mean total volatile fatty acid (VFA) concentrations (mmol/l) and the molar proportions of VFA (%) in the right ventral and right dorsal colon of three horses fed on three diets**

Measurement	Site		SD
	Right ventral colon	Right dorsal colon	
Total VFA concentration	58·2[a]	75·1[b]	5·77
Molar percentage			
Acetate	72·4[a]	78·3[b]	1·68
Propionate	18·8[a]	13·8[b]	1·57
Isobutyrate	0·9[a]	2·1[b]	0·23
n-Butyrate	7·4[a]	4·5[b]	1·15
Isovalerate	0·2[a]	1·0[b]	0·36
n-Valerate	0·1[a]	0·4[b]	0·20

[a,b] Mean values within a row with different superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 373–376.

exponential component for all three diets. The appearance of enriched NH_3-N in the right ventral colon and right dorsal colon following a single injection of [^{15}N]urea into the blood is also presented in Fig. 2.

Body urea-N pool size was significantly lower for the horses fed on the LN diet compared with that of horses fed on diets LNU and LNS. The mean irreversible loss rate of urea-N from plasma was significantly higher for the LNU diet than the LN or LNS diet. Mean irreversible loss rate of urea-N was higher for the LNS diet than the LN diet. Plasma urea-N, urea-N excretion and urea-N degradation were also significantly different for the three diets and followed a similar pattern to the irreversible loss rate of urea-N. However, the

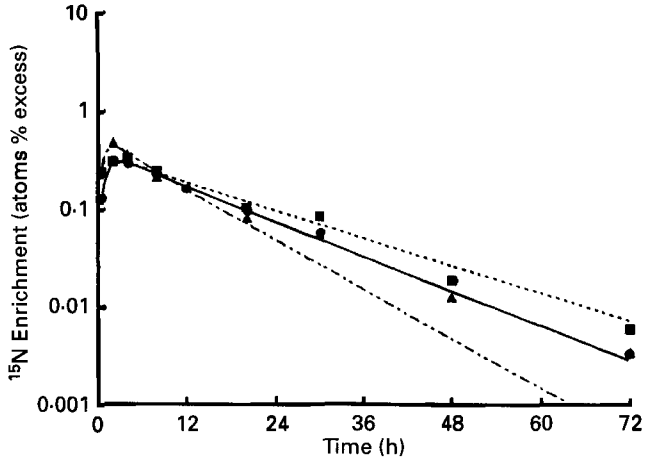

Fig. 1. Intra-gastric [^{15}N]urea: enrichment of plasma urea-N after a single intragastric dose of [^{15}N] to horse 1 (●), horse 2 (■), horse 3 (▲). The lines represent the predicted equations, horse 1 (——), horse 2 (--), horse 3 (----) and the symbols are the measurements.

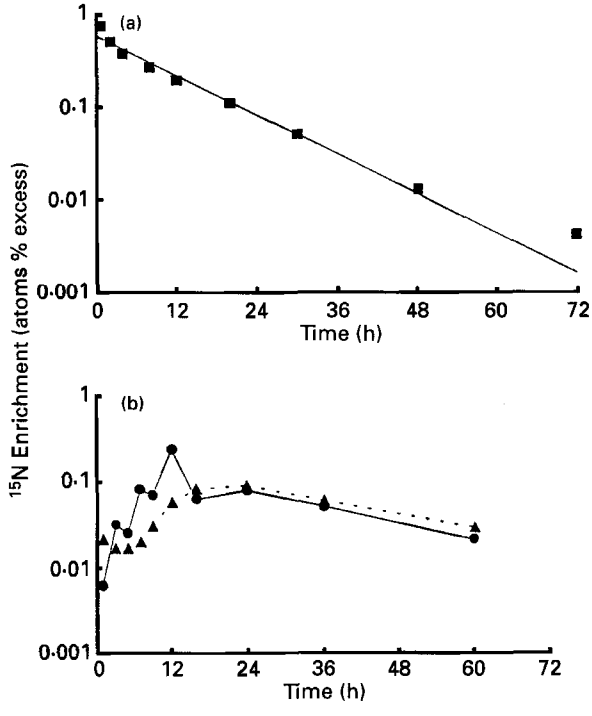

Fig. 2. Injection of [^{15}N]urea into blood. (a) Enrichment of plasma urea-N (■), and (b) ventral colonic ammonia-N (–●–) and dorsal colonic ammonia-N (–▲–) after a single injection of [^{15}N]urea into the blood of horse 2 on a low-N diet. (a) The solid line represents the predicted equation and the symbols are the measurements. (b) The symbols are the measurements and no equation accurately predicted the points measured.

Table 4. *Mean measures of urea metabolism of three horses fed on three different diets estimated using single injections of [^{15}N] urea into the blood**

Item	Diet			
	LN	LNU	LNS	SD
PUN (mmol/l)	2·4[a]	7·4[b]	4·9[c]	0·45
Body urea-N pool size (g)	21·4[a]	56·2[b]	48·5[b]	5·32
Body urea space (% live weight)	83	70	90	6·4
Urea irreversibly lost (g N/d)	32·6[a]	114·8[b]	85·7[c]	5·33
Urea excretion (g N/d)	6·4[a]	63·5[b]	46·6[c]	3·46
Urea degradation (g N/d)	26·1[a]	51·2[b]	39·1[c]	2·72
Proportion of urea irreversibly lost that enters the GIT (%)	80[a]	45[b]	46[b]	4·4

LN, low nitrogen; LNU, low nitrogen + urea; LNS, low nitrogen + soyabean meal; PUN, plasma urea nitrogen; GIT, gastrointestinal tract.

[a,b,c] Mean values within a row with different superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 374–377.

proportion of urea-N irreversibly lost that entered the gastrointestinal tract was significantly greater for horses fed on the LN diet than either the LNU or LNS diet. There was no difference between the LNU and LNS diets (Table 4).

Over the 5 d collection period, 35, 57 and 75 (SD 13·3)% of the intravenous [^{15}N]urea dose was excreted in urinary N ($P > 0·05$) and 27, 8 and 9 (SD 2·3)% was excreted in faecal N ($P < 0·05$) for horses fed on diets LN, LNU and LNS respectively. The percentage of [^{15}N]urea retained after 5 d of faecal and urinary collection was 38, 35 and 15 (SD 11·3)% for horses fed on diets LN, LNU and LNS ($P > 0·05$).

Single injection of [^{15}N]ammonium sulphate into the right ventral colon

An example of the enrichment of right ventral colonic NH_3-N with time following single administration of $(^{15}NH_4)_2SO_4$ into the right ventral colon is presented in Fig. 3 for diet LNU (horse 1). The $^{15}NH_3$ enrichment-with-time curves in right ventral colonic fluid were best described by equations with two exponential components for all three diets. The appearance of enriched bacterial-N in the right ventral colon, NH_3-N and bacterial-N in the right dorsal colon and plasma urea-N following administration of $(^{15}NH_4)_2SO_4$ into the right ventral colon are also presented in Fig. 3. None of the measures of NH_3 metabolism in the right ventral colon differed between diets (Table 5).

The protein precipitates obtained from the plasma samples collected 36 h after $(^{15}NH_4)_2SO_4$ administration into the right ventral colon for each of the three diets were all enriched with ^{15}N (0·005 to 0·01 atoms% excess).

Over the 5 d collection period 10, 19 and 37 (SD 20·8)% of the $(^{15}NH_4)_2SO_4$ dose administered into the right ventral colon was excreted in urinary N ($P > 0·05$) and 19, 12 and 23 (SD 6·6)% was excreted in faecal N ($P > 0·05$) for horses fed on diets LN, LNU and LNS respectively. The proportion of ^{15}N retained after 5 d of faecal and urinary collection was 71, 69 and 40 (SD 27·4)% for horses fed on diets LN, LNU and LNS ($P > 0·05$).

Rates of transfer of nitrogen between plasma urea and right ventral colonic ammonia pools

The mean rates of N transfer from plasma urea to ventral colonic NH_3 did not differ for the three diets (Table 6). There were no significant differences in the mean rates of N transfer from ventral colonic NH_3 to plasma urea between the three diets (Table 6).

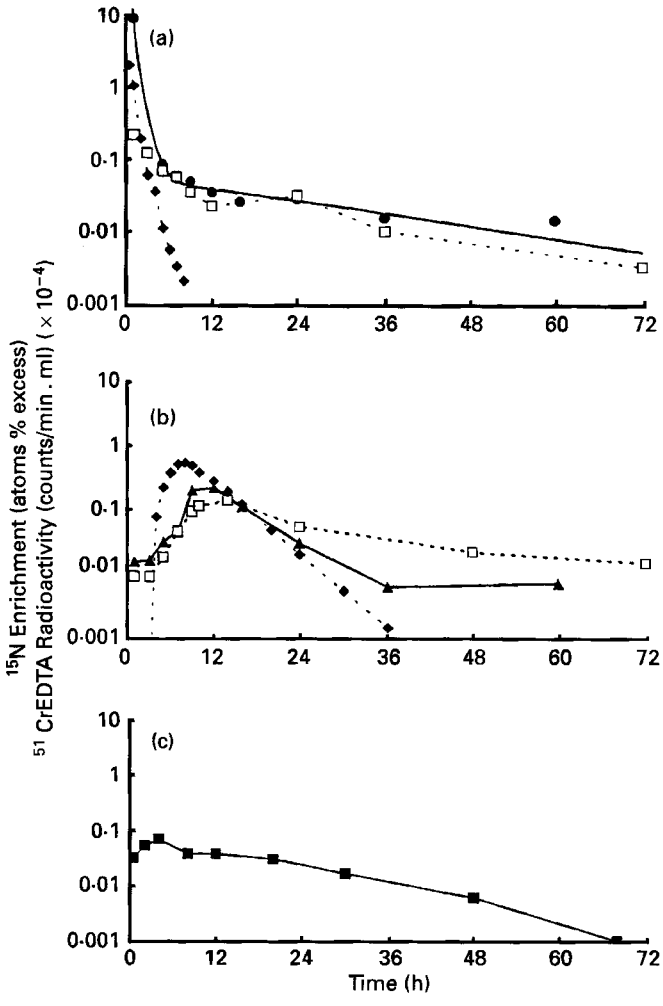

Fig. 3. Injection of $(^{15}NH_4)_2SO_4$ and ^{51}CrEDTA into the ventral colon. (a) Enrichment of ventral colonic ammonia-N (●) and bacterial N (–□–); (b) dorsal colonic ammonia-N (–▲–) and bacterial N (–□–); (c) plasma urea-N (–■–) after a single injection of $(^{15}NH_4)_2SO_4$ into the ventral colon of a horse fed on a low-N diet supplemented with urea. Injection of ^{51}CrEDTA was made simultaneously and its specific radioactivity with time after injection, in the water of the ventral and dorsal colon, is also given (–◆–). (a) The solid line represents the predicted equation and the symbols are the measurements. (b,c) The symbols are the measurements and no equation accurately predicted the points measured.

Water kinetics and transit times in the right ventral and dorsal colon

Although ^{51}CrEDTA was not absorbed from the colon, as negligible radioactivity was detected in urine, first-order kinetics generally did not apply to the disappearance curves of ^{51}Cr in ventral and dorsal colonic fluid. Either instantaneous mixing did not occur and/or recycling of liquid occurred in the pools sampled. It was possible on only two occasions to determine the water volume of the pool sampled in the right ventral colon and right dorsal colon and the outflow of liquid from these sites. It was calculated that the water volumes of the pools sampled in the right ventral colon and right dorsal colon for horse 1, diet LNU, were 3·7 and 6·8 litres and the outflows of fluid were 95·0 and 34·7 litres/d respectively. The water volumes of the pools sampled in the right ventral colon and right

Table 5. *Mean measures of ammonia metabolism in the right ventral colon of horses fed on three different diets, estimated using single injections of [^{15}N]ammonium sulphate into the right ventral colon, together with right ventral and right dorsal colonic ammonia-N concentrations (mg/l) and pH**

Item	Diet			
	LN (*n* 3)	LNU (*n* 3)	LNS (*n* 2)	SD
Right ventral colonic ammonia-N pool size (g)	2·7	2·0	2·0	0·82
Total flux rate of ammonia (g N/d)	66·1	42·1	55·8	16·10
Irreversible loss rate of ammonia (g N/d)	45·2	23·7	45·2	3·43
Recycling rate of ammonia (g N/d)	20·9 (*n* 3)	18·4 (*n* 3)	10·6 (*n* 3)	12·65
Right ventral colonic ammonia-N	44	53	57	16·8
Right dorsal colonic ammonia-N	159	168	167	6·6
Right ventral colonic pH	7·02	7·03	6·99	0·068
Right dorsal colonic pH	6·80	6·84	6·90	0·045

LN, low nitrogen; LNU, low nitrogen + urea; LNS, low nitrogen + soyabean meal.
* For details of diets and procedures, see Table 1 and pp. 373–377.

Table 6. *Estimates of rates of transfer of nitrogen between plasma urea and right ventral colonic ammonia pools at 24 h in horses fed on three different diets**

Item	Injection site†	Diet			
		LN (*n* 3)	LNU (*n* 3)	LNS (*n* 2)	SD
Plasma urea-N derived from ventral colonic ammonia-N (%)	RVC	35	14	41	20·4
Ventral colonic ammonia-N transferred to plasma urea-N (g N/d)	RVC	11·9 (*n* 2)	16·4 (*n* 2)	33·0 (*n* 2)	18·37
Ventral colonic bacterial N derived from ventral colonic ammonia-N (%)	RVC	45 (*n* 3)	21 (*n* 2)	21 (*n* 3)	18·4
Ventral colonic ammonia-N derived from plasma urea-N (%)	IV	17 (*n* 3)	41 (*n* 2)	18 (*n* 2)	7·0
Plasma urea-N transferred to ventral colonic ammonia-N (g N/d)	IV	8·4	11·5	10·0	5·72

LN, low nitrogen; LNU, low nitrogen + urea; LNS, low nitrogen + soyabean meal.
* For details of diets and procedures, see Table 1 and pp. 373–377.
† Estimates were computed using dilution values obtained when $(^{15}NH_4)_2SO_4$ was administered into the right ventral colon (RVC) or when [^{15}N]urea was administered intravenously (IV).

dorsal colon for horse 2, diet LNS, were 2·9 and 14·6 litres and the outflows of fluid were 91·8 and 56·1 litres/d respectively. There was no retrograde flow from the right dorsal colon to the right ventral colon.

DISCUSSION

Effectiveness of urea as a dietary supplement

The results of the present experiment demonstrate that dietary urea offers no nutritional benefit to mature horses consuming low-protein diets. Horses fed on a low-protein diet were able to degrade endogenous urea in the digestive tract. Endogenous urea is likely to

be important as a N source for the bacteria, thereby maintaining microbial fermentation in the large intestine and thus providing a supply of VFA to the horses. Although horses fed on the urea-supplemented diet degraded a greater quantity of urea in the digestive tract than did horses fed on the low-N or the soyabean-supplemented diet, this could not compensate for the lack of dietary crude protein.

The horses fed on the low-N and urea-supplemented diets were in a negative N balance and all lost weight. Several other studies have reported no improvement in N retention when urea was added to basal diets containing crude protein concentrations of between 42 and 59 g/kg to increase the crude protein concentrations to 91–117 g/kg (Reitnour & Treece, 1971; Johnson & Hart, 1974; Reitnour, 1978).

Metabolism of ingested urea

At 5 d after the administration of intragastric [^{15}N]urea, approximately 26% of the dose was not accounted for in either faecal or urinary N. It is likely that this unaccounted [^{15}N]urea was degraded to NH_3 in the large intestine. This NH_3 was probably incorporated into non-essential amino acids in the liver and these amino acids retained in the body as muscle and/or plasma proteins. Schubert *et al.* (1991) reported that in two lactating pony mares 68 and 72% of orally administered [^{15}N]urea was excreted in urinary N and 15 and 7% in faecal N. Approximately 90% of the dose was recovered after collecting faeces, urine, milk and body tissues (at slaughter).

Endogenous synthesis and excretion of urea

The horses fed on the urea-supplemented diet had higher values of urea metabolism than the horses fed on the low-N or the soyabean-supplemented diets. However, although a greater quantity of urea was degraded in the digestive tract of horses fed on the urea- and soyabean-supplemented diets, the percentage of urea lost from the blood that was degraded in the digestive tract was actually lower in these animals than in the horses fed on the low-N diet. Prior *et al.* (1974) also reported that ponies fed on diets containing 60 g crude protein/kg had lower irreversible loss and degradation rates of urea-N estimated with [^{14}C]urea than did ponies fed on diets containing crude protein levels of 90 g/kg or higher. These authors also found that a higher proportion of urea produced in the body was degraded in the digestive tract of horses consuming low-protein diets.

It is logical to expect that, as in other species, some of the urea degraded in the gut would be converted to NH_3, absorbed into the blood, reconverted to urea and a proportion recycled back to the gut. The extent of this recycling in the present experiment was so small that statistical analysis failed to detect it even though examination of Fig. 1 suggests that a second decay curve may have been present. Distinguishing between singly- and doubly-labelled urea may have detected this recycling.

In the present study the results suggest that renal conservation of N occurs in horses fed on low-N diets. This is supported by the fact that only 22% of the intravenous dose of [^{15}N]urea was excreted in urine in a 24 h period for horses fed on the low-N diet, whereas 41 and 52% of the dose was excreted in the same time period for horses fed on the urea- and soyabean-supplemented diets respectively. However, it should be noted that blood urea degraded in the large intestine will only be of value to the horse as a N source if colonic NH_3 is limiting microbial fermentation and growth. In ruminants a similar response to low N intakes has been observed to those in the present study (Cocimano & Leng, 1967; Robbins *et al.* 1974; Bunting *et al.* 1987).

Ammonia metabolism in the colon

In the present study the higher NH_3-N levels found in the right dorsal colon than those measured in the ventral colon were possibly due to the slow liquid flow rate through the dorsal colon and the accumulation of microbes and their degradation at this site, resulting in release of NH_3. Hecker (1971) also reported that the NH_3-N levels in the left and right dorsal colon were approximately three times higher than those in the left and right ventral colon.

The results obtained concerning NH_3 kinetics in the right ventral colon and the rates of N transfer between pools should be interpreted cautiously. In planning this experiment the assumption was made that instantaneous mixing of $(^{15}NH_4)_2SO_4$ would occur in the right ventral colon. However, the results from the dilution curves obtained after the single administration of ^{51}CrEDTA into the right ventral and right dorsal colon indicated that, generally, complete mixing was not instantaneous and/or recycling of liquid occurred. Consequently, the dilution curves for colonic NH_3-N obtained in this experiment could, in part, be due to movement of NH_3-N with the colonic fluid within the colon itself.

It is likely that the majority of N that recycles in the NH_3-N pool in the right ventral colonic fluid is derived from bacteria that die and are digested in that organ. NH_3 could be irreversibly lost from the right ventral colon by incorporation into microbial cells which are transferred to the dorsal colon, by direct absorption through the ventral colonic wall into the portal blood and by loss in colonic fluid which flows to the dorsal colon.

The results of the present study appear to indicate that there was extensive use of non-NH_3-N sources for microbial growth by the microbial population in the ventral colon of the horses. Maczulak *et al.* (1985) demonstrated that the preferred N sources of caecal bacteria were peptones and amino acids. In contrast, Glinsky (1976) found that between 42 and 89% of caecal bacterial N was derived from caecal NH_3-N.

It was not an objective of the present study to determine if microbial amino acids were absorbed into the circulation. However, it was demonstrated that right ventral colonic NH_3-N was incorporated into the plasma proteins of each horse fed on each of the three diets. It is quite possible that this was a result of the synthesis of non-essential amino acids in the liver. No mechanism for the active transport of amino acids from the mammalian colon has been demonstrated (Cordero & Wilson, 1961; Binder, 1970; Munck, 1981).

Flux of urea and ammonia between blood and digestive tract

The rates of transfer of N from the plasma urea pool to the right ventral colonic NH_3 pool were similar for all three diets. Apparently only 32, 22 and 25% of the urea-N that was degraded in the digestive tract entered the right ventral colonic NH_3-N pool or that proximal to the right ventral colon of horses fed on the low-N, urea-supplemented and soyabean-supplemented diets. Generally similar enrichments of NH_3-N were noted at the 1 h sampling time in both the right ventral and the right dorsal colon. This would indicate absorption of endogenous urea along the length of the large intestine.

There appeared to be a greater rate of transfer of N from the right ventral colonic NH_3 pool to the plasma urea pool in horses fed on the soyabean-supplemented diet than in horses fed on the low-N and urea-supplemented diets, although these differences were not significant. This result would appear to make sense as the bacteria in the horses fed on the soyabean-supplemented diet could use other forms of N (protein). Therefore, the NH_3 would not be used as extensively and would be recycled to the plasma. This is supported by the fact that a higher proportion of the $(^{15}NH_4)_2SO_4$ dose was excreted in urinary N of the horses consuming the soyabean-supplemented diet.

CONCLUSIONS

Although the quantity of urea degraded in the digestive tract of the horses fed on the urea-supplemented diet was greater than in the horses fed on the low-N or the soyabean-supplemented diet, this increase could not compensate for the lack in dietary crude protein. The horses remained in a negative N balance and lost as much weight as when they were fed on the low-N diet.

Due to the high proportion of endogenous urea which is degraded in the digestive tract of horses fed on a low-protein diet it would appear that dietary urea supplementation may only be of significant nutritional benefit to horses fed on a protein-free diet or a diet containing a high proportion of essential amino acids but deficient in non-essential amino acids. This situation would not occur under normal grazing conditions.

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