Effect of time of starch supply to the rumen on the dynamics of urea and ammonia net flux across the rumen wall of sheep

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Original article

Effect of time of starch supply to the rumen on the dynamics of urea and ammonia net flux across the rumen wall of sheep

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Abstract — The effects of rumen starch fermentation on urea and ammonia net fluxes across the ruminal wall were studied in four Texel wethers (67 ± 1.5 kg BW) fitted with catheters in both ruminal veins and in a mesenteric artery, blood flow probes on ruminal arteries, and a ruminal cannula. They were fed 500 g of orchardgrass hay every 12 h. A dose of 120 g of starch was added directly to the rumen either during the meal or 3 h later. On a daily basis, the time of starch supply did not modify the urea and ammonia net flux across the rumen wall. Within the feeding cycle, whatever the time of starch injection, the decrease in ruminal ammonia and pH and the increase in ruminal volatile fatty acids and CO_2 associated with starch fermentation were accompanied by a decrease in ammonia absorption and an increase in urea net transfer across the ruminal wall. This study confirms the existence of a synchronisation between urea net transfer across the rumen wall and rumen carbohydrate fermentation within a feeding cycle. Factors acting in the regulation of urea and ammonia net flux in the ruminal veins are discussed.

ammonia / urea / flux / rumen / sheep

Résumé — **Effet de l'heure d'un apport d'amidon dans le rumen sur la cinétique des flux d'urée et d'ammoniaque à travers la paroi du rumen de mouton.** Les effets d'une fermentation d'amidon dans le rumen sur les flux d'urée et d'ammoniaque à travers la paroi du rumen ont été étudiés sur 4 moutons Texel (67 ± 1.5 kg de poids vif) porteurs d'une canule du rumen, de cathéters dans les 2 veines ruminales et dans une artère mésentérique et d'une sonde débit métrique sur les 2 artères ruminales. Ils étaient alimentés avec 500 g de foin de dactyle distribués toutes les 12 h. Une dose de 120 g d'amidon était injectée dans le rumen soit au début du repas soit 3 h après. Le moment d'addition de l'amidon n'a pas modifié la moyenne journalière du flux net d'urée et d'ammoniaque à travers

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la paroi du rumen. A l'intérieur d'un cycle d'alimentation, quel que soit le moment d'injection de l'amidon, la diminution de la concentration en ammoniaque et l'augmentation de la teneur en acides gras volatils et en CO_2 du contenu du rumen, associés à la fermentation de l'amidon, s'est accompagnée d'une diminution de l'absorption d'ammoniaque et d'une augmentation du transfert net d'urée à travers la paroi ruminale. Ces résultats confirment l'existence d'une synchronisation entre le recyclage d'urée par diffusion à travers la paroi ruminale et la cinétique des fermentations ruminales. La régulation à court terme par les paramètres intraruminaux des flux nets d'urée et d'ammoniaque à travers la paroi du rumen est discutée dans le texte.

ammoniaque / urée / flux / rumen / mouton

1. INTRODUCTION

Since Nolan et al. [8], quantification of ammonia absorption from and endogenous urea entry into the rumen has been widely achieved using the isotope dilution technique, under steady state conditions. In contrast, few studies have been carried out in meal fed ruminants. This is however of particular interest regarding the role of the delay compartment that can represent endogenous urea for nitrogen digestion in the rumen. In previous studies we observed that urea net flux across the epithelium varies throughout an alimentary cycle [11], responding to short-term regulation by intraruminal traits [12]. For sheep fed hay, the highest values of urea net flux were recorded 5 h after the meal, when the ruminal concentration of ammonia was low while the concentrations of the end products of the carbohydrate fermentation were still high, at a time therefore when dietary nitrogen availability may have limited microbial synthesis. Our hypothesis was a synchronisation throughout the feeding cycle between carbohydrate fermentation in the rumen and transfer of urea across the ruminal wall. This hypothesis was tested in the present study by shifting the time of energy supply into the rumen from the meal to 3 h later, so that the imbalance between nitrogen and energy availability for the microbes was moved within the feeding cycle. Furthermore attempts were made to relate urea and ammonia net fluxes in the ruminal veins to the concentrations of fermentation end products in the ruminal fluid.

2. MATERIALS AND METHODS

2.1. Animals and diet

Four mature Texel wethers (67 ± 1.5 kg BW) were used. They were fitted with a ruminal cannula, chronic indwelling catheters in the ruminal veins and in a mesenteric artery, and blood flow probes around the ruminal arteries [13]. Animals were allowed 1-month recovery from surgery before allocation to the trial.

Throughout the experiment, the sheep were maintained according to the principles for care of laboratory animals. They were housed in individual pens ($1 \text{ m} \times 1.5 \text{ m}$) in a room under continuous lighting with controlled temperature (19 to 23 ºC). They were given 1000 g of chopped orchardgrass hay (860 g of DM·d^{-1}, 611 g of digestible OM·kg^{-1} of DM, and 23.6 g of N·kg^{-1} of DM) in two equal meals at 08:15 and 20:15. The sheep had free access to water and salt blocks.

Twice daily, a dose of 120 g of wheat starch diluted in water was introduced into the rumen, through the cannula, either at the beginning of the meal (at 08:15 and 20:15) or 3 h later (at 11:15 and 23:15). On a DM basis daily starch supply accounted for 20% of the DM entering the rumen (hay + starch). Each sheep received each of the two treatments; the experiment consisted of two periods with two sheep per treatment for each period. Net fluxes were measured after 15 d of adaptation to the treatment.

2.2. Sampling and analysis

Sampling procedures for blood and ruminal content have been described previously [11]. Blood samples were simultaneously withdrawn from the three catheters in 5-mL syringes containing anticoagulant. Twelve sets of venous and arterial blood were collected during the feeding cycle (08:00, 08:30, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:30, and 19:00).

All methods used in the analysis of blood samples (urea, ammonia, haemoglobin), rumen fluid (pH, ammonia, volatile fatty acids, urease activity) and CO_2 in gas content have been described previously [11].

Blood flow (BF) was measured continuously with an ultrasonic transit time flowmeter (Transonic Systems, Ithaca, NY) interfaced with a computer for data acquisition [13].

Periods of rumination were registered recording jaw movements with the technique described by Baumont et al. [2], so as to verify that experimental treatments did not affect the alimentary behaviour of the animals.

2.3. Calculations and statistics

Net whole blood exchange of metabolites across the ruminal wall was calculated as described by Rémond et al. [11]. Net fluxes of metabolites across the rumen wall were calculated as the sum of the net fluxes in the right and the left ruminal veins. A positive net flux indicates release of a nutrient, whereas a negative net flux implies uptake. The quantities of metabolites transferred across the rumen wall within the 12-h period were estimated by integrating time-variations in net fluxes [11]. Daily net flux of metabolites was estimated on the assumption that all 12-h periods were equivalent.

The effect of the treatments on daily means of studied traits was achieved using standard analysis of variance, with the model including animal, period and treatment effects. Period effect was never significant ($P > 0.10$), and not reported. The effect of time on studied traits was analysed statistically using repeated measures ANOVA of SAS GLM procedure [16]. Time-period and time-animal interactions were never significant and hence are not reported. The differences in instantaneous fluxes of nitrogenous compounds due to the treatments were tested by pairwise t-tests. The SAS REG procedure [16] was used to predict metabolites net fluxes from the traits measured.

3. RESULTS AND DISCUSSION

All catheters and blood flow probes remained patent throughout the experiment. The sheep consumed all their meal in less than 45 min. Rumination time (on average 410 min·d^{-1}) was not affected by the time of starch injection ($P = 0.58$) and was close to that (447 min·d^{-1}) reported by Baumont et al. [2]. The sheep drank the same amount of water (about 1.9 L/12 h) whatever the treatment ($P = 0.44$).

The patterns of intraruminal traits (ammonia and volatile fatty acid concentrations, CO_2 percentage of rumen gas, urease activity, and pH) throughout a feeding cycle are presented in Figures 1 and 2. All traits showed significant variations with time ($P < 0.05$). The time-variations in rumen pH, ammonia, volatile fatty acids (VFA) and CO_2 concentrations, were affected by the time of starch injection into the rumen ($P < 0.05$). Comparison of rumen trait kinetics showed that, whatever the treatment, starch injection was followed by an increase in CO_2 and VFA concentrations in the rumen content and a decrease in rumen pH and ammonia concentration. The greatest effect was observed 3 to 4 h after starch injection. As previously observed [11], the ureolytic activity of the ruminal contents increased during the meal but this trait was not significantly affected by the time of starch addition.
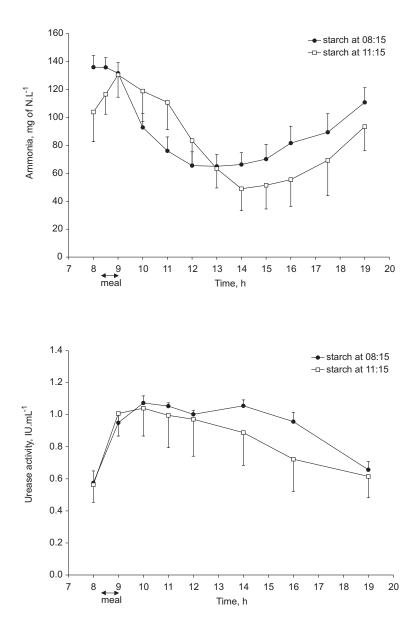
Figure 1. Effect of the time of starch supply on the variations in rumen ammonia concentration, and urease activity. Values are means of four sheep ± SE.

In agreement with previous observations made in sheep fed hay [1, 11], rumen BF increased rapidly during ingestion (Fig. 3). For both treatments the highest values were recorded at the end of the meal. Afterwards, although rumen BF at 11:00 and 14:00 tended to be different between treatments, the shape of the decrease in rumen

Figure 2. Effect of the time of starch supply on rumen pH, total volatile fatty acid concentration and CO_2 content of rumen gas. Values are means of four sheep $\pm$ SE.

BF was not greatly affected by the time of starch injection. No change was seen in daily ruminal BF by moving starch injection from the time of the meal to 3 h later ($P > 0.10$). In sheep fed the same diet without starch [11], the daily rumen BF was 402 L·d^{-1}. With starch addition, in the present experiment, it was on average 535 L·d^{-1}, suggesting a 40% increase in response to the additional OM fermented in the rumen.

The patterns of urea and ammonia net fluxes across the rumen wall are presented in Table I. Net release of ammonia varied throughout the feeding cycle ($P < 0.05$) and these variations were affected by the time of starch supply ($P < 0.05$). For both treatments ammonia net release showed patterns similar to those of ruminal ammonia: the highest fluxes were recorded at the end of the meal and the lowest about 3 h after starch injection. Consequently, there was a linear relationship between ammonia net release (y, mg of N·min^{-1}), and NH$_3$ concentration in the rumen (x, mg of N·L^{-1}): $y = 0.020 \times x + 0.881$ ($r^2 = 0.68$, $n = 96$). It is generally accepted that ammonia is ab-sorbed in its non-ionised form, however, as previously observed with a hay diet [11], ammonia net release was poorly related to non-ionised ammonia concentration in the rumen ($r^2 = 0.35$, $n = 96$). Modifications of local pH (against and within the epithelium) and subepithelial blood flow may affect ammonia absorption rate [14], and it was shown that increased concentrations of VFA and CO$_2$ in the rumen favour the absorption of ammonia during short-term experiments [3, 12].

As previously reported for sheep fed hay [11], large variations in urea net flux were observed throughout the alimentary cycle ($P < 0.01$). For both treatments the lowest values were recorded during the meal, but thereafter the variations of urea net flux were dependent on dietary manipulation. For the two treatments the greatest urea net uptake was recorded 3 to 4 h after starch injection, at a time when ammonia level in the rumen was the lowest while fermentation end-products (VFA, CO$_2$) were high. In contrast to what was reported for sheep fed hay [11], there was a poor relationship between
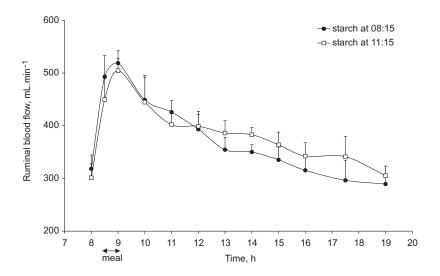

Figure 3. Effect of the time of starch supply on the variations in rumen blood flow. Values are means of four sheep ± SE.

Table I. Effect of wheat starch injection into the rumen, on the variations with time of the urea and ammonia net fluxes across the rumen wall of sheep fed twice daily. Each value is the mean of four sheep.
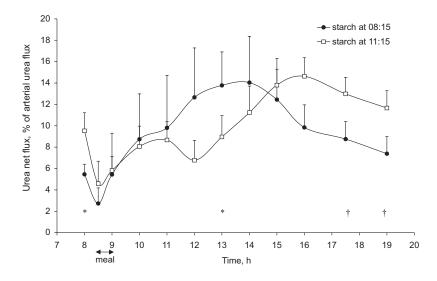
Item	Diet[a]	8	8.5	9	10	11	12	13	14	15	16	17.5	19	EMS[d]	Time	Time × Diet
						Time, h[b]									Statistics[c]	
Arterial concentration, mg of N·L^{-1}																
Urea	M	107.5[*]	110.9[†]	115.7[†]	111.7	104.9	98.4	92.3	90.4	87.8	86.4	92.0[†]	97.3[*]	9.28	0.0001	0.0001
	D	80.3	83.8	87.5	86.6	90.3	89.0	86.1	81.6	79.7	75.8	73.4	74.5			
Ammonia	M	1.1	0.8	0.8	1.1	1.1	1.1	1.1	1.0	1.0	1.1	1.2	1.2	0.13	0.5406	0.8375
	D	1.6	1.2	1.3	1.4	1.2	1.1	1.3	1.5	1.6	1.3	1.4	1.6			
Ruminal net flux, mg of N·min^{-1}																
Urea	M	−1.84[†]	−1.46	−2.86	−4.03	−4.05	−4.67	−4.41	−4.26	−3.67	−2.70	−2.36[*]	−2.07[†]	0.40	0.0001	0.0005
	D	−2.29	−1.72	−2.57	−3.10	−3.16	−2.42[*]	-2.97[†]	−3.49[†]	−3.99	−3.80	−3.24	−2.67			
Ammonia	M	3.56	3.39	3.90	2.98	2.16	2.30	2.62	2.41	2.52	2.32	2.69	2.74	0.15	0.0001	0.0100
	D	2.98	2.87	3.83	3.37	3.09[*]	2.46	1.94	1.71	2.06	2.00	2.37	2.55			
Ammonia + urea	M	1.72	1.93	1.04	−1.05	−1.88	−2.37	−1.78	−1.86	−1.15	−0.38	0.33	0.68[*]	0.32	0.0001	0.0001
	D	0.69	1.15	1.26	0.27	−0.07	0.04[*]	−1.03	−1.78	−1.93	−1.81	−0.88	−0.12			

[a] M = ruminal starch injection during the meal (08:15); D = ruminal starch injection 3 hours after the meal (11:15).
[b] Higher than the other diet at the same time, [*] = $P < 0.05$, [†] = $P < 0.10$.
[c] 'Repeated measures analysis of variance, within animals effects'.
[d] EMS = error mean square.

urea net flux (y, mg of N·min^{-1}) and NH$_3$ concentration in the rumen (x, mg of N·L^{-1}): y = 0.018 × − 4.05 (r^2 = 0.13, n = 96). This is in agreement with the observations of Rémond et al. [12] according to which urea net transfer across the rumen wall was not significantly affected by an increase in rumen NH$_3$ concentration of 100 mg of N·L^{-1} (the range of variation observed in the present study). Urease activity and CO$_2$ are known to promote urea net transfer across the rumen wall [12], and the best prediction of urea net flux from ruminal traits was: urea net flux (mg of N·min^{-1}) = 0.008 (rumen ammonia, mg of N·L^{-1}) – 0.047 (CO$_2$, % of rumen gas) – 1.330 (urease activity, IU·mL^{-1}) + 0.139 (r^2 = 0.47, P < 0.01, n = 64), with a partial regression coefficient of 0.32 for CO$_2$, 0.08 for urease activity and 0.07 for NH$_3$. Despite the combination of these rumen traits the accuracy of the prediction remained low compared to what was reported by Rémond et al. [11] in sheep fed hay, suggesting that others factors are involved in short-term regulation of urea net flux across the rumen wall.

With both treatments, the extraction rate of arterial urea by the rumen wall (urea net uptake expressed as a proportion of arterial flux) was increased during starch fermentation (Fig. 4). This extraction rate decreased linearly with the increase in rumen ammonia concentration (r^2 = 0.44, n = 96). This negative relationship is in agreement with the observations of Rémond et al. [13]. Since Houpt et Houpt [6] it is generally accepted that urea crosses the rumen epithelium by simple diffusion. If so, the permeability of epithelial cell membranes to urea is not modified in the short-term. Therefore, the most plausible explanation for this relationship would be a negative effect of rumen ammonia concentration on the speed of urea degradation by the bacterial population adhering to the rumen mucosa, which would affect the urea rumen-blood concentration gradient.

The net balance of urea and ammonia N fluxes across the rumen wall (ammonia + urea net flux) showed the highest values of N losses from the rumen at the end of the meal and the highest values of N gain for the rumen 3 to 4 h after starch injection (Tab. I). Rumen loss of N due to the absorption of ammonia from the feed protein rapidly degraded in the rumen is thus balanced by a gain of N (through urea cycling) when energy availability is high whereas feed N availability becomes low. In agreement with previous observations in sheep fed hay [11], throughout the feeding cycle, there was a close relationship between ruminal ammonia concentration (x, mg of N·L^{-1}) and the net balance of urea and ammonia N fluxes across the rumen wall (y, mg of N·min^{-1}): y = 0.0309 × − 3.1695 (r^2 = 0.62, n = 96). Figure 5 shows a compilation of the data from Rémond et al. [11] and the present experiment. The observed relationship suggested that the instantaneous N net balance of urea and ammonia exchange across the rumen wall results in a loss of N above 95 mg of ammonia-N·L^{-1} in the rumen, and a gain of N below this value.

The time of starch injection into the rumen did not modify the daily means of rumen traits (Tab. II). However starch supply during the meal tended to direct the fermentation to more propionate and less butyrate than starch supply 3 h after the meal (P < 0.10). In comparison with Rémond et al. [11], where sheep received the same diet without starch, higher rumen concentration of CO$_2$ (60 vs. 55% of gas content) and VFA (95 vs. 77 mM), and lower NH$_3$ concentration (88 vs. 115 mg of N·L^{-1}) were observed. This decrease in ammonia level is in agreement with what is observed with sugar-based supplements in sheep fed dried herbage diets [7, 10]. Despite the decrease in daily rumen ammonia concentration with starch addition, daily ammonia net release was similar between Rémond et al. [11] and the present work

Figure 4. Variations in urea extraction rate by the rumen wall according to the time of starch supply. Values are means of four sheep ± SE. Differences between diets: * $P < 0.05$, † $P < 0.10$.
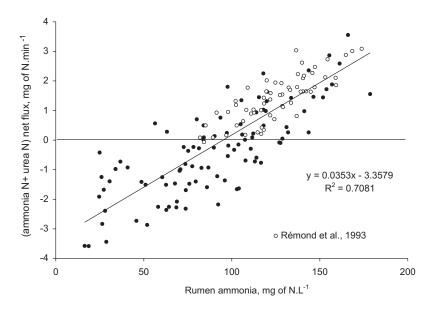

Figure 5. Relation between rumen ammonia concentration and the balance of urea and ammonia N net fluxes across the rumen wall.

(Tab. II). Feeding increasing levels of concentrate results in a large rise in the absorptive surface area of the rumen wall, through an increase in the size and number of papillae on the luminal surface of the rumen [5, 17]. Furthermore increased concentrations of VFA and CO_2 in the rumen (as observed with starch supply) favour the absorption of

ammonia during short-term experiments [3, 12]. The combination of these two effects (long-term for the surface area, short-term for fermentation end products) probably explains why ammonia net release did not differ between Rémond et al. [11] and the present work.

Daily transfer of urea across the rumen wall in the present work (Tab. II) was twice that reported by Rémond et al. [11] in sheep fed the same diet without starch supply. Similarly, Norton et al. [9] reported that adding barley to a hay diet increased urea transfer across the rumen wall from 1.4 to

2.4 g of $N \cdot d^{-1}$. In both cases, the increase in urea transfer occurred despite a decrease in uraemia. This increase may be related to the short-term effect of rumen traits as discussed above, but may also be the consequence of changes in the morphology of the rumen epithelium. Feeding concentrate not only increases the number and size of rumen wall papillae, but also the contact area between epithelium and connective tissues [15], and the surface of the epithelial capillary network [4]. High intake of easily fermentable carbohydrates is generally associated with an increase in the thickness of the

Table II. Effect of the time of starch injection into the rumen, on daily means of rumen traits, rumen blood flow, and nutrient concentrations and net fluxes in ruminal veins of sheep. Values are means of four sheep.

Item	Diet[a]		Statistics	
	M	D	SEM[b]	P
Ruminal traits				
pH	6.45	6.47	0.05	0.847
Ammonia, mg of $N \cdot L^{-1}$	91.9	83.4	8.93	0.433
Urease activity, $IU \cdot mL^{-1}$	0.93	0.83	0.20	0.695
CO_2, % ruminal gas	60.3	59.1	2.91	0.615
Total VFA, mM	94.6	96.0	4.93	0.833
Acetate, %	63.5	65.0	2.26	0.582
Propionate, %	19.3	14.4	2.66	0.073
Butyrate, %	11.2	14.0	2.08	0.141
Arterial concentration, mg of $N \cdot L^{-1}$				
Urea	97.9	81.6	10.68	0.172
Ammonia	1.07	1.38	0.21	0.185
Ruminal net fluxes				
Urea, g of $N \cdot d^{-1}$	–4.66	–4.39	0.60	0.572
Ammonia, g of $N \cdot d^{-1}$	3.92	3.65	0.78	0.616
Ammonia + urea, g of $N \cdot d^{-1}$	–0.74	–0.74	0.61	1.000

[a] M = ruminal starch injection during the meal; D = ruminal starch injection 3 h after the meal.
[b] SEM = standard error of the mean.

epithelium layers, which may increase the resistance to diffusion [5]. Therefore the increase in the surface area available for urea diffusion from blood to the rumen probably explain a large part of the increase in the ability of the rumen wall to eliminate urea observed when easily fermentable carbohydrates are added to a hay diet.

4. CONCLUSION

Within the feeding cycle, whatever its time of supply, the fermentation of starch produced an increase in urea net transfer across the rumen epithelium. During this period of high availability in energy, rumen ammonia concentration was low, and the rise in urea transfer across the rumen epithelium provided an additional source of N for microbial synthesis. Thereafter, when starch fermentation dropped (VFA and CO2 concentrations decreased), ammonia accumulated in the rumen, and urea net transfer decreased. Thus, at any time of the day, blood urea, which is mainly derived from rumen ammonia-N loss during the degradation of easily fermentable N, helps to maintain an ammonia concentration favourable for microbial growth when dietary N availability is low.

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