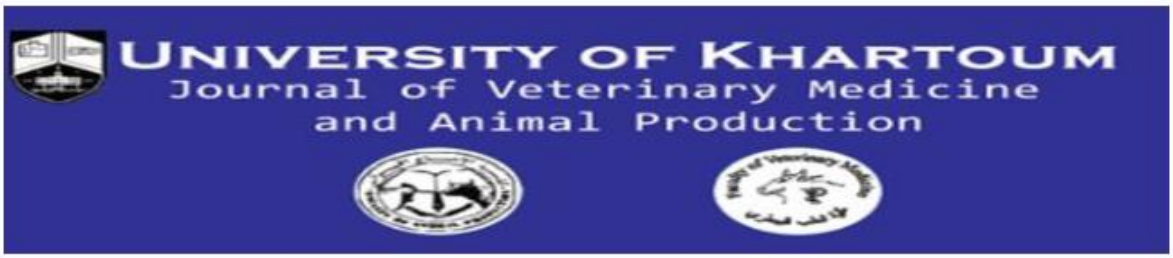
*U. of K. J. Vet. Med. Anim. Prod., Vol.9, Issue 1 (2018) page 17- 26*
*ISSN: 243456*




# Effect of pH on the Transport Capacity of $Na^+$ Across the Isolated Omasal and Ruminal Epithelia of Sheep

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## Abstract

This study was conducted in attempt to characterize the electroneutral transport of $Na^+$ across isolated omasmal and ruminal epithelia of sheep after treatment with pH and amiloride. The conventional Ussing chamber technique was used to measure the mucosal to serosal flux ($J_{ms}$), the serosal to mucosal flux ($J_{sm}$), the net flux ($J_{net}$), the short circuit current ($I_{sc}$), and tissue conductance ($G_t$) of isolated epithelia. A total of 12 pairs of omasmal and ruminal epithelia were used for each treatment, which categorized as: control group (pH 7.4), low pH group (pH 6.4), amiloride group (pH 7.4) and amiloride + low pH group (pH 6.4). The unidirectional transport values of $Na^+$ were significantly ($p<0.001$) higher in the omasum compared to the rumen. Lowering the pH to 6.4 significantly ($p<0.001$) enhanced the unidirectional transport of $Na^+$ in the omasum accompanied by marked changes in the electrochemical parameters ($I_{sc}$ and $G_t$); however, the enhancement of the unidirectional flux of $Na^+$ in the rumen did not reach the significant level. Application of amiloride in the mucosal side caused a significant ($p<0.001$) reduction in $J_{ms}$, $J_{sm}$ and $J_{net}$ of both epithelia. The findings confirmed that $Na^+$ transported by means of electroneutral transport systems in the omasum was similar to the system confirmed previously in the rumen. The electroneutral transport of $Na^+$ in the omasum is more efficient than the that in the rumen.

***Key words:*** *Sheep, $Na^+$ transport, pH, omasum, rumen*

## المستخلص

أجريت هذه الدراسة في محاولة لتوصيف كفاءة النقل المتعادل الكهربي لأيون الصوديوم ( $Na^+$) عبر الظهارة المعزولة للخراف بعد المعالجة بـإستخدام الأس الهيدروجيني و مركب أميلوريد amiloride. تم إستخدام تقنية Ussing chamber التقليدية لقياس معدل التدفق المخاطي إلى المصلي ($J_{ms}$) والتدفق المصلي إلى المخاطي ($J_{sm}$)ومعدل التدفق الصافي ($J_{net}$) ، تيار الدائرة الكهربية القصيرة ($I_{sc}$) وتوصيل الأنسجة الطلائية المعزول ($G_t$) . تم إستخدام 12 زوجا من ظهارة الورقية والكرش لكل معاملة ،والتي صنفت على النحو التالي: مجموعة شاهد (الأس الهيدروجيني 7.4) ، ومجموعة منخفضة الأس الهيدروجيني (الأس الهيدروجيني 6.4) ومجموعة amiloride (الأس الهيدروجيني 7.4) و مجموعة amiloride + أس هيدروجيني منخفض (amiloride + أس الهيدروجيني 6.4). كانت قيم النقل أحادي الاتجاه لـ $Na^+$ أعلى معنويًا ($P<0.05$) في الورقية مقارنة مع الكرش. أدى خفض الأس الهيدروجيني بشكل كبير إلى 6.4 إلى تعزيز ($P <0.05$) النقل أحادي

الإتجاه لـ $Na^+$ في حالة الورقية مصحوبًا بتغيرات ملحوظة في القياسات الكهروكيميائية ($I_{sc}$) و ($G_t$) ؛ ومع ذلك ، فإن تعزيز التدفق أحادي الإتجاه ل $Na^+$ في الكرش لم يصل إلى مستوى كبير ذو قيمة معنوية إحصائياً . تطبيق amiloride في الجانب المخاطي للظهارة تسبب في إنخفاض معنوى كبير ($P < 0.001$) في $J_{ms}$ و $J_{sm}$ و $J_{net}$ في كل من ظهارة الورقية والكرش. أكدت النتائج أن أيونات ($Na^+$) فى الورقية تم نقلها بواسطة أنظمة النقل المتعادل الكهربي الشبيهة بالنظام المؤكد في السابق في الكرش التى تم إثباتها. يعتبر النقل الكهربائي لأيونات ($Na^+$) فى الورقية أكثر كفاءة من نظيره فى الكرش.

***كلمات مفتاحية:*** *الضأن، نقل أيون الصوديوم، الأس الهيدوجينى، الورقية، الكرش*

## Introduction

Large amounts of $Na^+$ are provided into the forestomach of the ruminants from the saliva, due to their active salivary glands and/or in feed. The ruminal epithelia are able to transport up to 50% of the salivary secreted $Na^+$ (Dobson, 1959), which clearly shows the importance of the rumen in electrolyte balance and homeostasis. It is clear from *in vitro* studies that $Na^+$ net ($J_{net}^{Na+}$) transport is considerably higher than short circuit current ($I_{sc}$) projecting that most of $Na^+$ is transported by the means of electroneutral transport systems (Ferreira *et al.*, 1972; Harrison *et al.*, 1975; Martens and Gäbel, 1988). Martens and Gäbel, (1988) suggested a $Na^+/H^+$ exchanger indirectly coupled to $Cl^-/HCO_3^-$ as a main mechanism for electro-silent transport of $Na^+$ and $Cl^-$ in the rumen, which gives a suitable interpretation for the interaction between $Na^+$ and $Cl^-$ transported across the ruminal epithelium as reported previously (Ferreira *et al.*, 1972; Chien and Stevens, 1972; Martens and Blume, 1987). However, *in vivo* studies showed an enhancement of $Na^+$ transport in response to the increase in the luminal $K^+$ concentration in sheep (Martens and Hammer, 1981).

The unidirectional flux rates of $Na^+$ are known to be stimulated by the presence of short chain fatty acids (SCFA) (Gäbel *et al.*, 1988; Diernaes *et al.*, 1994). Noteworthy, this stimulatory effect is coupled by the enhancement of active transport of $Cl^-$ across the rumen epithelium of bovine, but not ovine (Diernaes *et al.*,1994; Sehested *et al.*, 1996). According to Sehested *et al.*, (1996), this stimulatory effect may be due to the metabolic effect of SCFA. The same authors concluded that the produced $CO_2$ due to the metabolism of SCFA could stimulate the apical $Na^+/H^+$- and $Cl^-/HCO_3^-$ exchangers running in parallel via increasing $H^+$ and $HCO_3^-$ gradients.

The absorption of electrolytes ($Na^+$, $K^+$ and $Cl^-$), SCFA and water has been detected in the omasum of sheep and goats (von Engelhardt and Hauffe, 1975), and calves (Edrise *et al.*,1986). However, our knowledge about the underlying transport mechanisms of these substances is very limited compared to the rumen (Martens and Schweigel, 2000; Gäbel *et al.*, 2002). Martens and Gäbel (1988) stated that there is a possible interaction between $Na^+$ transport and the transport of anions ($Cl^-$, $HCO_3^-$ and SCFA) in the omasum of sheep. However, the same authors stated that $Na^+$ transport is predominantly mediated via electroneutral $Na^+/H^+$-exchanger (NHE) and only to small extend electrogenic transport as represented by $I_{sc}$ in *in vitro* study with isolated epithelium of sheep omasum.

Schulthei ß and Martens, (1999) showed that the electrogenic $Na^+$ transport was not sensitive against 1mmol/l of amiloride mucosal concentration (as NHE inhibitor) and not stimulated by serosal addition of 1µmol/l of aldosterone. In addition, the same authors found that the removal of mucosal divalent cations ($Ca^{++}$ and $Mg^{++}$) considerably enhanced $I_{sc}$ and $J_{net}^{Na+}$, which supports the assumption that the electrogenic $Na^+$ transport in sheep omasum significantly differs from the classical $Na^+$ transport in frog skin or rabbit colon (Biber and Mullen 1976; Frizzell *et al.*, 1976). Thus, these observations led to a model of $Na^+$ transport, which consists of electroneutral $Na^+$ transport via $Na^+/H^+$ exchanger and electrogenic $Na^+$ transport through an unknown cation channel. However, inhibition of electroneutral $Na^+$

transport with amiloride reduces $J_{ms}$ and $J_{net}$ $^{Na+}$ significantly, but $J_{net}$ was in the presence of 1mmol/l amiloride always larger than the $I_{sc}$. Obviously, the concentration of amiloride was either not high enough for complete inhibition of $Na^+/H^+$ exchanger or a further electroneutral mechanism exists. Hence, it was hypothesized that most of $J_{sm}$ $Na^+$ is electroneutral and possibly transcellular, which is definitely in contrast to the current models of $Na^+$ transport of absorptive epithelia. Therefore the aim of the present study is to further characterize the electroneutral transport of $Na^+$ in the omasum and the rumen with special emphasis on the gap between $J_{net}$ and $I_{sc}$ after treatment with pH and amiloride

## Materials and Methods

**Experimental animals:** Omasal and ruminal epithelial tissues were obtained from sheep (*Ovis aries*) of various breeds, weights and ages from both sexes. The animals had free access to a lick stone and tap water, and they were fed on hay *ad libitum* at least 3 weeks before the experiment. The chemical composition and the nutritive value of hay feed are shown in Table 1.

**Table 1.** Chemical composition and nutritive value of hay feed

| **Dietary Component** | **%** |
|---|---|
| DM | 93.5 |
| CP (% DM) | 8.8 |
| NDF (% DM) | 56.5 |
| ADF (% DM) | 34.0 |
| Ash (% DM) | 4.9 |
| NE (MJ/kg DM) | 5.5 |
| *DM: Dry matter, CP: Crude protein, NDF: Neutral Detergent Fibre, NDF: Acid Detergent Fibre,* NE*: Net energy for lactation* | |

**Isolation, preparation and handling of the epithelial tissues:** The preparation and the incubation of omasal and ruminal epithelia have been described in detail by Martens *et al.*,(2004) and Gäbel *et al.*,(1991), respectively. Two to three minutes after stunning and exsanguination of the sheep, the forestomach and the abomasum were removed from the abdominal cavity. The omasum was separated from the reticulum and the abomasum, opened with a longitudinal cut along the omasal canal, everted and carefully cleaned with warm buffer solution. Six to eight large leaves were removed from the wall of the omasum with pair of scissors and carefully cleaned by immersion in a buffer solution until the solution remained clean. Whilst immersed in a buffer solution, mucosal sheets on the two surfaces of the leaves were cautiously separated by blunt dissection and cut into pieces for use in Ussing chambers. Regarding rumen, a piece from the ventral ruminal sac (approximately 300 $cm^2$) was removed. The piece was rinsed with warm (38°C) basic buffer solution until the solution remained clear. Then, the epithelium was manually stripped off the muscle layer and transferred to the laboratory in warmed (38°C) basic buffer solution. In laboratory, the epithelium was cut into pieces of about 4×4 cm and mounted between the two halves of the Ussing chamber. Then the epithelia were transferred to a transport experimental buffer solution in the Ussing chamber (Table 2), which was continuously gassed with 95% $O_2$ + 5% $CO_2$ and kept at 38°C. The time period necessary for the preparation, transportation and mounting of the epithelia was approximately 30–45 min.

**Experimental design:** All experiments were conducted under the short-circuit condition in the Ussing chamber. A chamber consisted of two equal halves (Fig. 1); between them the

epithelium has to be mounted. Thus, dividing the chamber into two equal compartments, one which represents the blood side (serosal or basolateral side) and the other represents lumen side (mucosal or apical side).

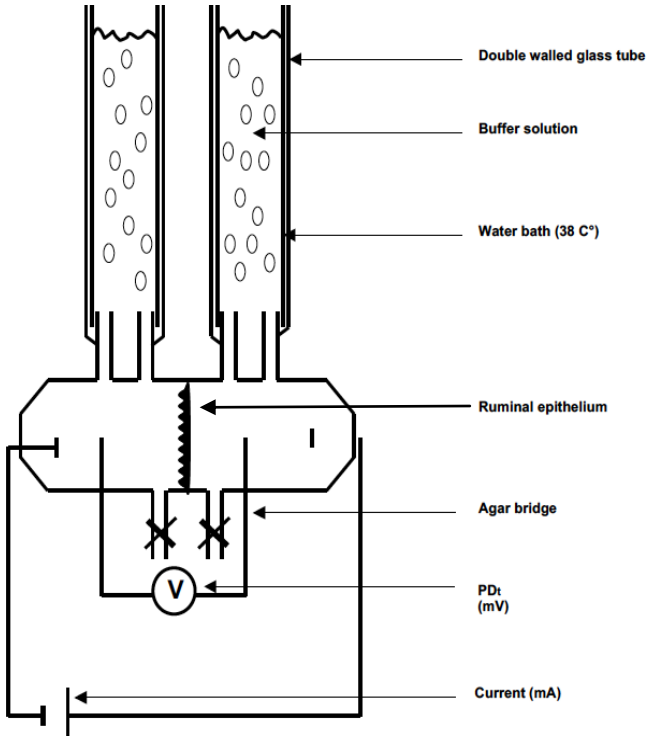


**Fig.1** Ussing chamber model

The experiments started after an equilibration period of the epithelial tissues with the experimental buffer solution (Table 2) for not less than 30 min to ensure a relatively stable state for all the electrophysiological parameters. After this equilibration period, only tissues with $G_t$ not more than 6.0 mS·cm-2 and $I_{sc}$ not less than 1.0 µeq·cm-2·h-1 were used in all experiments. Under these conditions, the experimental omasum epithelial tissues remain stable for relatively long period of time with a slight decrement of $I_{sc}$ per hour (Martens and Gäbel, 1988). For the determination of the ion fluxes (mucosal to serosal flux, $J_{ms}$ and serosal to mucosal flux, $J_{sm}$), the mounted tissues were paired when the electrophysiolgical parameters ($G_t$ and $I_{sc}$) did not differ by more than 25% after equilibration (30 min). For each set of experiment 6 pairs of epithelia (when 2 clamps were used) or 12 pairs (when 4 clamps were used) were available at the same time.

To study the effect of the pH, the tissues were allocated into two groups; the control group in which the pH of the buffer solution (Table 2) in both luminal and serosal sides was adjusted to 7.4 ± 0.1 using TRISMA (tris-hydroxymethyl-aminomethan), and low pH group in which the pH was adjusted to 6.4 ± 0.1 at the luminal side using 1 mmol/l HCL and 7.4 ± 0.1 in the blood side (TRISMA). Amiloride (1 mmol/l) was added to the luminal side in attempt to inhibit NHE.

**Table 2.** Composition of the transport and experimental buffer solutions

| **Substance** | **Transport buffer** | **Experimental buffer** |
|---|---|---|
| NaCl | 115.00 | 60.00 |
| KCl | 5.00 | - |
| $NaHCO_3$ | 25.00 | 25.00 |
| $NaH_2PO_4 \cdot H_2O$ | 0.40 | - |
| $Na_2HPO_4 \cdot H_2O$ | 2.40 | - |
| $KH_2PO_4 \cdot H_2O$ | - | 1.00 |
| $K_2HPO_4 \cdot H_2O$ | - | 2.00 |
| Glucose ($C_6H_{12}O_6 \cdot H_2O$) | 5.00 | 10.00 |
| MOPS ($C_7H_{15}NO_4S$) | - | 8.00 |
| Na-Acetate ($C_2H_3NaO_2 \cdot 3H2O$) | - | 25.00 |
| Na-Propionate ($C_3H_5NaO_2$) | - | 10.00 |
| Na-Butyrate ($C_4H_7NaO_2$) | - | 5.00 |
| $CaCl_2 \cdot 2H_2O$ | 1.20 | 1.00 |
| $MgCl_2 \cdot 6H_2O$ | 1.20 | 1.00 |
| Gas | ($95\%O_2 + 5\% CO_2$) | ($95\%O_2 + 5\% CO_2$) |

**Measurement of $Na^+$ flux rates:** For the determination of $Na^+$ flux rate, radioactive isotope $^{22}Na$ (Amersham Buchler, Braunschweig) was used. $Na^+$ net flux rate was calculated as a difference between $J_{ms}$ and $J_{sm}$ ($J_{net} = J_{ms} - J_{sm}$). After the equilibration period, pairing of the epithelia, and marking of the mucosal and serosal sides, 70 kBq of $^{22}Na$ were added either to the marked mucosal side (for determination of $J_{ms}$) or to the marked serosal side (determination of $J_{sm}$). The side to which the radioactive isotope was added was referred as "hot side", while the other ones was referred as "cold side". After 10 min of the radioisotope addition, 100 µl from the experimental buffer solution were taken from the hot side. These samples were referred to "$H_1$". The same protocol was done at the end of the experiment and the samples were referred to "$H_2$". $H_1$ and $H_2$ were used to calculate the specific activity. Periodic samples of 2 ml from the cold side of the experimental buffer solution were taken in interval of 30 min. In the mean 3-5 flux periods were done. To keep the volume in the reservoir constant throughout the experiment, each 2 ml sample from the cold side was replaced by the same volume from the respective experimental buffer. $^{22}Na$ was assayed using a well-type crystal ɤ- counter (LKB; Wallace-Perkin Elmer, Überlingen, Germany).

**Statistical analysis:** Statistical analysis was carried out by using SPSS program version 10 for Windows (Jandel, Chicago, IL, USA). The comparison between the treatment groups was performed by an analysis of variance (ANOVA). Results are given as means ± SEM (standard error of the mean).

## Results

Fig.2 represents the effects of luminal pH on unidirectional $Na^+$ transport rate of the omasum and rumen epithelial cells. In all treatments (control: 7.4, low pH: 6.4 and amiloride group), the unidirectional transport of $Na^+$ was significantly ($p<0.001$) higher in the omasum compared to the rumen values. Moreover, $J_{ms}^{Na+}$ was significantly ($p<0.001$) higher than the values of the $J_{sm}^{Na+}$ in the both omasum and ruminal epithelia.

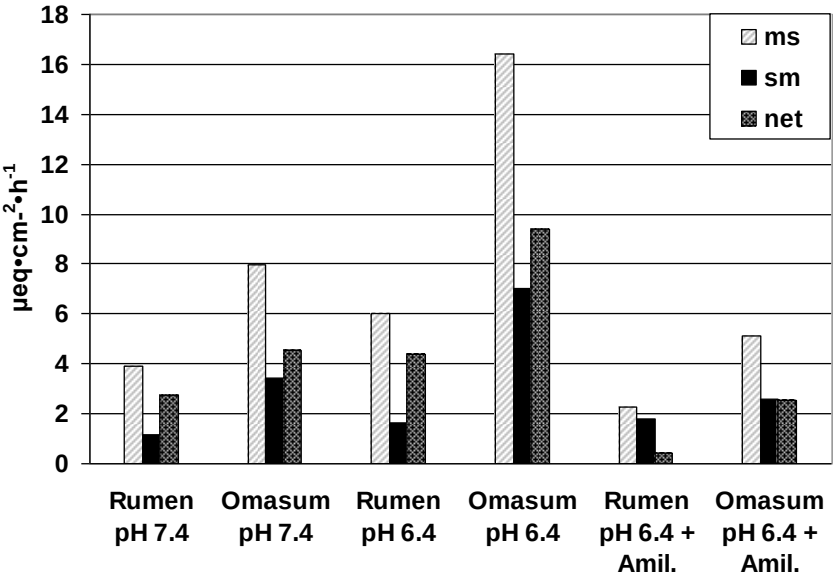


**Fig.2** Effect of low pH (6.4) and amiloride (1 mmol/l) at the mucosal side of ruminal and omasal epithelium on $Na^+$ transport

Table 3 indicates that the lowering the pH to 6.4 significantly ($p<0.001$) enhanced the unidirectional transport of $Na^+$ in the omasum (almost increased by double rate); however, the level of increase was not statistically significant in the rumen (almost increased by 30%). Significant changes ($p<0.001$) in the omasum were noticed also in the electrochemical parameters ($I_{sc}$ and $G_t$); the significant increment in $Na^+$ absorption seems to be via an electroneutral mechanism. The significant effect of the luminal pH on $J_{ms}^{Na+}$ support the assumption of enhanced SCFA uptake in the undissociated form, release of protons by dissociation of SCFA in the epithelial cells, hence, increased activity of $Na^+/H^+$ exchanger. The $J_{net}$ after treatment with amiloride is higher than $I_{sc}$ in the omasum epithelium (Table 3).

**Table 3.** Effect of decreasing the luminal pH (pH 6.4) on the unidirectional Na transport rate in omasal epithelium with and without addition of 1 mmol/l amiloride at luminal side (Mean ± SEM).

| Treatment | $J_{ms}^{Na+}$ (µeq.cm-2.h-1) | $J_{sm}^{Na+}$ (µeq.cm-2.h-1) | $J_{net}^{Na+}$ (µeq.cm-2.h-1) | $I_{sc}$ | $G_t$ (mS.cm-2) |
|---|---|---|---|---|---|
| Control (pH 7.4) | 7.99 [b] ± 0.66 | 3.42 [b] ± 0.28 | 4.57 [b] ± 0.48 | 1.43 [b] ± 0.10 | 2.86 [a] ± 0.28 |
| Low pH (pH 6.4) | 16.44 [a] ± 1.88 | 7.02 [a] ± 0.84 | 9.41 [a] ± 1.23 | 1.51 [a] ± 0.07 | 2.81 [a] ± 0.23 |
| Amiloride (pH 7.4) | 4.02 [c] ± 0.29 | 2.15 [c] ± 0.18 | 1.86 [c] ± 0.22 | 1.05 [c] ± 0.08 | 3.20 [b] ± 0.31 |
| Amiloride (pH 6.4) | 5.10 [c] ± 0.42 | 2.58 [c] ± 0.26 | 2.53 [c] ± 0.37 | 0.95 [d] ± 0.05 | 3.17 [b] ± 0.27 |

*Means within the same column bearing different superscripts are significantly different at p≤0.05*

## Discussion

The SCFA are the natural products of carbohydrate fermentation in the forestomach of ruminants and in the colon of many mammals. Their transport across the epithelium could occur in the dissociated (ionized) and/or undissociated (protonated) form (Gäbel *et al.*, 2002). The positive interaction between SCFA and $Na^+$ transport is generally known and the underlying mechanisms are well established (Fig. 3): SCFA are taken up across luminal membrane in the protonated (lipophilic) form by diffusion. Protons released intracellularly by dissociation of SCFA (pK 4.8). Also, the decrease in pH in the cytosol (pHi) activates $Na^+/H^+$-exchanger and mediates Na uptake and proton extrusion. (d) $Na^+$ is pumped out of the cell by $Na^+/K^+$ ATPase in the basolateral membrane (Gäbel and Martens, 1991; Gäbel *et al.*, 2001).

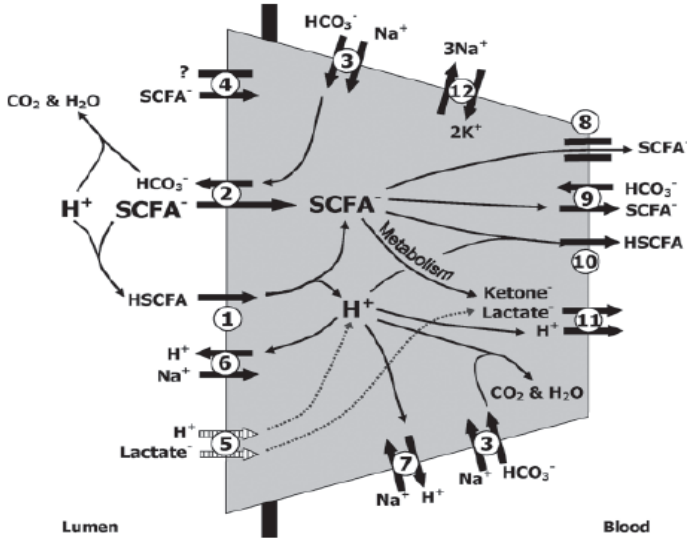


**Fig. 2** Model on organic acid transport in ruminal epithelial cells (Aschenbach *et al.*, 2011).

On the other hand, Sehested *et al.,* (1996) reported that 60-70% of the active $Na^+$ transport in the rumen epithelium in the presence of the SCFA is amiloride sensitive and mediated via $Na^+/H^+$- exchanger (NHE). Despite the well documented positive effect of SCFA on the electroneutral $Na^+$ transport in the rumen (Dierneas *et al.,* 1994), the reciprocal effect of the $Na^+$ on SCFA is controversy. Neither amiloride nor total replacement of $Na^+$ influence SCFA transport in sheep rumen (Kramer *et al.,* 1996). However, some studies reported an inhibitory effect of amiloride on SCFA transport in the caecum and proximal colon of guinea pig (von Engelhardt *et al.,* 1993), and some a direct relationship between the SCFA transport across the ruminal epithelia and the concentration of the $Na^+$ (Sehested *et al.,* 1996).

It is well known that the lowering the pH enhance the absorption of SCFA (pK ~ 4.8) in the undissociated form. The protonated SCFA dissociate intracellularly providing protons, hence, lowering the pHi. Consequently, $Na^+/H^+$ exchanger will be stimulated resulting in enhancement of electroneutral $Na^+$ absorption. The current result confirms this assumption since it was demonstrated that the significant increase in $Na^+$ absorption was due to enhancement of the electroneutral component, as confirmed by addition of 1 mmol/l amiloride to the luminal side. Marten and Gäbel, (1988) demonstrated that the largest part of $Na^+$ transport process across the isolated omasum epithelia occurs by means of an electroneutral component, which is amiloride sensitive. The current results agree with what was reported by (Marten and Gäbel, 1988) which showed that there is a considerable amount of $Na^+$ transported by amiloride in-sensitive mechanism. Noteworthy, the present results and the results of the previous study (Marten and Gäbel, 1988) demonstrated that not only $J_{ms}$ but also $J_{sm}$ are significantly inhibited by apical application of amiloride. Since the addition of amiloride was done after 3 flux periods, the significant reduction in $I_{sc}$ in the current study in the omasum might be due to the time-dependent effect as demonstrated by Marten and Gäbel (1988).

## Conclusions

The present study indicates a higher unidirectional flux of $Na^+$ in the omasum compared to the ruminal values. This assumption is strengthened by the effect of mucosal amiloride application, which caused a significant reduction of $J_{ms}$, $J_{sm}$ and $J_{net}$. Previous studies in different polarized epithelial cells indicated that NHE located in the apical and basolateral sides of these epithelia in multiple isoforms (NHE1, NHE2, NHE3 and NHE4) (Knickelbein *et al.,* 1990; Montrose *et al.,* 1997; Vilella *et al.,* 1992; Busche *et al.,* 1997). These isoforms could be expressed in the omasal epithelia more than the ruminal epithelia.

## Acknowledgments

We are grateful to K. Wolf and U Tietjen for expert technical assistance. This work was supported by "Deutsche Forschungsgemeinschaft" (GA and DAAD).

## References

Aschenbach JR, Penner GB, Stumpff F, Gäbel G. (2011). Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J Anim Sci. 89:1092-1107.

Biber TU and Mullen TL. (1976). Saturation kinetics of sodium efflux across isolated frog skin. Am. J. Physiol. 231(4): 995-1001.

Busche B, Bartels J, Genz AK and von Engelhardt W. (1997). Effect of SCFA on intracellular pH and intracellular pH regulation of guinea-pig caecal and colonic enterocytes and of HT29-19a monolayers. Comp Biochem Physiol A 118: 395-398.

Chien WJ, Stevens CE. (1972). Coupled active transport of Na and CI across forestomach epithelium. Am J Physiol. 223:997-1003.

Diernaes L, Sehested J, Moller PD and Skadhauge E. (1994). Sodium and chloride transport across the rumen epithelium of cattle in vitro: effect of short-chain fatty acids and amiloride. Experimental Physiology. 79: 755–762.

Dobson A. (1959) Active transport through the epithelium of the reticulo-rumen sac. J Physiol. 146: 235-251.

Edrise BM, Smith RH and Hewitt D. (1986). Exchange of water and certain water-soluble minerals during passage of digesta through the stomach compartments of young ruminanting bovines. Br. J. Nutr. 55: 157-167.

Ferreira HG, Harrison FA, Keynes RD and Zurich L. (1972). Ion transport across an isolated preparation of sheep rumen epithelium. J. Physiol. 222:77–93.

Frizzell RA, Koch MJ and Schultz SG. (1976). Ion transport by rabbit colon. I. Active and passive components. J. Membr. Biol. 27(3): 297-316.

Gäbel G and Martens H. (1991). Transport of Na and Cl across forestomach epithelium: mechanisms and interactions with short-chain fatty acids. In: Tsuda T, Sasaki Y and Kawashima R (editors). Physiological Aspects of Digestion and Metabolism in Ruminants. Orlando, Florida: Academic Press, pp. 129–151.

Gäbel G, Smith E, Martens H. (1988). Stimulation of rumen Na absorption by short chain fatty acids. Pfltigers Arch (Suppl. 1) 411:R107.

Gäbel G , Müller F , Pfannkuche H and Aschenbach JR. (2001). Influence of isoform and DNP on butyrate transport across the sheep ruminal epithelium. J. Comp. Physiol. B 171:215–221.

Gäbel G , Aschenbach JR and Müller F . (2002). Transfer of energy substrates across the ruminal epithelium: Implications and limitations. Anim. Health Res. Rev. 3:15–30.

Harrison FA, Keynes RD, Rankin JC and Zurich L. (1975). The effect of ouabain on ion transport across isolated sheep rumen epithelium. J. Physiol. 249: 669-677.

Knickelbein RG, Aronson PS and Dobbins JW. (1990). Characterization of Na+/H+ exchangers on villus cells in rabbit ileum. Am J Physiol 259: G802-G806.

Kramer T, Michelberger T, Gürtler H, Gäbel G. (1996). Absorption of short-chain fatty acids across ruminal epithelium of sheep. J Comp Physiol B. 166:262-69.

Martens H and Hammer U. (1981). Absorption von Natrium und Magnesium aus dem vorubergehend isolierten Pensen von Schafen wahrend intrravenoser Infusion von Aldosteron. Dtsch. Tierarztl. Wschr. 88: 404-407.

Martens H and Blume I. (1987). Studies on the absorption of sodium and chloride from the rumen of sheep. Comp. Biochem. Physiol. A 86: 653-656.

Martens H and Gäbel G. (1988). Transport of Na and Cl across the epithelium of ruminant fore stomachs: rumen and omasum. A review. Comparative Biochemistry and Physiology. A 90: 569–575.

Martens H and Schweigel M. (2000). Pathophysiology of grass tetany and other hypomagnesuim implications for clinical management. Vet. Clin. North Am. Food Anim. Pract. 16(2): 339-368.

Martens H, Krutzfeld T and Wolf K. (2004). Sodium transport across the isolated epithelium of sheep omasum is influenced by luminal ammonia. J. Vet. Med. A Physiol. Pathol. Clin. Med. 51(2): 46-51.

Montrose MH and Chu S. (1997). Transepithelial SCFA gradients regulate polarized Na/H exchangers and pH microdomains in colonic epithelia. Comp Biochem Physiol A 118: 389-393

Schultheiss G and Martens H. (1999). Ca-sensetive Na transport in sheep omasum. Am. J. Physiol. 276(39): G1331-G1344.

Sehested J, Diernaes L, Moller PD and Skadhauge E. (1996). Transport of sodium across the isolated bovine rumen epithelium: interaction with short-chain fatty acids, chloride and bicarbonate. Experimental Physiology. 81: 79–94.

Shen Z, Seyfert HM, Lhrke B, Schneider F, Zitnan R, Chudy A., Kuhla S, Hammon HA, Blum JW, Martens H, Hagemeister H and Voigt J. (2004). An energy-rich diet causes rumen papillae proliferation associated with more IGF type 1 receptors and increased plasma IGF-1 concentrations in young goats. J. Nutr. 134:11–17.

Vilella S, Guerra L, Helmle-Kolb C and Murer H. (1992). Characterization of basolateral Na/H exchange (Na/H-1) in MDCK cells. Pflugers Arch 420: 275-280.

Von Engelhardt W, Burmester M, Hansen K, Becker G, Rechkemmer G. (1993). Effects of amiloride and ouabain on short-chain fatty acid transport in guinea-pig large intestine. J Physiol. 460:455- 66.

Von Engelhardt W and Hauffe R. (1975). Role of the omasum in absorption and secretion of water and electrolytes in sheep and goats. Page 216 in Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner, ed. Univ. New England Publ. Unit, Armidale, New South Wales, Australia.