

## Physiological Approach to Improve Efficiency of Nitrogen Utilization in Ruminants

<sup>1</sup>Khalid Abdoun and <sup>2</sup>Holger Martens

<sup>1</sup>Department of Animal Production, Faculty of Food and Agriculture Science, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia

<sup>2</sup>Institute of Veterinary Physiology, Free University of Berlin, Oertzenweg 19b, 14163 Berlin, Germany

**Abstract:** It was the aim of this study to characterize the factors which could enhance urea recycling into the rumen and consequently improve N utilization and reduce the N release into the environment. The sources for animal nitrogen are dietary nitrogen and endogenous nitrogen (mainly urea nitrogen). Utilization of dietary nitrogen can be improved by nutritional approaches such as dietary energy-nitrogen synchronization and increasing the portion of Un-degradable Dietary Protein (UDP). This study focused on trials which could improve endogenous N-utilization through manipulation of urea recycling. The study was conducted on isolated rumen epithelial tissue of sheep using conventional Ussing-chamber technique. Feeding conditions prevailing a ruminal pH of 6.0-6.4 seems to be optimal for better urea N utilization and reduction of nitrogen release into the environment. Feeding conditions leading to lower ruminal pH values (<6) can be expected to reduce the use of endogenous urea-N for the synthesis of microbial protein and hence to increase urea excretion via urine with an adverse environmental impact.

**Key words:** Ruminant, urea recycling, nitrogen utilization, efficiency Kingdom of Saudi Arabia, Germany

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### INTRODUCTION

It has long known of the ability of camels, cows or sheep to shift the excretion of urea from the kidney (Read, 1925; Schmidt-Nielsen and Osaki, 1958) to the gastrointestinal tract. The transport of urea through the rumen epithelium was first demonstrated *in vivo* and *in vitro* many years ago.

In the rumen, dietary cellulose is broken down by bacteria that utilize urea-nitrogen for synthesis of microbial proteins. After passage into the duodenum, the amino acids of these proteins are absorbed and reach the liver where new urea for secretion into the rumen can be formed.

Recycling of nitrogen via urea secretion into the rumen thus, allows these animals to survive on low-protein diets while producing milk and meat for human consumption (Abdoun *et al.*, 2006; Kennedy and Milligan, 1980; Reynolds and Kristensen, 2008). The quantities of nitrogen recycled widely vary and might account for up to 25% of the nitrogen ingested or up to 90% of urea turnover (Reynolds and Kristensen, 2008; Harmeyer and Martens, 1980; Lapierre and Lobley, 2001; Marini *et al.*, 2006). It was the intention of this study to characterise the physiological factors that could influence nitrogen utilization efficiency in ruminants.

### MATERIALS AND METHODS

This study was conducted under short circuit conditions using the conventional Ussing-chamber technique with isolated epithelial tissues from sheep rumen. All buffer solutions contained (mmol L<sup>-1</sup>) 140 Na<sup>+</sup>, 5 K<sup>+</sup>, 1 Ca<sup>2+</sup>, 1 Mg<sup>2+</sup>, 104 Cl<sup>-</sup>, 1 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 2 HPO<sub>4</sub><sup>-2</sup>, 10 glucose and 40 gluconate with 1 phenyl-phosphorodiamidate (urease inhibitor) and 1 urea. In HCO<sub>3</sub><sup>-</sup>-containing solutions, 25 mmol L<sup>-1</sup> gluconate was replaced by 25 mmol L<sup>-1</sup> HCO<sub>3</sub><sup>-</sup>.

In Short Chain Fatty Acids (SCFA) containing solutions, 40 mmol L<sup>-1</sup> gluconate was replaced by 25 mmol L<sup>-1</sup> acetate, 10 mmol L<sup>-1</sup> propionate and 5 mmol L<sup>-1</sup> butyrate. These concentrations are well-tolerated by tissues *in vitro* and reflect the physiologically relative proportions found in the rumen.

<sup>14</sup>C-labelled urea (46.25 kBq) was added to the hot side of the epithelium and three flux periods of 20 min were performed after an equilibration time of 40 min.

Samples from the hot side were taken before the first and after the last flux period for the calculation of the specific radioactivity and assayed by using a β-counter (LKB Wallace-Perkin-Elmer; Uberlingen/Germany). The sample volume was replaced by the corresponding buffer.

Table 1: Means ( $\pm$ SE) of effects of short chain fatty acids on bidirectional urea flux rates across the rumen epithelium in the absence of  $\text{CO}_2/\text{HCO}_3^-$

Groups	$J_{ms}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{sm}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{ns}^{urea}$ (nmol/cm <sup>2</sup> /h)	N/n
pH 7.4-SCFA	20.45 $\pm$ 01.28 <sup>a</sup>	18.88 $\pm$ 01.50 <sup>a</sup>	1.57 $\pm$ 01.92 <sup>a</sup>	3/9
pH 6.4-SCFA	21.07 $\pm$ 02.01 <sup>a</sup>	22.86 $\pm$ 02.36 <sup>a</sup>	-1.79 $\pm$ 01.35 <sup>a</sup>	3/9
pH 6.4+SCFA	83.63 $\pm$ 16.41 <sup>b</sup>	82.41 $\pm$ 12.08 <sup>b</sup>	1.22 $\pm$ 10.35 <sup>a</sup>	3/9

Means ( $\pm$ SE) in the same column with different letters are significantly different at  $p < 0.05$ . N = Number of animals; n = number of tissues for each treatment

Table 2: Means ( $\pm$ SE) of effects of  $\text{CO}_2$  and mucosal pH on bidirectional urea flux rates across the rumen epithelium in the absence of SCFA

Groups	$J_{ms}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{sm}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{ns}^{urea}$ (nmol/cm <sup>2</sup> /h)	N/n
pH 7.4, 5% $\text{CO}_2$	28.28 $\pm$ 1.28 <sup>a</sup>	25.82 $\pm$ 1.500 <sup>a</sup>	2.46 $\pm$ 1.920 <sup>a</sup>	3/9
pH 6.4, 5% $\text{CO}_2$	68.12 $\pm$ 2.01 <sup>b</sup>	63.97 $\pm$ 2.360 <sup>b</sup>	4.15 $\pm$ 1.350 <sup>a</sup>	3/9
pH 7.4, 10% $\text{CO}_2$	43.62 $\pm$ 6.41 <sup>c</sup>	39.21 $\pm$ 12.08 <sup>c</sup>	4.41 $\pm$ 10.35 <sup>a</sup>	3/9
pH 6.4, 10% $\text{CO}_2$	122.51 $\pm$ 7.63 <sup>d</sup>	127.27 $\pm$ 5.560 <sup>d</sup>	-4.76 $\pm$ 4.360 <sup>a</sup>	3/9

Means ( $\pm$ SE) in the same column with different letters are significantly different at  $p < 0.05$ . N = Number of animals; n = number of tissues for each treatment

Table 3: Means ( $\pm$ SE) effects of mucosal pH on urea flux rates across the rumen epithelium in the presence of  $\text{CO}_2$  and SCFA

pH	$J_{ms}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{sm}^{urea}$ (nmol/cm <sup>2</sup> /h)	$J_{ns}^{urea}$ (nmol/cm <sup>2</sup> /h)	N/n
Mucosal pH 7.4	34.61 $\pm$ 3.780	26.15 $\pm$ 1.900	8.46 $\pm$ 3.240	4/16
Mucosal pH 6.4	151.68 <sup>c</sup> $\pm$ 16.39	155.06 <sup>c</sup> $\pm$ 15.65	-3.38 $\pm$ 16.44	4/16

<sup>c</sup> $p < 0.05$  compared with control. N = Number of animals; n = number of tissues for each treatment

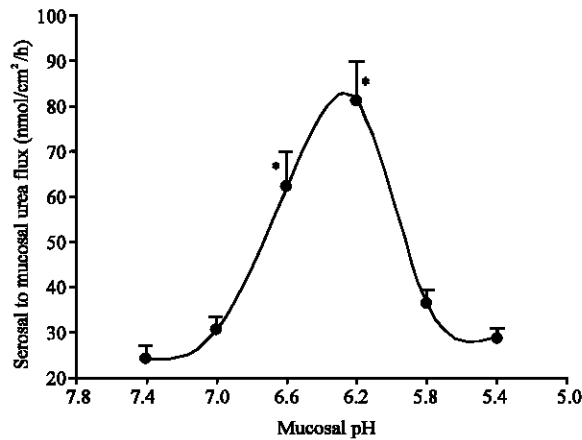


Fig. 1: Effect of decreasing mucosal pH on  $J_{sm}^{urea}$  flux rates across the rumen epithelium in the presence of SCFA and absence of  $\text{CO}_2/\text{HCO}_3^-$ . N = 2; n = 8. \* $p < 0.05$  compared to pH 7.4

## RESULTS AND DISCUSSION

Lowering of luminal pH had minor effects on urea flux rates (Table 1). Only after the physiological situation was simulated more completely by the addition of SCFA and  $\text{CO}_2$  at a pH of 6.4, large stimulatory effects were observed on urea flux (Table 1-3), corresponding to the well-documented effects observed *in vivo* (Harmeyer and Martens, 1980; Remond *et al.*, 1993; Thorlacius *et al.*, 1971; Engelhardt and Nickel, 1978).

Interestingly, a stepwise reduction of pH in the presence of SCFA (without  $\text{CO}_2$ ) from 7.4-5.4 led to a bell-shaped modification of urea transport (Fig. 1). Transport rates were low at pH 7.4 and 7.0, rising to maximal levels at pH 6.2 followed by a sharp drop to the original level when pH was lowered to  $< 5.8$ . The inhibition

of urea transport by low luminal pH of  $< 6.0$  may be of significant practical importance. Thus, the attempt to raise urea recycling by increasing intraruminal fermentation with the production of  $\text{CO}_2$  and SCFA by feeding highly fermentable carbohydrates can result in failure (Gozho *et al.*, 2008) which is to be expected since ruminal pH frequently drops to values below 5.7 under these conditions.

## CONCLUSION

Feeding conditions with low ruminal pH values ( $< 6$ ) can be expected to reduce the use of endogenous urea-N for the synthesis of microbial protein on the one hand, whereas on the other hand, urea excretion via urine is increased with an adverse environmental impact. A better understanding of the factors that increase or decrease the gastrointestinal recycling of urea *in vitro* and *in vivo* thus, appears to be of central importance in reducing production costs and the release of nitrogen into the environment.

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