Aus dem Institut für Veterinär-Physiologie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Transport of HCO_3^- in Sheep Omasum: Effects of Na and SCFA

Inaugural-Dissertation
zur Erlangung des Grades eines Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Driton Çaushi
Tierarzt aus Gjakovë/Kosovo

Berlin 2014
Journal-Nr. 3716

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Holger Martens
Zweiter Gutachter:	Univ.-Prof. Dr. Kerstin Müller
Dritter Gutachter:	Univ.-Prof. Dr. Johanna Plendl

Deskriptoren (nach CAB-Thesaurus):
Horses, sweet itch, eczema, Culicoides, treatment, cytokines, ELISA, allergic reactions, plasma cells, B lymphocytes, ligands

Tag der Promotion: 21.11.2014

Gefördert durch die Margrete-Markus-Charity und durch ein Stipendium des Deutschen Akademischen Austauschdienst (DAAD)

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

ISBN: 978-3-86387-557-2
Zugl.: Berlin, Freie Univ., Diss., 2014
Dissertation, Freie Universität Berlin
D 188

Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

Dedicated to my mother

Table of Contents

List of Abbreviations

AE = Anion exchanger

AgCl = Silver chloride

ATP = Adenosine triphospate

°C = Celsius centigrade

CA = Carbonic anhydrase

Ca = Calcium

CFTR = Cystic fibrosis transmembrane regulator

Cl = Chloride

CLD = Congenital chloride diarrhea

cm^2 = Square centimeters

CO_2 = Carbon dioxide

DMSO = Dimethyl sulfoxyde

DRA = Down Regulated Adenoma

g = Gram

G_t = Tissue conductivity

h = Hour

H^+ = Proton

HCO_3^- = Bicarbonate ion

H_2CO_3 = Carbonic acid

H_2O = Water

H_2SO_4 = Sulfuric acid

HSCFA = Undissociated short chain fatty acid

I_{sc} = Short circuit current

J_{ms} = Mucosal to serosal flux

J_{net} = Net flux

J_{sm} = Serosal to mucosal flux

K = Potassium

kg = Kilogram

l = Liter

log = Logarithm

Mg = Magnesium

µA = Microampère

µeq = Microequivalent

µm = Micromol

min = Minute

MJ = Megajoule

ml = Millilitre

mM = Millimolar

mmol = Millimole

ms = Mucosal-serosal

mV = Millivolt

n = Number of epithelia

N = Number of animals

Na = Sodium

NaCl = Sodium chloride

NBC = Sodium Bicarbonate Cotransporter

NDCBE = Na^+- driven Cl^-/HCO_3^- exchanger

NH_3 = Ammonia

NH_4^+ = Ammonium-Ion

NHE = Sodium/hydrogen exchanger

O_2 = Oxygen

p = p value, probability

PD = Potential difference

PD_t = Transepithelial potential difference

pH = Potentia hydrogenii

pH = Intracellular potentia hydrogenii

pK-value = The negative base-10 logarithm of the acid dissociation constant of a solution.

R_c = Cell resistance

R_s = Paracellular resistance

R_t = Transepithelial resistance

RT-PCR = Reverse transcriptase – Polymerase Chain Reaction

SCFA = Short chain fatty acids

HSCFA = Undissociated short chain fatty acids

SEM = Standard error of the mean

SLC = Solute carrier

sm = Serosal-mucosal

1. INTRODUCTION

Animals belonging to the suborder **Ruminantia** possess a uniquely developed digestive tract. The forestomachs, which are an enlargement of the front part of the stomach, represent a large fermentation chamber where the microbial degradation of food takes place. It is actually through this activity of the microorganisms which colonize the forestomachs that these animals utilize the low digestible energy in plants. The forestomachs consist of the rumen, the reticulum and the omasum.

The omasum is the third and smallest compartment of the forestomach. Initially, the earliest studies suggested that its main functions are mechanical, namely that its laminae serve as a grinding mill for the food particles (Ellenberger, 1881). It has been, since then, accepted, that the main functions of the organ are a) transport of food particles from the rumen to the abomasum, b) prevention of larger particles from leaving the reticulo-rumen and c) absorption of water and electrolytes.

A net absorption of various electrolytes (Na^+, K^+, Mg^{2+}, NH_4^+, short chain fatty acids - SCFA and HCO_3^-) and water in the omasum of sheep has been established. Furthermore, previously conducted in vitro studies (Tiling, 1997; Wegeler, 2008) have shown that this epithelium has the unique capability to absorb parallel a base (HCO_3^-; pK = 6.1) and an acid (undissociated short chain fatty acid = HSCFA; pK = 4.8). In addition, given the normal in vivo transepithelial concentration gradients of HCO_3^- (mucosal to serosal) and Cl^- (serosal to mucosal), a net secretion of Cl^- was observed (Hauffe and von Engelhardt, 1975; Tiling, 1997). As a result, an exchange of HCO_3^- and Cl^- through anion exchangers in series in the apical and basolateral membrane was proposed as a transport mechanism for these two anions: HCO_3^- absorption and Cl^- secretion (Tiling, 1997; Niebuhr, 2003; Wegeler, 2008).

The parallel luminal uptake of HCO_3^- via HCO_3^-/ Cl^- exchange and of HSCFA by lipid diffusion requires on the one hand the availability of Cl^- in the subapical compartment of the multilayered omasal epithelium for HCO_3^-/Cl^- exchange and on the other hand potent mechanism(s) of intracellular pH (pH_i) regulation to ensure that transcellular HCO_3^- transport can proceed without formation of gas ($H^+ + HCO_3^- = H_2O + CO_2$) despite the acidic challenge resulting from the uptake of HCSFA and the release of H^+.

The intracellular release of protons from luminal HSCFA uptake induces an increase of Na^+/H^+ exchange (NHE) activity and it is assumed that the activity of this exchanger is the first line of defence of pH_i (Ali et al., 2006). Consequently, the luminal Na^+ concentrations, with Na^+ providing the driving force for the activity of the NHE, is of major importance as a protective mechanism on the one side and the luminal presence and absorption as HSCFA can be considered as an acidifying challenge on the other side for pH_i.

2. LITERATURE REVIEW

2.1. Anatomical and histological aspects of the omasum in sheep

The omasum is located on the floor of the intrathoracic part of the abdominal cavity, at the level of the 8th and 10th ribs. It is an almost spherical organ, slightly flattened on the sides. The right face (Facies parietalis) of the omasum touches the diaphragm, the gall bladder and the liver, while the left face (Facies ventralis) touches the rumen. It is connected to the reticulum through the reticulo-omasal opening (Ostium reticulo-omasicum) and to the abomasum through the omaso-abomasal opening (Ostium omaso-abomasicum). Its mucosal side is covered by non-glandular cornified stratified squamous epithelium and its thickness measures approximately 77 micron (Lubis and Oshea, 1978). The epithelia form leave-like, concave free borders structures of four different sizes called omasal laminae. The surface of the laminae is covered by very small papillae of different structure, size and shape, depending on their site.

Different numbers of laminae in sheep omasum are reported, varying from 35 (McSweeney, 1988) to 64-88 (Chandrasekar, 1992). The laminae have three thin smooth muscle layers which consist of an intermediate layer and two lateral layers. The intermediate layer penetrates into the underneath connective tissues until it attaches to the inner and outer layers of the tunica muscularis, while the lateral layers run parallel to the free border of the laminae (Yamamoto et al., 1991a).

Although the weight of the omasum is considered to be only 10% of the total weight of the forestomachs of sheep (Warner and Flatt, 1965), this organ has a very efficient absorptive capacity, calculated to be up to 30% of the total absorptive capacity of the reticulo-ruminal epithelia (Hauffe and von Engelhardt, 1975), due to the presence of the laminae and hence, enlargement of the surface area.

It has also been observed that the epithelium covering the laminae and particularly the interpapillary space is very thin, with a very rich network of blood vessels underneath in the subepithelial layers (Flavilli, 1937; Yamamoto et al., 1991b, 1994).

2.2. Physiological functions of the omasum

Being the last part of the non-secretory forestomach of ruminants, the omasum mainly assists in a) transport of ingesta from the forestomach into the abomasum, b) prevention of outflow of larger particles from the reticulo-rumen and c) absorption of water and various electrolytes.

2.2.1. Passage of ingesta

Due to its contracting capacity, the omasum functions as a two-stage pump, aspirating initially reticular contents into the omasal canal and pumping the more fluid contents into the omasal body, and finally expressing the omasal body contents into the abomasum (Stevens et al., 1960). The passage of the main part of ingesta from reticulum to omasum is thought to be attributed mainly to the negative pressure in the omasal canal and less from the relaxation of the omasal body, during the peak of the second reticular contraction. A second, minor passage of ingesta occurs after the contraction of the omasal canal (Ehrlein and Hill, 1969; Ehrlein, 1979). This cycle of contractions is linked to the motility of the reticulo-rumen and occurs 2 – 3 times in two minutes (Ehrlein and Hill, 1969).

2.2.2. Prevention of outflow

The prevention of outflow of larger particles from the reticulo-rumen into the omasum relies on the particle density of the reticulum contents, where the larger and less-dense ones are returned into the rumen during the reticular contraction and the smaller and dense particles are aspirated into the omasum (Kaske et al., 1987, 1991). This sieving mechanism increases the retention time of particles in the reticul-ruminal compartment and hence the time for microbial fermentation and rumination until the size of the particles is small enough for passage (Kaske et al., 1987, 1991).

2.2.3. Absorptive properties

A feature shared by epithelia, beside their protective function, is their ability to transport water and solutes (Powell et al., 1985; Martens, 1995). The transepithelial transport has two possible routes: the transcellular route (across the plasma membrane of the epithelial cells) and/or the paracellular route (across tight junctions between epithelial cells). Transcellular transport involves the passage of substances through the apical and basolateral membrane. These membranes act as two resistances (R_a+R_b), which together form the cell resistance (R_c) (Powell, 1981). This type of transport across membranes can be passive, driven by chemical or electrical gradients (downhill), as is the case with the transport of undissociated short chain fatty acids (HSCFA). Furthermore, passive transport can be mediated via carriers as Na/H exchanger or ion channels. Active transport requires pumps as Na/K-ATPase, which hydrolyses ATP and permits uphill transport against chemical and/or electrical gradients.
Paracellular transport on the other hand, consists in the movement of solutes through the tight junctions between the cells and is in all cases passive and hence depends on electrical or chemical gradients. Paracellular resistance (R_s) is determined by tight junctions, and, along with the cell resistance constitutes the tissue resistance (R_t).

Depending on their resistance, epithelial tissues can be classified into leaky (when R_t is <1000 Ω•cm^2) or tight epithelia, when the resistance is higher than that value (Powell, 1981). Tissue conductance (G_t), which represents the reciprocal of the resistance, can be increased through the activation of channels or pumps in the cell membrane.

The omasum epithelium, according to its paracellular resistance, is considered to be moderately tight (Schultheiss and Martens, 1999).

2.2.4. Transport across the omasal epithelium

Early studies have shown that the omasum has the capability to absorb various electrolytes as Na^+, K^+, ammonia, SCFA and water (Engelhardt and Hauffe, 1974; Edrise and Smith, 1979). These in vivo data clearly show the absorptive capacity of the omasum which is remarkably high in calves. Furthermore, the net secretion of Cl^- must be considered as a surprising observation. This secretion is linked to HCO_3^- absorption which is a particular transport mechanism of the omasum (Engelhardt and Hauffe, 1975b).The transport capacity of the major ions of the omasum is summarized in Table 1.

Table 1: In- and outflow, including absorption/secretion of water, Na^+, K^+, SCFA and Cl^- in omasum of sheep, goat and calves.

Substance	Inflow	Outflow	Absorption (% of inflow)
Sheep and Goats (ENGELHARD und HAUFFE, 1976)			
Water [l/d]	5.37	4.66	13.1
Na [mmol/d]	397	294	26
SCFA [mmol/d]	ca. 500	ca. 250	~50
Cl [mmol/d]	95	186	-196
Bull Calves (EDRISE et. al., 1986)			
Water [l/d]	22.4	12.8	43
Na [Mol/d]	2.5	1.14	54
K [Mol/d]	0.55	0.47	15
Cl [Mol/d]	0.22	0.62	-282

2.2.4.1. Cl^- and HCO_3^- transport in sheep omasum

Earlier research has shown a net secretion of Cl^- in the omasum of sheep, by examining the contents of different segments of the forestomach (Pfeffer et al., 1966). Studies conducted in cows delivered similar findings, consisting at the same time of a decrease in the HCO_3^-

concentration (Ekman and Sperber, 1953). All these studies, including those *in vivo*, found a correlation between Cl^- secretion and HCO_3^- absorption (Engehardt und Hauffe, 1975b; Edrise and Smith, 1979; Edrise and al. 1986; Oyaert and Bouckaert, 1961). However, most of the *in vitro* studies have found a net absorption of Cl^- under Ussing chamber conditions with a high concentration of Cl^- in the buffer solution on both sides of the tissue (Harrison, 1971; Höfelmeier, 1991; Martens and Gäbel, 1988). In contrast, in an *in vitro* Ussing chamber study, carried out by Tilling under physiological concentrations of Cl^- (high at the serosal side) and HCO_3^- (high at the mucosal side), a chloride secretion was observed.

Studies centered directly on bicarbonate transport in the omasum have been very limited. Research conducted by Engelhardt and Hauffe (1975a) in sheep with fitted sleeves in the omasal-obamasal orifice revealed high transport rates of HCO_3^-, at 3,9 $mM \bullet h^{-1}$, which were the highest rates along with those of SCFA absorption. The first direct proof of HCO_3^- absorption came from in vitro research by Niebuhr (2003), using a combination of the conventional Ussing-chamber technique (Ussing, 1949) and the pH-Stat method.

Cl^-/HCO_3^- exchangers

Intracellular pH, pH_i, in mammalian tissues is regulated through the activity of a range of acid-base transporters. Bicarbonate transporters, among others transport proteins, are involved in this process in epithelia. To date, the known bicarbonate transporters belonging to a superfamily of proteins have been classified into three functional groups (McMurtrie et al., 2004, Cordat, Casey, 2009; Romero et al., 2013,):

1) Na^+ independent Cl^-/HCO3 exchangers (AE), gene family SLC4
2) Na^+-coupled Cl^-/HCO3 exchangers and co-transporters (NDCBE, NBC), gene family SLC4
3) Anion exchangers of the gene family SLC26

Na^+ independent Cl^-/HCO_3^- exchangers (AE)

These exchangers belong to the SLC4 gene family and mediate electroneutral exchange of monovalent anions, the preferred substrates being Cl^-, HCO_3^-, although they can transport OH^- too (Jennings ML, 1976). The physiologically relevant transport in living cells is the exchange of Cl^- for HCO_3^-, and the transmembrane chemical gradients for these two ions determine the direction of net transport (Romero, Fulton et al., 2004).

Three isoforms belong to this functional group (AE1, AE2 and AE3).

AE1 mediates Na^+ independent anion exchange in red blood cells and the renal collecting duct, AE2 is widely expressed in non-excitable tissues, where it has been proposed to be a housekeeping Cl^-/HCO_3^-, and AE3 splice variants have been found in different organs and tissues including the heart and brain (Pushkin and Kurtz, 2006). The AE2 mRNA, in fact, has been already detected in sheep omasum (Wegeler, 2008).

Na^+-coupled Cl^-/HCO_3^- exchangers and cotransporters (NDCBE, NBC)

This group of exchangers belongs likewise to the SLC4 family. There are two Na^+/HCO_3^- electrogenic cotransporters (NBCe1 and NBCe2), an electroneutral Na+/HCO_3^- cotransporter (NBCn1), and the Na^+- driven Cl^-/HCO_3^- exchanger – NDCBE (Romero, Fulton et al., 2004). Members of this functional group have been found in various organs. In omasum, the direction of exchange for HCO_3^- and Cl^- seems to be chemical gradient dependent, therefore a presence of a Na^+/HCO_3^- cotransporter seems less probable. The observed interaction between the electroneutral Na^+ transport (Na^+/H^+ exchanger) (Schultheiss, 1995; Schultheiss and Martens, 1999) and CO_2/HCO_3^- is presumably due to availability of H^+ supplied by activity of carbonic anhydrase.

Anion exchangers of the gene family SLC26

This functional group includes 11 members, with SLC26A10 likely being a pseudogene (Dorwart et al., 2008). Based on their functional similarities, SLC26 transporters have been grouped into three groups. Group 1 (SLC26A1 and SLC26A2) are selective sulphate transporters, group 2 (SLC26A3, SLC26A4, and SLC26A6) are coupled Cl^-/HCO_3^- exchangers, and group 3 members function as ion channels and include SLC26A7 and SLC26A9 (Dorwart et al., 2008). The transport modes of SLC26A8 and SLC26A11 are not known, and SLC26A5 does not appear to function as anion exchanger in mammals (Schaechinger TJ, Oliver D., 2007). Group 2 Cl^-/HCO_3^- exchangers are distributed in the luminal membrane of secretory epithelia and mediate Cl^- absorption and HCO_3^- secretion. SLC26A3 (DRA = Down Regulated Adenoma) is an electroneutral exchanger predominantly found in the digestive system. A dysfunctional SLC26A3 plays an important role in congenital chloride diarrhea (CLD), a disease with a high Cl^- content and low pH as its main clinical feature (Höglund P, 2006). SLC26A4 is an exchanger with a poorly understood mode of transport, expressed in the kidney, cochlea, thyroid and salivary gland and its mutations are related to Pendred syndrome and non-syndromic hearing loss, while SLC26A6 is found in the intestines and pancreatic duct, with a role in the overall epithelial fluid and electrolyte secretion (Dorwart et al., 2008).

2.2.4.2. Na^+/H^+ exchanger (NHE)

NHE, sodium proton exchangers, is a family of proteins known for their vital role in cellular physiology and pathophysiology. Their most important functions include regulation of intracellular pH and cell volume as well as functions in transepithelial transport. They accomplish these functions by extruding proton from, and taking up Na^+ ion into the cell. Response to pharmacological compounds exhibits variations and supports the notion of an extended family of NHE molecules. Following the pivotal work of Murer et al. (1976), where

the first isoform has been described, at least nine isoforms of this protein have been identified in mammalian cells. Each isoform is designated with a numeric suffix (1, 2, 3, etc.) reflecting the chronological order of its cloning. The first NHE is designated NHE1 and the most recent one is designated NHE9 (Yun et al., 1995; Noel and Pouysségur, 1995; Masereel et al., 2003; Goyal et. al., 2003; de Silva et al., 2003). The isoforms differ in tissue localization, sensitivity towards inhibitors and mode of transcriptional and posttranscriptional regulation. Accordingly, they participate in a wide range of physiological processes taking place in the cell. NHE1 is ubiquitously expressed and plays a central housekeeping role in intracellular pH (pH_i) and cell volume homeostasis (Orlowski and Grinstein, 1997; Counillon and Pouysségur, 2000). In contrast, the isoforms 2-5 have a more limited distribution and are more specialized in functions. NHE8 and 9 are the most recently discovered members of this extended family of proteins (Goyal et al., 2003; de Silva et al., 2003).

The NHE's can be divided into plasma membrane and intracellular, organellar isoforms (Zachos et al., 2005). The established plasma membrane isoforms include NHE1-5 (Sardet et al., 1989; Tse et al., 1991, 1992 and 1993; Orlowski et al., 1992; Klanke et al., 1995; Baired et al., 1999). The plasma membrane isoforms are further divided into those having the ability to cycle and recycle between intracellular endosomes and plasma membrane, such as NHE3 (Janecki et al., 1998) and NHE5 (Szaszi et al., 2002) and those that permanently reside on the plasma membrane including NHE1, 2, and 4 (Pizzonia et al., 1998; Cavet et al., 2001). The organellar isoforms include NHE6 and 7 which have been localized in the recycling endosomes and trans-Golgi network, respectively (Numata and Orlowski et al., 2001; Brett et al., 2002). NHE8 and 9 are considered to be organellar isoforms although their intracellular localisation has not yet been established (Zachos et al., 2005). Interestingly, a role for NHE1, NHE2 and NHE3 has been proposed in mediating HCO_3^- uptake in the kidney of mouse models.

Electroneutral Na^+ transport via NHE has been demonstrated very early in *in vitro* studies in bovine, goat (Chien and Stevens, 1971) and sheep rumen epithelium (Martens et al., 1991). In a recent study, Graham et al. (15) have demonstrated the expression of mRNA of NHE1-3 and NHE8 by reverse transcription with the polymerase chain reaction (RT-PCR) in bovine rumen epithelium. The authors showed with immunostaining that NHE1 is apically localized in the stratum granulosum of the multilayered squamous rumen epithelium and discussed that NHE1 mediates electroneutral Na^+ uptake across the apical membrane. These findings were not confirmed by a study of Rabbani et al. (2011) which provided conclusive evidence on the crucial role of NHE3 in mediating transepithelial transport of Na^+ across the rumen epithelium of sheep and bovine (Rabbani et al., 2011) and in sheep omasum too (Dölle, 2008).

Structure of NHE

All mammalian NHE share a common structure with some 400 amino acids in the N-terminal half of the protein spanning the plasma membrane 12 times. The transmembrane domain is responsible for amiloride-sensitive Na^+/H^+ exchange function. A further region of approximately 400 amino acids in the carboxy-terminal half of the protein constitutes the cytoplasmatic domain with regulatory functions (Wakabayashi et al., 1992; Weinman et al., 2005).

Regulation of NHE

The NHE1 and NHE3 have been studied in more detail and the mRNA of these isoforms has been detected in omasal epithelium of sheep (Etschmann, unpublished). Hence, the following short summary will be restricted to important aspects of the regulation of NHE1 and NHE3.

Acute regulation

a. Modifier site: The activation of Na^+/H^+ exchange by pH_i is considerably steeper than can be explained by simple Michaelis-Menten kinetics. Aronson et al. (1982) suggested an additional cytoplasmatic binding side for protons known as the H^+ modifier site. Truncation studies of the cytoplasmatic domain have shown that Na^+/H^+ exchange activity and the function of the modifier site are preserved until almost complete removal of the cytoplasmatic domain (Wakabayashi et al., 1992). These functions (NHE and modifier site) are located in the N-terminal half of the protein.

b. Set point: The cytoplasmatic domain is the target of protein kinases and binds various regulatory factors. Deletion of the cytopasmatic domain shifts the NHE1 activity to an acidic range (Wakabayashi et al., 1992) and mutation of histidine in the cytoplasmatic domain of NHE3 lowers the set point by 0.3 – 0.6 pH units (Cha et al., 2003). In addition, NHE activity is regulated by interactions with other proteins such as NHE regulatory factor 1 (NHERF-1). Phosphorylation is not required (NHE 1; Wakabayashi et al. 1994).

c. NHE trafficking: NHE3 is not only located in the apical membrane, but substantial amounts are located in subapical vesicles which can be inserted in the luminal membrane and hence, increasing transport capacity (V_{max}; see review Zachos et al. 2005). There is evidence that PI 3-kinase is involved in vesicle trafficking (Cheyron et al., 2003; Blazer-Yost and Nofziger, 2005) as well as NHERF-2 (Lee-Kwon et al., 2003).

d. Regulation by NHERF-1: There is compelling evidence that NHE3, the PDZ protein NHERF and ezrin-PKa form a multi-protein complex, which is linked to actin (Weinman et al., 2005). The acute regulation of NHE by phosphorylation is mediated via NHERF (Weinman et al., 2005b; Donowitz et al., 2005).

Chronic regulation

a. Intestine: Chronic exposure of epithelial cells to low pH increased NHE3 mRNA and protein abundance. Musch et al. (2001) exposed human colonic monolayers (C2/bbe) to SCFA and observed a time and concentration dependent increase of NHE3 activity, of NHE3 protein and mRNA. The results were confirmed in the colon of rats fed a pectin rich diet.

b. Kidney: It has been known for decades that the kidney has the potential to respond in a precise manner to minor changes of acid-base-metabolism. The cascade of regulation has been studied in more detail in kidney cell lines. In a recent study of Li et al. (2004), the proline rich tyrosine kinase (PyK2) was shown to function as an intracellular “acid sensor”. The signalling cascade involved binding of PyK2 to c-Src kinase, with phosphorylation and activation of this enzyme, followed by activation of the MAPK (mitogen-activated protein kinase) and the JNK signalling pathway. Ultimately, the pathway leads to an increase of transcription of NHE3. Hence, kidney epithelial cells exhibit an “acid sensor” and a cascade of regulation (Li et al., 2004).

2.2.4.3. Short chain fatty acids (SCFA) transport

SCFA provide 70-80 % of the energy requirements in ruminants (Siciliano-Jones and Murphey, 1989; Bergman, 1990). They are one of the main fermentation products that cover the energy demand for maintenance and production activities. The rumen is recognized as being the main compartment for SCFA production and absorption (85% of SCFA are absorbed in the rumen, according to Engelhardt and Hauffe, 1975). It is well established that SCFA in the rumen are transported via a carrier-mediated transport mechanism across the apical (Gäbel et al. 2002) and basolateral (Tyagi et al. 2002) membrane, and a similar mechanism was proposed for the omasum. However, recent in vitro studies with the omasum support the idea that SCFA are taken up predominantly in a protonated form (Ali et al., 2005) and there is no evidence that SCFA are taken up by the anion exchanger in the apical membrane (Tiling, 1997). The uptake of the protonated form of SCFA transport requires automatically a mechanism for proton extrusion and recycling, which is proposed to be provided by NHE (Ali et al., 2005). The basolateral exit of SCFA as an anion is mediated by a large anion conductance (Georgi et al., 2013).

2.3. Putative Transport Model of HCO_3^-

The omasum represents an important site for the absorption of ions and water in the ruminant’s forestomach. This capacity is enhanced by its enlarged surface area in the leaves (laminae) and the histological structure of the epithelium. The absorptive properties of this

organ have been conclusively confirmed and demonstrated for H_2O, SCFA, as well as for electrolytes such as Na^+,K^+, HCO_3^- and Cl^- secretion (Figure 1).

The scheme of the proposed model presented in Figure 1, which has been established from previous studies (Tiling, 1997; Wegeler, 2008; Beisele, 2008), describes the significant transport mechanisms and pathways on which the current study is based.

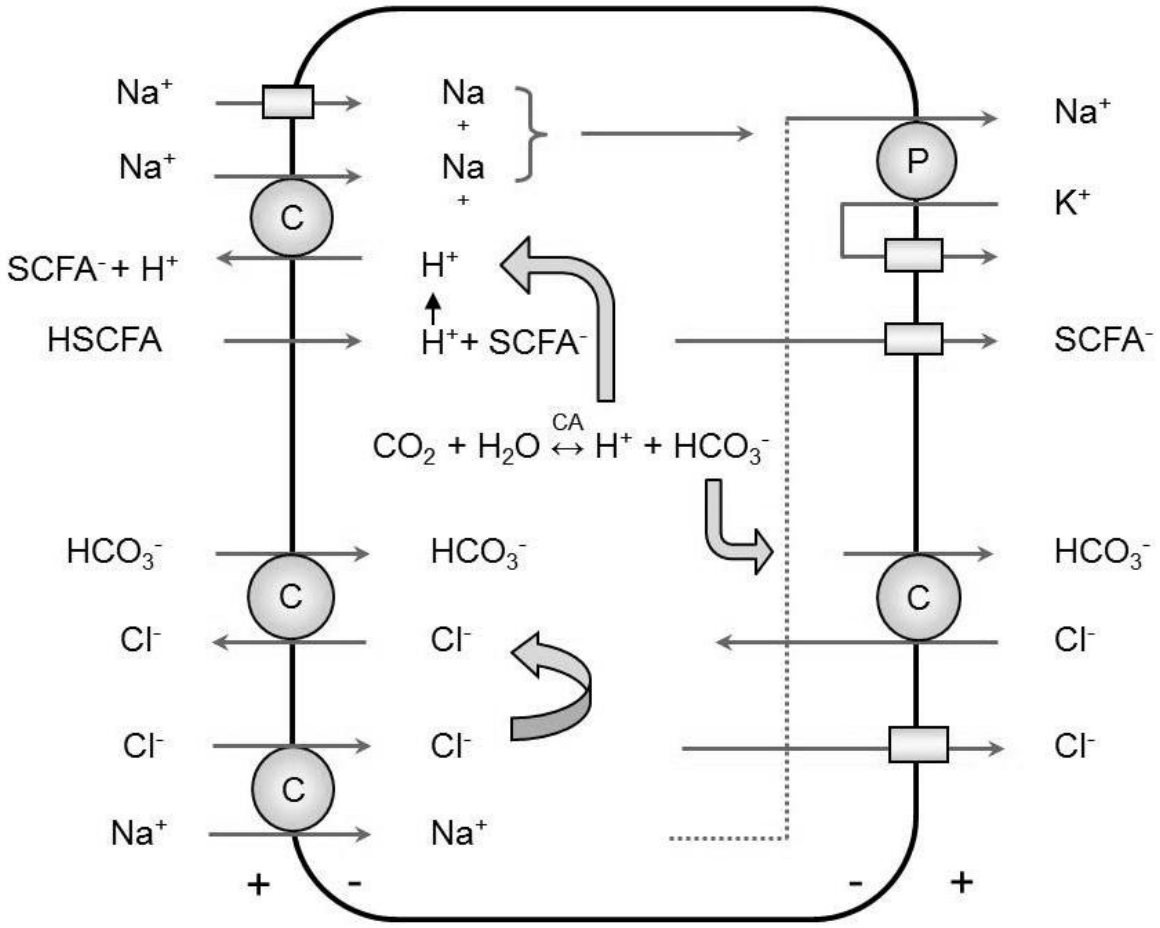

Figure 1: Tentative model of ion transport in sheep omasal epithelium. C = carrier; P = pump (Na/K-ATPase); CA = carbonic anhydrase. The cylindrical scheme represents a channel. For details see text.

In this model, HCO_3^- is exchanged apically and basolaterally with Cl^- via a HCO_3^-/Cl^- exchanger. The proposed direction of HCO_3^- flux (absorption) and Cl^- flux (secretion) via anion exchange occurs with corresponding gradients of the both anions.

The apical Cl^-/HCO_3^- exchange requires for the proposed exchange Cl^- in the subapical compartment. The apical NaCl cotransporter mediates apical Na^+ and Cl^- uptake and Cl^- is recycled via Cl^-/HCO_3^- exchanger. The parallel transcellular transport of HCO_3^- and HSCFA underlines the necessity of a constant pH_i. It is proposed that the luminal NHE represents the first and probably most important mechanisms of pH_i regulation.

2.4. Objectives

According to the transport model of ions depicted in Figure 1 the present study has the intention to study the transport of HCO_3^- which requires as the underlying working hypothesis a constant regulation of pH_i. The regulation of pH_i is challenged by three factors and changes of pH_i are very likely caused by:

- Decrease of luminal Na concentration and hence reduced NHE3 activity and
- Absorption of SCFA which are predominantly taken up across the apical membrane in the undissociated form and release intracellular H^+.
- Activity of carbonic anhydrase.

Corresponding *in-vitro* experiments were designed for the measurement of HCO_3^- transport and for testing the significance of these factors (Na^+, HSCFA, activity of carbonic anhydrase) in tissues of sheep fed a conventional hay diet (see Material and Method).
Adaptation to diet is a known phenomenon of forestomachs epithelia (Bannink et al., 2012; Martens et al., 2012). In a first approach the effect of SCFA was achieved with tissues from concentrate fed animals (see Material and Methods).

3. MATERIAL AND METHODS

3.1. Experimental animals and feeding regime

German dairy sheep of different sex were used. Animals were 9–10 months old at the time of the experiment, and their weight ranged between 33 and 40 kg at the beginning of the experiment. Experiments were conducted in accordance with German law for the care and use of experimental animals, as attested by the Animal Welfare and Ethics Representative of the Veterinary Faculty/FU Berlin. No procedures were conducted with live animals. The sheep were slaughtered according to German slaughter regulations (after stunning; permit no. T0064/99 from the Landesamt für Gesundheit, Berlin), and the material used for scientific purposes was taken from the omasum of the dead animals.

Before the beginning of experimentation the sheep were fed hay ad libitum for at least 8 weeks, in group housing arrangement, in order to adapt them to a low-energy feeding regime. During this period the daily hay intake was about 1-1.5 kg. A full description of the experimental procedures and feeding regime has recently been described in detail (Etschmann et al., 2009).

Briefly, prior to implementation of the experimental protocol, all animals were fed pure hay which contained per kg dry matter (DM) 88 g crude protein, 28 g fat, 293 g crude fiber, 89 g ash, 14.5 g potassium, 0.32 g sodium and 9.3 MJ metabolizable energy (ME).

At the beginning of the experimental period, hay intake was 1000 g per animal and day (93.5 % dry matter) and was offered in two portions at 7.00 a.m. and 3.00 p.m. equaling an intake of 7.5 MJ ME, which is slightly above the requirement for the maintenance of sheep (40 kg body weight) according to the Gesellschaft für Ernährungsphysiologie (GfE) (40).

The animals were then fed on two different experimental diets for at least 3 weeks: (A) hay ad libitum (hay-fed sheep) or (B) hay ad libitum + 780 g concentrate per day in two equal portions at 7:00 a.m. and 3:00 p.m. (concentrate-fed sheep). In order to have a better control over hay intake, animals for concentrate feed experiments were separated into individual boxes two weeks before the concentrate feeding regime, and were given 1.5 kg of hay daily. At the beginning of concentrate feeding regime, the animals were given increasing amounts of concentrates (day 1. 2 x 100 g, day 2. 2 x 200 g, day 3. 2 x 300 g) and from the fourth day on, a constant amount of 390 g, twice a day (7.00 a.m. and 15.00 p.m.) and once 1 kg of hay at 07:00 a.m. (see Table 2 and Table 3 for feed composition). The animals had free access to a lick stone and tap water.

Table 2: Composition of concentrate

Feed ingredients	**%**
Moisture	10.9
Dry matter	89.1
Crude ash	6.42
Crude protein	18.03
Crude fiber	9.65
Calcium	0.65
Phosphorus	0.59
Magnesium	0.27
Potassium	1.35
Sodium	0.42
Chloride	0.46
Sulphur	0.21
ADF (Acid detergent fibre)	13.28
NDF (Neutral detergent fibre)	25.46
ADL (Acid detergent Lignin)	3.99
Vitamin A	7200 IE*
Vitamin D3	1800 IE*
Selenium	0.5 mg*
Copper	10 mg*

*Information from the producer

DCAB (Dietary Cation/Anion Balance) + 299 meq/kg TS

Table 3: Composition of hay

Feed ingredients	**%**
Moisture	6.5
Dry matter	93.5
Crude ash	4.9
Crude protein	8.8
Crude fiber	29.3
Calcium	0.65
Potassium	1.44
Sodium	0.032
ADF (Acid detergent fibre)	34
NDF (Neutral detergent fibre)	56.5
ADL (Acid detergent Lignin)	4
Metabolized energy (ME)	9.3 MJ/kg
Net energy content for lactation (NEL)	5.5
Undegraded feed protein (nXP)	120.3 g/kg
Undegradable protein (UDP)	17.6 g/kg
Ruminal nitrogen balance (RNB)	-5.2 g/kg
Non fiber carbohydrates (NFC)	22.5

3.2. Isolation, preparation and handling of epithelial tissues

Preparation and incubation of the omasal epithelium have been described in detail by Martens et al. (2004). Animals were stunned by captive bolt and killed by exsanguination before tissues were removed for experiments (permit: T0064/99). All experiments were performed according to German laws for the protection of animals. Two to three minutes after stunning and exsanguinations of sheep, the forestomachs and the abomasum were removed from the abdominal cavity of the animal. The omasum was separated from the reticulum and the abomasum and was opened with a longitudinal cut along the omasal canal, then everted and carefully cleaned with warm buffer solution. Six to eight large leaves were removed from the wall of the omasum with a pair of scissors, carefully cleaned by immersion in a buffer solution until the solution remained clean. While they were immersed in buffer solution, mucosal sheets on the two surfaces of the leaves were cautiously separated by blunt dissection and cut into pieces ready to be used in Ussing chambers. Then they were transferred to a buffer solution, continuously gassed with 95% O_2 + 5% Cl^-/ and kept at 38°C and taken to the laboratory. The time course necessary for the preparation, transportation and mounting of the epithelia in this study was around 30-45 minutes.

3.3. In-vitro assays

The experiments were carried out with isolated epithelial tissues using the conventional Ussing-chamber technique (Ussing, 1949) and pH-Stat method (Figure 2).

3.3.1. Ussing-chamber technique

This method was modified many times to fit the forestomachs epithelial tissues (Ferreira et al., 1966; Stevens, 1964). The chamber consist of two equal halves, between them the epithelia are mounted dividing it into two equal half chambers (luminal = apical = mucosal, and the blood side = basolateral = serosal).The exposed area of the epithelium was 3.14 cm^2. To minimize the edge damage, silicon rings are used on both sides between the epithelium and the chamber. The chamber is connected to two glass cylinders by rubber tubes. Each of the cylinders contains 16 ml buffer solution. The Ussing-chamber conditions provide the means of controlling the tissue vitality through parameters such as short-circuit current (I_{sc}) and tissue conductance (G_t). Typically, the ion composition of the buffers is the same in both sides of the epithelium (Ussing and Zerahn, 1951; Stevens, 1964; Ferreira, Harrison et al., 1966). In our arrangement nevertheless, this principle was changed. The purpose of the chosen design was the measurement of HCO_3^- transport under in vivo ion

gradients. The solution on the mucosal side contained HCO_3^- and was continuously gassed with 90% O_2 + 10% CO_2, whereas the solution on the serosal side was HCO_3^- free and was continuously gassed with 100% O_2. Warm water (38°C) was circulated between the walls of the glass cylinder by the aid of a pump from a water bath. In this manner, the temperature of the buffers remained always around 38°C (for buffer composition see appendix).

3.3.2. Electrical measurements

The electrical measurements, Isc, PD_t and G_t, were continuously obtained with the aid of a computer-controlled voltage-clamp device. Agar bridges were positioned near each surface of the tissue and connected to AgCl electrodes for the measurement of the transepithelial potential difference. Others bridges were inserted into the chambers approximately 3 cm from the surface of the tissue so that a uniform density of current flow can be assumed. In all experiments the tissues were incubated for 10-15 minutes from the mounting point under open circuit condition (in this technique the potential difference remained unchanged) and then under short circuit conditions (the transepithelial potential difference was clamped to 0 mV) until the end of the experiment. Under these conditions the short-circuit current (I_{sc}) is equivalent to the sum of all electrogenic ions movement across the epithelial tissue. The tissue conductance (G_t) and the transepitheilal resistance (R_t) were determined by changes in PD_t according to Ohm's law. The short-circuit current (I_{sc}) can be calculated from the resistance (R_t) and potential difference (PD_t) values according to Ohm's law ($I_{sc} = PD_t / R_t$).

3.3.3. pH-Stat

The combination of pH-stat method with Ussing-chamber method was done with the purpose of measuring HCO_3^- transport directly, which is impossible with the standard Ussing-chamber arrangement. The pH-Stat method carries out continuous measurement of the pH and automatic titration on the serosal side glass cylinder. This way, by titrating acid the pH is kept at the same value (7.4 in this study). A solution of 0.01 M H_2SO_4 was used for titration. The

electrolyte composition of the titration solution corresponds to that of the serosal side buffer.

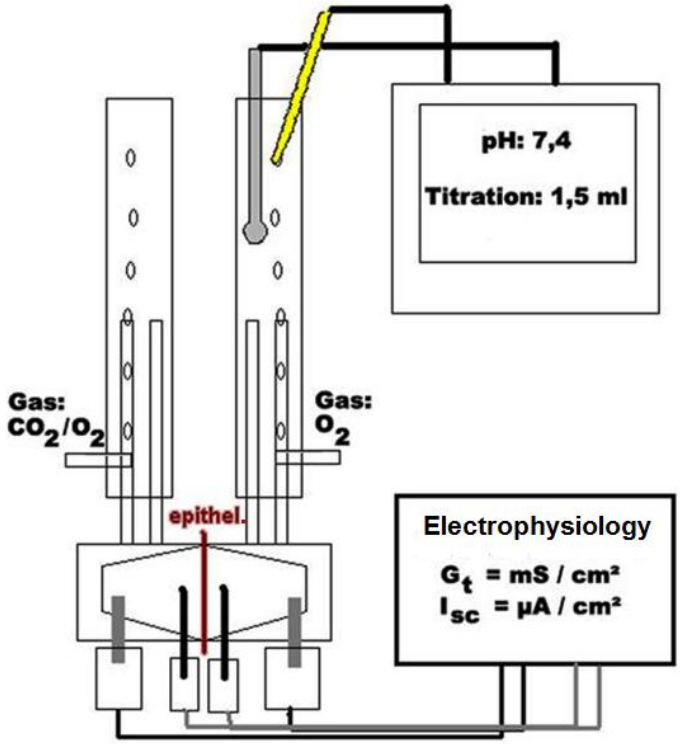

Figure 2: Scheme of the experimental design. The epithelium is mounted between the two halves of the Ussing chamber. The electrodes close to the epithelium are sensing the transepithelial potential difference, PD_t, and the two electrodes at the end of the chambers are used for applying the short circuit current, I_{sc}. H_2SO_4 is titrated to the serosal side according the change of pH = HCO_3^- transport.

3.3.4. Calculation of transport rates

As a consequence of HCO_3^- transport from the mucosal side to the serosal side of the epithelium, there is a continuous alkalization of the solution in the serosal side. The pH was measured by a pH electrode and the corresponding amount of acid (H_2SO_4) was titrated.

The calculation of the amount of the titrating substance and the corresponding transport rates is derived from the following expressions:

$$H_2SO_4 \Leftrightarrow 2H^+ + SO_4^{2-}$$

Formula 1: Titrating substance

$$2H^+ + 2HCO_3^- \Leftrightarrow 2H_2CO_3 \Leftrightarrow 2H_2O + 2CO_2 \uparrow$$

Formula 2: Neutralization reaction

$H_2SO_4 : HCO_3^-$

1 : 2

Formula 3: Ratio

The actual volume of the substance used for titration over time is given by the titration program and this can be used to calculate the amount of substance spent, according to:

$$\frac{n_1}{V_1} = \frac{n_2}{V_2} \qquad n_2 = \frac{n_1 \cdot V_2}{V_1}$$

Formula 4: Calculation of the amount of the titration substance

Where:
n_1 = amount mol of the titration substance
V_1 = 1000 ml
n_2 = sought amount of substance mol
V_2 = volume of the spent titration substance

The values were recorded in 1 minute intervals and the corresponding amount of acid was titrated.
The principle for this process is the Henderson–Hasselbalch equation:

pH = pK + log (c[Base] / c[Acid])

Formula 5: Henderson–Hasselbalch equation

c = concentration of the substance in the brackets

3.4. Experimental design

All the experiments were maintained under the short-circuit conditions and were started after an equilibration period of not less than 30 minutes, so that all the electrophysiological parameters became relatively stable. After this incubation period, only the epithelial tissues with conductance (G_t) not more than 6.0 $mS \bullet cm^{-2}$ and short circuit current (I_{sc}) not less than 1.0 $\mu eq \bullet c^{-2} \bullet h^{-1}$ were used in all experiments. Under these conditions the experimental omasum epithelial tissues remain stable a relatively long period of time (Martens and Gäbel, 1988). Another equilibration phase of around 10 minutes followed before the pH meters and titrators were inserted into the serosal side glass cylinders. Finally, after another 2-3 minutes, titrators were turned on and the measurement of HCO_3^- transport and titration began (Table 4).

Table 4: Time course of the experiment

Trial period	Description	Explanation
0. - 30. Minute	Forerun 1	Time from the beginning of the measurement up to achieving a steady state, mainly relatively high transport rates. Not included in the calculations.
31. - 90. Minute	Flux 1	First measurement period, all epithelia incubated under standard conditions. Data (1 measurement/minute) are transmitted.
90. Minute	Treatment	Depending on experimental design: Addition of an Inhibitor, respectively buffer change in the mucosal side.
91. - 120. Minute	Forerun 2	Time between treatment and the new steady state. Not included in the calculations. Mainly a fall of transport rates.
121. - 180. Minute	Flux 2	Second measurement period. Data (1 measurement/minute) are transmitted.
181. - 240. Minute	Flux 3	Third measurement period. Data (1 measurement/minute) are transmitted.

3.5. Buffer solutions

Chemicals used for the preparation of the buffer solution in this study were of analytic grade. The different experimental buffer solutions used in this study are summarised in tables in the appendix (chapter 8). The osmolarity of all the buffer solutions was adjusted to 300 ± 10 mOsmol/l using mannitol and the pH was adjusted to 7.4 ± 0.1 using TRISMA. Bicarbonate-containing buffer solutions were continuously gassed with carbogen (90% O_2 + 10% CO_2), meanwhile those without HCO_3^- (bicarbonate free buffer solutions) were gassed with pure O_2. All chemicals were of analytical grade and purchased from Sigma (including the inhibitors).

3.6. Inhibitors

3.6.1. Amiloride

Amiloride is a non-specific NHE inhibitor (Benos, 1982). Its inhibitory effects were reported on the ruminal as well as on the omasal epithelia (Martens and Gäbel, 1988). It was prepared immediately before the experiments by using Dimethylsulfoxid (DMSO) as a dissolving agent. The inhibitor was added to the mucosal side (1 $mmol \cdot l^{-1}$) in an attempt to challenge the pH_i and HCO_3^- by blocking the NHE.

3.6.2. Ethoxyzolamide

Ethoxyzolamide is a carbonic anhydrase inhibitor. It was added to the mucosal side in a concentration of 0.1 $mmol \cdot l^{-1}$ in order to investigate the effect of endogenously produced HCO_3^- on HCO_3^- transport in the omasum.

3.6.3. Levetiracetam

Levetiracetam is a Na^+ independent Cl^-/HCO_3^- co-transport inhibitor (Leniger et al., 2004). It was added to the mucosal side of the epithelium to investigate the possible role of a Na^+ independent Cl^-/HCO_3^- in HCO_3^- transport.

3.7. Statistics

Omasal epithelial tissues were available from each animal. A total number of 6 Ussing chambers combined with the pH Stat method were used. The tissues were mounted in the Ussing chambers and then divided between control and treatments per experiment: Six tissues were used in parallel and control (3) and treatment (3) were performed in tissues from the same animal for compensation of the variation between the animals.

"N" refers to the number of experimental animals, for most experiments at least 3 animals per each treatment, while "n" refers to the number of epithelial tissues per treatment groups and are considered as independent observations.

All evaluations were carried out by using SPSS program, version 12.0 for Windows. Results are given as means ± SE. Significance testing was performed between paired values from the same experiment by using Friedman repeated measures analysis of rank, with the Student-Newman-Keuls (SNK) method used for pairwise multiple comparisons. Where only two columns of values were tested, the data were tested for normality and compared by using the paired Student *t*-test. *P* values of < 0.05 were considered significant.

4. RESULTS

4.1. Control of parameters of the methods

4.1.1. Ussing chamber

The electrophysiological parameters of the Ussing chamber method have low relevance for measurement of HCO_3^- transport, but Isc and G_t were used as control for viability of the tissue.

As explained in Material and Methods we used asymmetric solutions between serosal and mucosal side, with chemical gradients from the mucosal to serosal side and vice versa. This could create a challenge for the tissue so as means of control we used electrophysiology to observe the vitality and stability of the tissues during the course of the experiments. The electrophysiological parameters used for this purpose are short-circuit current (I_{sc}), tissue conductance (G_t) and the transepithelial potential difference (PD_t).

The short-circuit current is equivalent to the sum of all electrogenic ions movement across the epithelial tissue. Due to the gradients in the solutions we used, it is not possible to define the whole ionic background of the observed current. Both HCO_3^- and Cl^- with their gradients contribute to this current and of course the electrogenic Na transport. It is important to notice that the current is high at the start of the experiment and declines very rapidly for some 60 min after mounting the tissue. As observed with almost all tissues in Ussing chamber experiments I_{sc} decreased with time (Figure 3). The negative current represents under the experimental condition a cation transport from mucosal to serosal side or an anion secretion in the opposite direction. As mentioned above the ionic background of I_{sc} is not known.

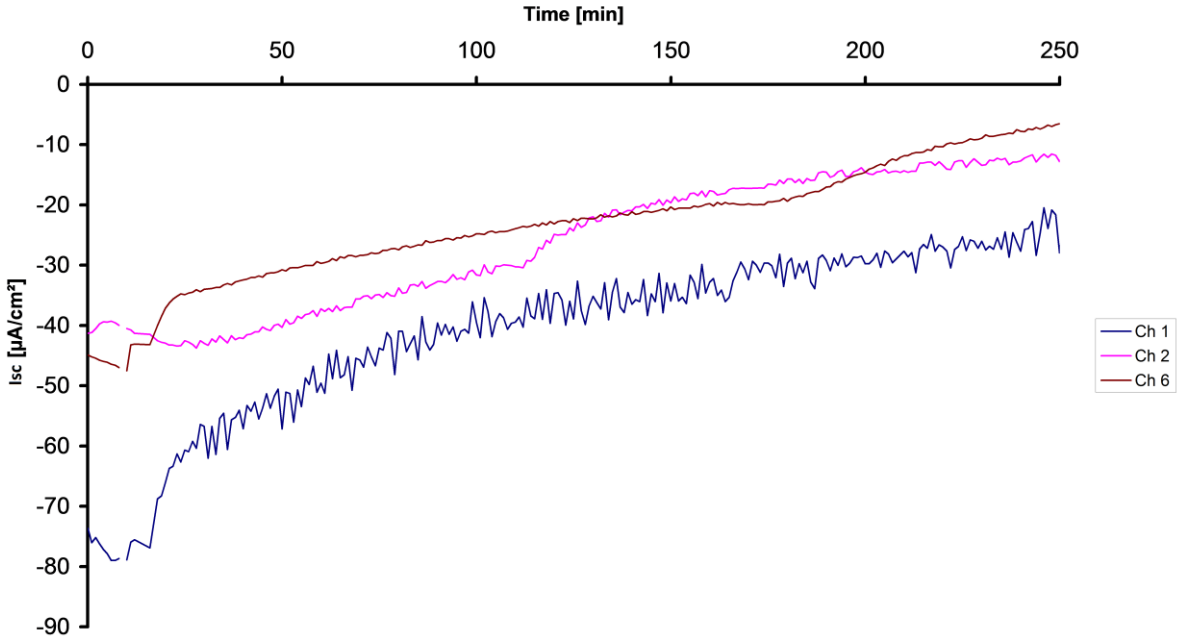

Figure 3: Original trace of I_{sc} of three tissues from one sheep during the time course of an experiment. The experiment was finished at 250 minutes

The time course of tissue conductance is shown in Figure 4. G_t decreased after mounting and remained relatively stable during the rest of the experiment.

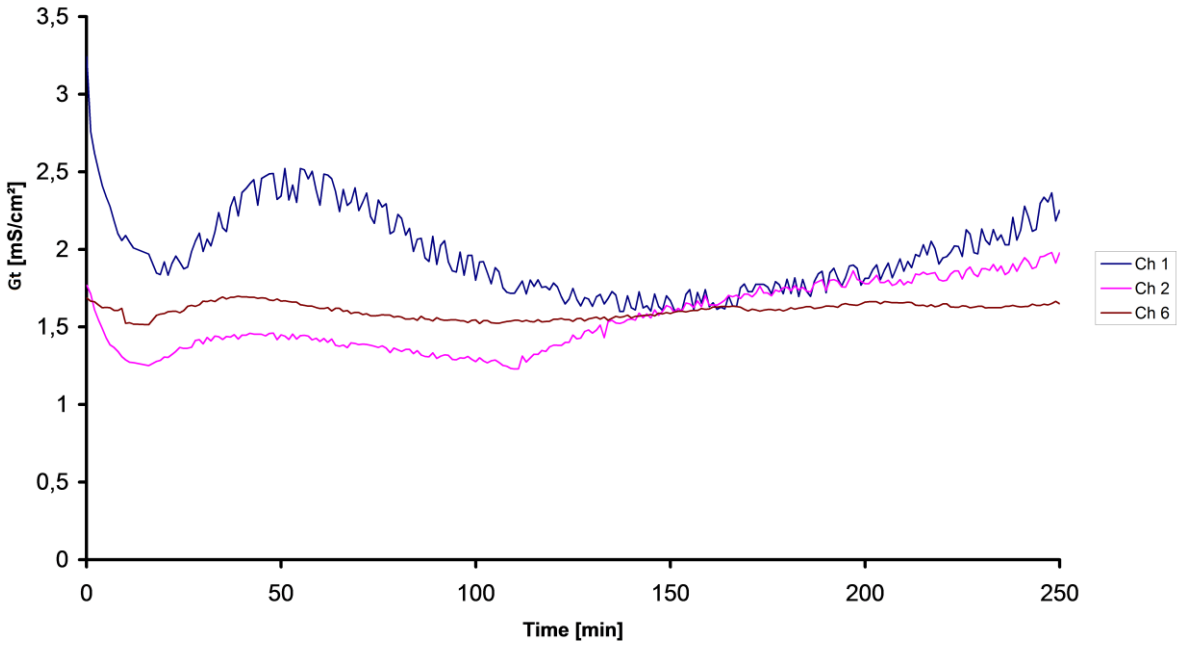

Figure 4: Tissue conductance (G_t) measured in [mS/cm^2], in three separate omasal epithelia of one sheep during the time course of the experiment.

Figure 5 exhibits the potential difference of the tissue. The PD_t is a virtual parameter, because it is calculated from I_{sc} and G_t. The PD_t would have this magnitude under open circuit conditions. Because the G_t was relatively constant (see Figure 4), PD_t mirrors the I_{sc}.

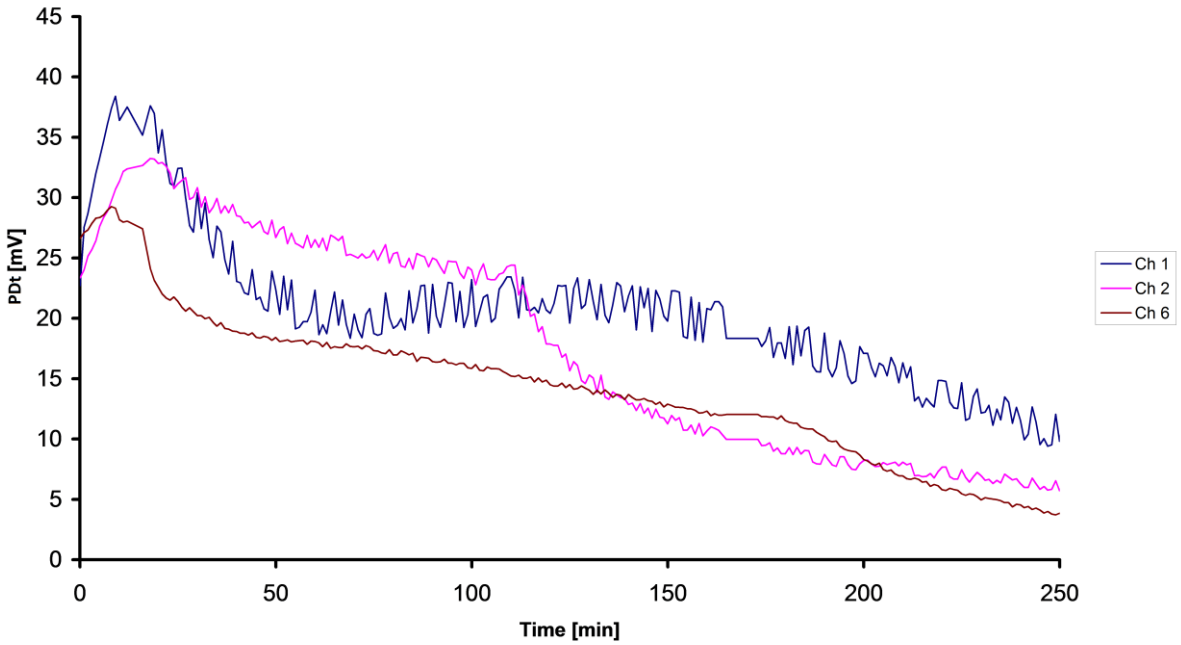

Figure 5: Transepithelial potential difference (PD_t) measured in [mV], in three separate omasal epithelia of one sheep during the time course of the experiment.

The electrophysiological parameter values for short-circuit current (I_{sc}), tissue conductance (G_t) and the transepithelial potential difference (PD_t) (figures 3 – 5) were typical for this type of experiment and simply considered as control parameter. The most relevant observation is the stable tissue conductance (Figure 4) during the time course of the experiment (240 minutes), which indicates stability of integrity of the tissue. Hence, G_t was used as internal control, but not included in the data set.

4.1.2. pH – Stat

The principle of pH-Stat method is to keep the pH constant in the studied compartment. Transport of HCO_3^- causes an increase of pH of the unbuffered solution in the serosal compartment. This deviation was kept constant by continuous titration with acid 0.01 M H_2SO_4 during the measurements. Figure 6 shows that the pH was constant and it is very important that the amount of titrated H_2SO_4 per time unit was almost constant which clearly indicates steady state transport of HCO_3^-.

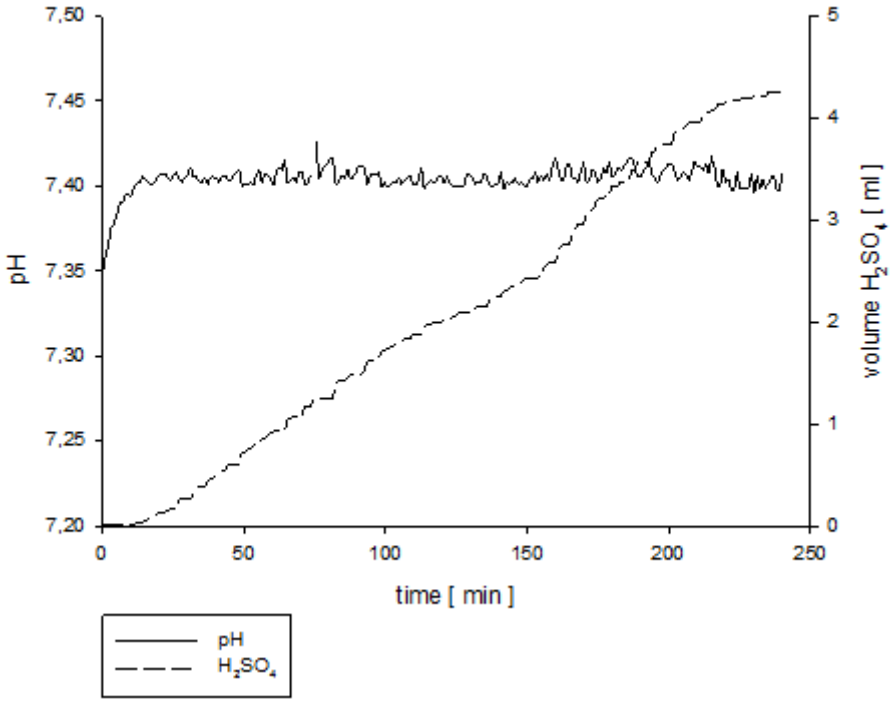

Figure 6: The pH of the serosal compartment (free of HCO_3^-) was kept constant by continuous titration with H_2SO_4. The amount of added H_2SO_4 represents the transport of HCO_3^-.

4.2. Transport of HCO_3^-: Unidirectional transport rates

In this experimental setup the epithelial tissues were allocated in two groups. The control group was exposed to our standard buffers [HCO_3^- on the mucosal side, no HCO_3^- on the serosal side (buffer 1 and 2)] and the second group was exposed to experimental condition [no HCO_3^- on the mucosal side and HCO_3^- on the serosal side (buffer 1 and 2, in this case though in opposite order)]. As expected, the physiological transport rates (absorption) were significantly higher in the mucosal-serosal direction and accounted for 4.04 ± 1.30 µeq·cm^{-2}·h^{-1}. The opposed flux rate of 2.68 ± 0.72 µeq·cm^{-2}·h^{-1} was significant lower (p = 0.046), with a difference of 1.36 µeq•cm-2• h^{-1} (Table 5).

Table 5: Unidirectional transport rates of HCO_3^- across the isolated omasal epithelium

Treatment	HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Absorption (J_{ms})	4.04 ± 1.30	3/7
Secretion (J_{sm})	2.68 ± 0.72	3/8
P	0.046	

4.3. HCO_3^- transport and intracellular pH

HCO_3^- transport through the epithelium requires a constant pH_i. In different experiment setups, by using a NHE blocker (amiloride), different feeding regimes as well as different SCFA concentrations, we studied the effect of perturbation of pH_i on HCO_3^- transport.

4.3.1. Effect of SCFA and Amiloride on HCO_3^- transport

4.3.1.1. Effect of SCFA

SCFA uptake in the omasum occurs mainly in the undissociated form (HSCFA) and it induces the activity of NHE (Ali et al., 2006). Accordingly, it is presumed the intracellular release of protons from luminal HSCFA uptake is an acidifying challenge for pH_i and could affect HCO_3^- transport. Based on the model (Figure 1), it was assumed that an increase in SCFA concentration should reduce HCO_3^- absorption in the omasal epithelium, as it has been shown in recent experiments by Beisele (2008) where HCO_3^- fluxes between control (no SCFA) and 25 mmol·l^{-1} SCFA was compared. The uptake of the undissociated SCFA reduced HCO_3^- transport significantly.

In a new experimental setup, the two groups of epithelial tissues were incubated with two different solutions on the mucosal side (64 mmol· l^{-1} SCFA and 100 mmol· l^{-1} SCFA). The HCO_3^- transport rates under these treatments are summarized in Table 6.

An increase in SCFA concentration reduced HCO_3^- transport from 5.67 ± 0.94 µeq·cm^{-2}·h^{-1} by 24.6 % to 4.28 ± 1.52 **µeq·cm^{-2}·h^{-1}**, although this effect was not significant.

Table 6: Effect of mucosal SCFA concentration on HCO_3^- transport (hay-fed sheep)

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
64 mmol·l^{-1} SCFA	5.67 ± 0.94	3/9
100 mmol·l^{-1} SCFA	4.28±1.52	4/12
P	0.50	

4.3.1.2. Effect of Amiloride

Amiloride is described as a NHE non-specific inhibitor. Its inhibitory effect was reported on the ruminal as well as omasal epithelia (Martens and Gäbel, 1988). According to the model (Figure 1), NHE plays an important role in pH_i regulation by recycling H^+ taken up with the undissociated form of SCFA. By blocking this exchanger cells lack this pH regulation mechanism, thereby, a decrease of pH_i and hence, a change in the transport rate of HCO_3^- is expected. Table 7 shows that amiloride (1 mmol·l^{-1}) reduced Jms HCO_3^- significantly ($p < 0.015$), from 5.67 ± 0.94 µeq·cm^{-2}·h^{-1} in control tissue to 2.32 ± 0.87 µeq·cm^{-2}·h^{-1} in tissues treated with amiloride.

Table 7: Effect of 1 mmol· l^{-1} amiloride added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed; 64 mmol· l^{-1} SCFA).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	5.67 ± 0.94	3/9
Amiloride	2.32 ± 0.87	3/6
P	0.015	

In a further experiment the tissues were challenged with a higher concentration of SCFA [100 instead of 64 mmol·l^{-1} (Table 8)]. The addition of amiloride induced a significant reduction of HCO_3^- transport rates ($p = 0.047$) from 4.28 ± 1.52 µeq·cm^{-2}·h^{-1} to 2.72 ± 1.96 µeq·cm^{-2}·h^{-1} upon this challenge of mucosal SCFA, which supports our assumption on the effect of SCFA on HCO_3^- transport. It is worth to mention that the transport rates of HCO_3^- under control conditions (mucosal SCFA 100 mmol·l^{-1}) is numerically lower than the rates of the control

tissues with 64 mmol·l^{-1} (see table 7 and 8). It is worth to mention that amiloride decreased the HCO_3^- transport in both sets of experiments (see table 7 + 8) to the same magnitude.

Table 8: Effect of amiloride (1 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed; 100 mmol·l^{-1} SCFA).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	4.28±1.52	4/12
Amiloride	2.72±1.96	4/12
P	0.047	

4.3.2. Effect of feeding regimen on HCO_3^- transport

In a similar set, in order to evaluate the possible impact of the feeding regime on HCO_3^- absorption capacities of the omasum, we repeated the experiments with tissues from concentrate fed animals (Table 9). Surprisingly, amiloride (1 mmol·l^{-1}) reduced the HCO_3^- transport rates only by 9.93 % from 7.35 ± 1.91 µeq·cm^{-2}·h^{-1} to 6.62 ± 2.48 µeq·cm^{-2}·h^{-1}. This effect was not significant.

Table 9: Effect of 1 mmol/l amiloride added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (concentrate-fed; 64 mmol·l^{-1} SCFA).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	7.35 ± 1.91	2/4
Amiloride	6.62 ± 2.48	2/6
P	0.219	

4.3.3. Inhibition of carbonic anhydrase with ethoxyzolamide

The previous experiments have shown that inhibition of NHE or challenging the tissue with SCFA decreased significantly HCO_3^- transport, but this effect was modulated in tissues of concentrate fed animals. This lead us to the assumption that there may be other mechanisms of pH_i regulation involved and one possible mechanism could be the production of HCO_3^- by carbonic anhydrase, which could contribute to the buffer capacity of the cell. To test this hypothesis, a new set of experiments was conducted, in which the effect of 0.1 mmol·l^{-1} ethoxyzolamide added to the mucosal side was investigated. Table 10 shows that

ethoxyzolamide significantly (p = 0.034) decreased J_{ms} HCO_3^- ~~(p = 0.034)~~, from 7.33 ± 1.24 µeq•cm^{-2}• h^{-1} in the control group to 5.53 ± 1.01 µeq•cm^{-2}• h^{-1} in the experimental group.

Table 10: Effect of ethoxyzolamide (0.1 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed; 64 mmol· l^{-1} SCFA).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	7.33 ± 1.24	3/7
Ethoxyzolamide	5.53 ± 1.01	4/11
P	0.034	

Furthermore, in our intention to understand the interaction between NHE function and that of carbonic anhydrase in relation to HCO_3^- transport, in another set of experiments the tissue were exposed to two inhibitors by combining 0.1 mmol· l^{-1} ethoxyzolamide with 1 mmol· l^{-1} amiloride, added to the mucosal side. J_{ms} HCO_3^- in the experimental group fell by 27.1 % compared to the control group. This decrease was significant (p = 0.049), but no additional effect of amiloride was observed on HCO_3^- transport rates of the experimental group (Table 11).

Table 11: Effect of ethoxyzolamide (0.1 mmol· l^{-1}) and amiloride (1 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed; 64 mmol· l^{-1} SCFA).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	6.59 ± 1.35	2/6
Ethoxyzolamide + Amiloride	4.80 ± 1.57	2/7
P	0.049	

4.4. Effect of Na^+ concentration on HCO_3^- transport

As it has been shown in the previous experiments with amiloride, there is an interaction between Na^+ transport via NHE and HCO_3^- transport in the omasum.

The rumen fluid exhibits large variations of Na^+ concentrations and hence, it was our assumption that this should have an effect on NHE, and consequently on HCO_3^- transport. It was therefore our intention to demonstrate this effect under different concentrations of Na^+. For this purpose, we reduced the Na^+ concentration on the mucosal solution. Na^+ was replaced with K^+, in order to have a constant osmolarity (300 mmol·l^{-1}) and at the same time simulate the *in vivo* physiologically reciprocal changes of these two cations.

All epithelia were incubated under standard conditions in 50 mmol·l^{-1} HCO_3^- and 145 mmol·l^{-1} Na^+ buffer solution The experimental group chambers were filled with a buffer solution with 50 mmol·l^{-1} Na^+, while the control group received the standard mucosal buffer (145 mmol·l^{-1} Na^+).

Table 12 displays the effect of reduced Na^+ concentration on HCO_3^- transport. The transport rates fell significantly ($p = 0.012$), from 7.09 ± 1.30 µeq•cm^{-2}• h^{-1} in the control group to 3.48 ± 0.82 µeq•cm^{-2}• h^{-1} in the experimental group, a reduction of 3.61 µeq•cm^{-2}• h^{-1}.

Table 12: Effect of reduced Na^+ concentration (50 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	7.09 ± 1.30	4/12
50 mmol·l^{-1} Na^+	3.48 ± 0.82	4/12
P	0.012	

In order to further evaluate the effect of mucosal Na^+ on HCO_3^- absorption, the experiment was repeated with a lowered Na^+ concentration compared to the previous experiment. The experimental group chambers were filled with a 20 mmol·l^{-1} Na^+ buffer solution, while the control group chambers contained the standard mucosal buffer (145 mmol· l^{-1} Na^+).

The further reduction of Na^+ concentration had a significant effect on the HCO_3^- transport rates (Table 13), namely a significant reduction ($p < 0.027$) by 48.9% in the experimental group from 3.27 ± 1.13 µeq·cm^{-2}·h^{-1} to the low Na group of 1.67 ± 0.98 µeq·cm^{-2}·h^{-1}. It must be must emphasized, that the epithelia used in this experiment generally expressed low transport rates.

Table 13: Effect of reduced Na^+ concentration (20 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed)

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	3.27 ± 1.13	4/12
20 mmol·l^{-1} Na^+	1.67 ± 0.98	4/12
P	0.027	

4.5. Effect of Levetiracetam on HCO_3^- transport

One of the three major functional groups of Cl^-/HCO_3^- exchangers is the one comprising the Na^+-coupled Cl^-/HCO_3^- exchangers (AE), gene family SLC4. Research on a new anticonvulsant, Levetiracetam (LEV), have found it to have inhibitory effects on the Na^+ dependent Cl^-/HCO_3^- exchangers (Leniger et al., 2004).

In our last set of experiment, with the aim of examining the possible role of the Na^+-coupled Cl^-/HCO_3^- exchangers in HCO_3^- absorption in the omasum, we tested the effects of this inhibitor on HCO_3^- transport in omasal epithelium. The effect of LEV (1 mmol· l^{-1}) was examined by its addition to the mucosal side of the epithelium. Addition of LEV induced slight numerically, but not significant reduction of HCO_3^- transport rates (6.65 ± 1.86 µeq·cm^{-2}·h^{-1} to 6.23 ± 1.73 µeq·cm^{-2}·h^{-1}**;** table 14).

Table 14: Effect of LEV (1 mmol· l^{-1}) added to the mucosal side on HCO_3^- transport across the omasal epithelia of sheep (hay-fed).

Treatment	J_{ms} HCO_3^- (µeq·cm^{-2}·h^{-1})	Number of sheep/ tissue
Control	6.65 ± 1.86	2/5
LEV	6.23 ± 1.73	2/5
P	0.311	

5. DISCUSSION

5.1. Absorptive properties of the omasum and HCO_3^- transport

Earlier *in vivo* studies from various research groups, as well as previous *in vitro* studies from our institute have recognized the importance of the omasum as a compartment of water and ion absorption, including HCO_3^-, within the forestomachs of ruminants (Edrise et al., 1986, von Engelhardt and Hauffe, 1975; Oyart and Buckaert, 1961; Martens and Gäbel, 1988; Tiling, 1997; Niebuhr, 2003; Wegeler, 2008; Beisele, 2008). The current study aims to further clarify and characterize HCO_3^- absorption in the omasal epithelium of sheep using the Ussing chamber technique in combination with the pH-stat method. In addition, perturbations of pH_i are induced and their interactions with HCO_3^- transport were investigated.

It should be mentioned that contrary to the traditional Ussing chamber technique setup, where the ion composition of the buffers is the same in both sides of the epithelium, we worked under ion gradients simulating *in vivo* conditions. This could present a challenge for the tissue, however, as electrophysiology data (particularly G_t) and the relatively constant HCO_3^- transport rates show (Fig. 5, Fig. 6), this was clearly not the case.

5.2. Unidirectional HCO_3^- transport measurements

The capacity of the omasal epithelium for an uptake of HCO_3^- and secretion of Cl^-, and therefore the presence of an ion exchanger has been proposed by several earlier studies (Tiling, 1997; Niebuhr, 2003; Wegeler, 2008). According to the cell model presented in Figure 1, the transport of HCO_3^- in both directions should be possible by changing the chemical gradient at the corresponding sides of the epithelium. Indeed, the dependence on gradients of the substrates has been shown in all known anion exchangers, as well as the possibility to experimentally induce transport in this manner (Tiling, 1997). The findings from experiments, whereby the gradients were changed, confirmed this assumption. However, the transport rates of HCO_3^- through the opposed sides of the epithelium were different, namely the absorption flux rates (J_{ms}) were significantly higher than the secretion flux rates (J_{sm}). The observed discrepancy is related to the presence of a Na^+-Cl^- cotransporter on the apical membrane (Fig 1). This cotransporter increases the subapical availability of Cl^-, which is recycled through the HCO_3^-/ Cl^- exchanger (Fig. 1).

5.3. HCO_3^- transport and the interaction with intracellular pH

5.3.1. Effect of SCFA

As it has been shown from earlier studies SCFA uptake in the omasum occurs mainly in the undissociated form (HSCFA) (Ali, 2005, Ali et al., 2006). It is postulated that through the intracellular release of protons the pH_i is lowered. This acidifying challenge for the pH_i enhances the activity of Na^+/H^+ exchanger (Gäbel, Bestmann et al., 1991; Diernas, Sehested et al., 1994; Ali et al., 2006).

These findings encouraged to design an experimental setup to investigate the role of different SCFA concentrations on HCO_3^- transport in omasum epithelium.

By using a SCFA concentration of 100 $mmol \cdot l^{-1}$ HCO_3^- transport across the epithelium decreased by 24.5%. Although this fall in transport rates was not significant, these results provide further support for our hypothesis. Through a high SCFA concentration a large amount of protons enters the epithelial cells. This on the other hand, lowers the pH_i, whereby HCO_3^- acts as a buffer, resulting in this way with its lower transport rate to the serosal side of the epithelium.

5.3.2. Effect of Amiloride

The presence of an amiloride sensitive Na^+/H^+ exchanger (NHE) in the apical membrane of the omasal epithelial cells has been documented in earlier studies (Martens and Gäbel, 1988; Ali, 2005), and as concluded by later research, the isoform in question is NHE3 (Dölle, 2008).

Mucosal addition of amiloride had the intention to examine the interactions between pH_i and HCO_3^- transport, namely the interdependence of the Na^+/H^+ exchanger and the HCO_3^-/Cl^- exchanger. The assumption leading to this investigation was that by inhibiting the Na^+/H^+ exchanger the apical extrusion of protons would be blocked, and therefore cause a fall in the pH_i. Consequently, HCO_3^- absorbed from the apical side should react with the unrecycled protons, thereby disturbing HCO_3^- transport through the epithelium. For this reason a buffer solution with a 64 $mmol \cdot l^{-1}$ concentration SCFA was used, which are predominantly taken up in the undissociated form and hence stimulate Na^+/H^+, consequently representing a challenge for pH_i after inhibition of NHE by amiloride.

The findings from the current study demonstrated the high importance of pH_i in the transport of HCO_3^- in the omasum and the crucial role of the Na^+/H^+ exchanger in maintaining a stabile pH_i. The resulting fluxes for HCO_3^- (J_{ms}) after the addition of amiloride displayed a significant fall in HCO_3^- transport ($p < 0.05$) compared to the control tissues (Table 9).

5.3.3. Effect of feeding regime

Functional adaptation of the forestomach epithelium due to dietary changes is a well-recognised process in ruminants. Earlier studies conducted on Na^+ transport in the rumen epithelium of sheep using Ussing chamber experiments have revealed a significant increase in net Na^+ transport after a change from hay feeding to a mixed hay/concentrate diet (Uppal et al., 2003, Etschmann et al., 2009). Similar observations were made in the omasal epithelium of sheep, where Na^+ and SCFA transport was significantly enhanced after the introduction of a concentrate diet (Ali, 2005).

Following the findings acquired from our amiloride experiments with hay-fed animals, it was our hypothesis that due to functional adaptations, HCO_3^- transport capacities of the omasal epithelium after a concentrate diet would be enhanced. However, the transport rates of HCO_3^- were within the range of the hay-fed animals (see for example table 9, 10 or 11). A very likely explanation for the missing stimulation of HCO_3^- is its concentration (50 mmol·l^{-1}) in both experiments. When the transport capacity of the assumed anion exchanger is not saturated in hay-fed animals a possible higher number of exchangers in concentrate-fed animals as a consequence of adaptation would not cause a change in transport rates. If this hypothesis is correct the activity of the anion exchanger is probably not altered under the current conditions (50 mmol·l^{-1} HCO_3^-).

A surprising and very important observation was the small effect of amiloride on HCO_3^- transport in tissues from concentrate-fed sheep. The physiological significance of the NHE as a first line of defence of pH_i has been emphasized, but obviously other – unknown - mechanisms of regulation of pH_i are activated and are able to maintain pH_i and consequently HCO_3^- transport. This is from a practical point of view a very important observation because it indicates mechanisms of compensation, which could be crucial *in vivo* for example at high K intake and hence low Na concentrations and consequently low NHE activity (see below).

5.3.4. Inhibition of carbonic anhydrase with ethoxyzolamide

One of the most important mechanisms regulating pH_i in mammalian cells is the carbonic anhydrase system. Carbonic anhydrases (CA) are a family of zinc metalloenzymes involved in many physiological processes, whereby catalyzing the reversible reaction of CO_2 hydration to bicarbonate and a proton is one of its most important functions as a buffering system. It was our hypothesis that CA could be a possible alternative regarding buffer mechanisms in the omasal epithelium. The inhibition of this enzyme should have an effect on pH_i and consequently influence HCO_3^- transport through the epithelium. To date, a variety of inhibitors has been described, ethoxyzolamide being one of the common chemotypes (Supuran, 2010).

The results from the current research suggest a significant effect of the inhibitor on pH_i and hence, HCO_3^- transport. The transport rates for HCO_3^- in the experimental group were significantly reduced by 24%.

In a second experimental setup we decided to provide a further acidifying challenge for the epithelial cells by combining ethoxyzolamide with amiloride. However, no additional effect from the use of amiloride was observed.

The current findings support the assumption of the importance of CA as a buffer mechanism for the pH_i and HCO_3^- transport in the omasum. It should be mentioned that inhibition of CA by ethoxyzolamide is not absolute (Chegwidden and Carter, 2000). The absence of an increased effect from the combination of ethoxyzolamide and amiloride suggests that other buffer mechanisms most probably play a role in pH_i regulation or a decrease of pH_i after addition by ethoxyzolamide which is not further changed by amiloride.

5.3.5. Effect of luminal concentration of Na^+

It has been known that the absorption of Na^+ from the rumen is mediated by an active transport mechanism (Dobson, 1959). This has been supported by all the subsequent *in vitro* studies (Chien, Stevens, 1972; Harrison et al., 1975; Martens et al., 1991), which have further revealed that the flux in net Na^+ (J^{Na}_{net}) is considerably higher than the (Na-dependent) short-circuit current (I_{sc}). The discrepancy between I_{sc} and J^{Na}_{net} has led to the assumption of two parallel transport mechanisms for Na^+, namely electrogenic and electroneutral (Chien, Stevens, 1972; Martens et al., 1991). These mechanisms enable the rumen epithelium to cope with the wide range of ruminal Na^+ concentrations between 21 mmol/l (Martens et al., 1987) at Na deficiency and 145 mmol/l (Bailey, C. B., 1961). At low Na concentrations, Na is mainly transported via the electrogenic pathway, whereas, at higher Na concentrations, the electroneutral Na/H exchange mechanism is predominant.

The luminal concentration of Na^+ is important for the normal HCO_3^- transport through the omasal epithelium. According to our model (Fig. 1), this is due to the indirect coupling of this transport with two Na^+ transport systems - Na^+/H^+ exchanger and Na^+/Cl^- cotransporter. By lowering the luminal Na^+ concentration to 50 $mmol \cdot l^{-1}$ (145 $mmol \cdot l^{-1}$ in the control group) the NHE activity was reduced and hence, there was an effect on HCO_3^- transport, which was reduced significantly. This effect is most probably due to the accumulation of intracellular protons caused by uptake of undissociated SCFA, which by reacting with HCO_3^- disturb its normal transport through the epithelium. Furthermore, this shortage of Na^+ should reduce the activity of the Na^+/Cl^- cotransporter, which in turn, due to the low intracellular availability of Cl^- for the HCO_3^-/Cl^- exchanger perturbs the apical uptake of HCO_3^-. The reduction of the luminal Na^+ concentration to 20 $mmol \cdot l^{-1}$ did not result with a further significant fall in the HCO_3^- transport rates. These findings are in accordance with the previous experiments with amiloride.

K^+ is the most abundant mineral in plants and is rapidly dissolved in the forestomach fluid (Scott, D., 1967). An increase of K^+ intake causes an increase of K^+ and a concomitant decrease of Na^+ concentration in the rumen fluid which keeps the sum of both cations almost constant (for details see Lang and Martens, 1999). Hence a low Na concentration is very often observed and could impair HCO_3^- transport in the omasum.

The data about an impairment of HCO_3^- transport relies on the hypothesis that the induced decrease of pH_i is enhancing the reaction of $H^+ + HCO_3^- = H_2O + CO_2$ and consequently impairs HCO_3^- transport. The released CO_2 will diffuse out of the epithelial cells into the serosal and mucosal department of the Ussing chamber method (figure 2) and will be eliminated very rapidly into the air by the vigorous gas perfusion of both compartments. This fast removal of CO_2 is not possible *in-vivo.* Rather diffusion of CO_2 *in-vivo* into the mucosal compartment means into the omasal fluid und further transport with this fluid into the abomasum where it is most likely released as gas (see below). Diffusion of CO_2 into the blood is without severe consequences because the blood passes the liver and is flowing to the lungs where it immediately diffuses into the alveolae and is expired.

5.3.6. Effect of Levetiracetam on HCO_3^- transport

Molecular biology investigation conducted on omasum epithelium has revealed the presence of mRNA of two anion exchangers, namely DRA (Down regulated adenoma); AE2 (Anion exchanger 2); two isoforms of carboanhydrase CA1 (Carboanhydrase 1); CA2 (Carboanhydrase 2) and the anion channel of CFTR (Cystic fibrosis transmembrane regulator) (Wegeler, 2008). One major functional group of anion exchangers which was not included in this investigation is the one consisting of Na^+-coupled Cl^-/HCO_3^--exchangers, gene family SLC 4.

In the current study, the presence of a Na^+-coupled Cl^-/HCO_3^- exchanger in the omasum epithelium, as a possible mediator of HCO_3^- transport was examined by using levetiracetam, a Na^+-coupled Cl^-/HCO_3 exchanger inhibitor. The addition of levetiracetam failed to produce a reduction of the HCO_3^- transport rates.

It can be concluded that this group of anion exchangers most probably does not play a significant role on HCO_3^- transport in the omasum.

5.3.7. Transporters of HCO_3^-

The studies clearly show a transport of HCO_3^- across the isolated omasal epithelium. At least ten different mechanisms of HCO_3^- have been described in two recent reviews (Cordat, Casey, 2009; Romero et al., 2013). Electroeneutral, electrogenic and Na-linked HCO_3^- transport mechanisms have been distinguished. The results of the current study support the assumption of an electroneutral anion exchange as has been depicted in Figure 1.

The mRNA of two anion exchangers, namely DRA (Down regulated adenoma) and AE2 (Anion exchanger 2) has been detected so far in the omasal epithelium (Wegeler, 2008). Immunostaining of these exchangers is not available and their possible role and location (apical or basolateral membrane) for HCO_3^- transport are still unknown.

5.4. Conclusions

One important absorptive function of the omasum is the transport of HCO_3^- from lumen to blood side. This absorption prevents flow of HCO_3^- into the abomasum and hence, production of CO_2. The parallel absorption of HCO_3^- and SCFA in the abomasum requires a constant pH_i. It was hypothesized that the pH_i depends on the activity of Na transport and H^+ extrusion via NHE3 in the apical membrane. The activity of NHE3 was blocked by amiloride or reduced by low luminal Na concentration. The obtained results support the working hypothesis. Perturbations of pH_i by absorption of SCFA or by inhibition of the carbonic-anhydrase support the physiological significance of this parameter (pH_i). Furthermore, the small reducing effect (not significant) of SCFA on HCO_3^- transport in tissues of concentrate-fed sheep hints on the capability of the omasum to adapt to feeding conditions. This adaptation appears to be important because outflow of ruminal fluid into and consequently the load of the omasum with ingesta are increased with higher intake of concentrate (Tamminga et al., 1988) and possibly contribute to CO_2 production from HCO_3^- in the abomasum. Concentrate feeding causes an adaptation of the forestomachs epithelia (Martens et al., 2012; Bannink et al., 2012) including the omasum (Martens et al., 2004). This effect was confirmed, because amiloride reduced HCO_3^- transport only to small extend in tissues from concentrate-fed animals. It is proposed to include the absorptive function of the omasum (HCO_3^- and SCFA), its possible impairment and assumed adaptation capability into the discussion of the displacement of the abomasum (Martens, 1998).

6. SUMMARY

6.1. Summary

Transport of HCO_3^- in sheep omasum: Effects of Na and SCFA

In-vitro the transport of HCO_3^- in the mucosal–serosal direction (absorption), J_{ms}, was studied across the isolated epithelium of sheep omasum. The conventional Ussing chamber technique was combined with the pH-stat method. The mucosal side of the tissues was incubated with a buffer solution of 50 mmol·l^{-1} HCO_3^- and gassed with 10% CO_2. The buffer solution of the serosal side was HCO_3^- and buffer free. The pH (7.4) of the serosal side was kept constant by titration of H_2SO_4. The amount of titrated H_2SO_4 was considered as amount of transported HCO_3^-.

a) The current model of anion transport across the omasal epithelium with two anion exchangers in series in the apical and basolateral membrane was confirmed. The omasal epithelium has the capability to transport HCO_3^- in both directions according the applied ion gradients. However, the flux from mucosal to serosal side was significantly larger than the transport in the reversed direction.
b) The transport of HCO_3^- depends on the activity of Na/H exchanger (NHE3) in the apical membrane. Inhibition of NHE3 by mucosal amiloride (1 mmol·l^{-1}) or reduction of mucosal Na^+ concentrations reduced HCO_3^- transport significantly. It is concluded that the NHE3 mediated extrusion of H^+ is of predominant importance for regulation of the intracellular pH, pH_i, and hence HCO_3^- transport.
c) The manipulation of pH_i by transport of SCFA (uptake of the undissociated SCFA in the mucosal-serosal direction) reduced HCO_3^- transport. However, this effect was only of minor importance in tissues from concentrate-fed sheep.
d) Inhibition of carbo-anhydrase by carboxyzolamide (0.1 mmol·l^{-1}) caused a significant decrease of HCO_3^- transport.
e) The suggested anion exchanger of HCO_3^- transport was examined by using levetiracetam, a Na^+-coupled Cl^-/HCO_3 exchanger inhibitor. The addition of levetiracetam (1 mmol·l^{-1}) failed to produce a reduction of the HCO_3^- transport rates.
f) It is concluded that the transport of HCO_3^- is mediated by two anion exchangers in series and that the regulation of pH_i is of paramount importance of undisturbed HCO_3^- transport. There is evidence for adaptation of the omasal epithelium to diet.
g) It is proposed that the absorption of HCO_3^- should be included in the discussion of the pathogenesis of displaced abomasum.

6.2. Zusammenfassung

HCO_3^- Transport des Blättermagens vom Schaf: Effekte von Natrium und kurzkettigen Fettsäuren (SCFA)

In-vitro wurde der Transport von HCO_3^- in der mucosal–serosalen Richtung, J_{ms}, (Absorption) durch das Epithel des Blättermagens von Schafen untersucht. Die konventionelle Ussing Kammer Methode wurde mit der pH-stat Technik kombiniert. Die mucosale Seite des Epithels wurde mit einer Pufferösoung mit 50 mmol·l^{-1} HCO_3^- inkubiert und 10% CO_2 begast. Die Pufferlösung der serosalen Seite enthielt kein HCO_3^- und keinen Puffer. Der pH (7.4) der serosalen Seite wurde konstant gehalten duch die Titration mit H_2SO_4. Die Menge der titrierten H_2SO_4 wurde als transportierte Menge HCO_3^-,J_{ms}, angesehen.

a. Die erhaltenen Ergebnisse bestätigen das zur Diskussion vorgeschlagene Modell des Aniontransports durch das Epithel des Blättermagens vom Schaf: Der Transport wird durch zwei Anionenaustauscher in Serie in der apikalen und basolaterlaen Membran vermittelt. Das Epithel ermöglicht den Transport von HCO_3^- in beiden Richtungen entsprechend den vorgegebenen Gradienten. Der Transport von J_{ms} ist jedoch signifikant höher als in der entgegengesetzten Richtung, J_{sm}.
b. Der Transport von HCO_3^- wird durch die Aktivität des Na/H Austauschers (NHE3) in der apikalen Membran beeinflusst. Eine Hemmung des NHE3 mit Amilorid (1 mmol·l^{-1}) oder Verringerung der mukosalen Na Konzentration reduzieren den HCO_3^- Transport signifikant. Diese Effekte lassen die Schlussfolgerung zu, dass der Heraustransport von H^+ mit Hilfe des NHE3 von großer Bedeutung für die Regulation des intrazellulären pH, pH_i, und als Konsequenz auch für den HCO_3^- Transport ist.
c. Die Beeinflussung des pH_i durch SCFA (Aufnahme der undissoziierten SCFA (J_{ms}) verringert den HCO_3^- Transport. Diese Wirkung ist jedoch von geringer Bedeutung für den HCO_3^- Transport in Geweben von Schafen, die mit Kraftfutter gefüttert wurden.
d. Die Hemmung der Carboanhydrase mit Ethoxyzolamid (0.1 mmol·l^{-1}) verursacht eine signifikante Abnahme des HCO_3^- Transports.
e. Levetiracetam hemmt den Na-gekoppelten Cl^-/HCO_3^-. Die muksoale Zugabe dieses Inhibitors (1 mmol·l^{-1}) beeinflusste den HCO_3^- Transport nicht.
f. Die Ergebnisse lassen die Schlussfolgerung zu, dass der HCO_3^- Transport durch zwei Anionenaustauscher in Serie in der apikalen und basolateralen Membran vermittelt wird und maßgeblich vom pH_i beeinflusst wird. Es liegen Hinweise vor,

dass Adaptationsvorgänge in Abhängigkeit von der Fütterung (Kraftfutter) erfolgen und den HCO_3^- Transport stabilisieren.

g. Es wird vorgeschlagen, dass der Transport von HCO_3^- und dessen Beeinflussung in die Diskussion der Pathogenese der Labmagenverlagerung einbezogen wird.

7. REFERENCES

A

Ali, O., Shen, Z., Tietjen, U., Martens, H. (2006)
Transport of acetate and sodium in sheep omasum: mutual, but asymmetric interactions
J Comp Physiol [B]: 1-11
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16468047.

Ali, O. H. A. (2005)
In vitro studies of ion transport in sheep omasum: interaction between Na, Cl and short chain fatty acids
Berlin, Freie Universität. 138 S.
https://bib.vetmed.fu-berlin.de/ResourceList/details/17091

Aronson, P. S., Nee, J., Suhm, M. A. (1982)
Modifier role of internal H^+ in activating the Na^+- H^+ exchanger in renal microvillus membrane vesicles
Nature 299 (5879): 161-3
http://www.ncbi.nlm.nih.gov/pubmed/7110335

B

Baird N. R., Orlowski J., Szabó E. Z., Zaun H. C., Schultheis P. J., Menon A. G., Shull G. E. (1999)
Molecular cloning, genomic organization, and functional expression of Na^+/H^+ exchanger isoform 5 (NHE5) from human brain
J. Biol. Chem. 274 (7): 4377-82
http://www.ncbi.nlm.nih.gov/pubmed/?term=Baired+1999+nhe

Bannink A., Gerrits, W.J.J., France J., Dijkstra J. (2012)
Variation in rumen fermentation and the rumen wall during the transition period in dairy cows.
Animal Feed Science and Technology 172, 80-94
http://www.animalfeedscience.com/article/S0377-8401(11)00499-8/abstract

Benos. D. J. (1982)
Amiloride: a molecular probe of sodium transport in tissues and cells
Am. J. Physiol. 242 (3): C131-45
http://www.ncbi.nlm.nih.gov/pubmed/7039345

Bergman, E. N. (1990)
Energy contributions of volatile fatty acids from the gastrointestinal tract in various species
Physiol Rev 70: 567-590

Blazer-Yost. B. L., Nofziger, C. (2005)
Phosphoinositide lipid second messengers: new paradigms for transepithelial signal transduction
Pflugers Arch. 450 (2): 75-82
http://www.ncbi.nlm.nih.gov/pubmed/15614575

Brett, C. L., Wei, Y., Donowitz, M., Rao, R. (2002)
Human Na^+/H^+ exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria
Am. J. Physiol. Cell Physiol. 282 (5): C1031-41
http://www.ncbi.nlm.nih.gov/pubmed/?term=Brett+2002+nhe

C

Cavet, M. E., Akhter, S., Murtazina, R., Sanchez de Medina, F., Tse, C. M., Donowitz, M. (2001)
Half-lives of plasma membrane Na(+)/H(+) exchangers NHE1-3: plasma membrane NHE2 has a rapid rate of degradation
Am. J. Physiol. Cell Physiol. 281 (6): C2039-48
http://www.ncbi.nlm.nih.gov/pubmed/11698263

Cha, B., Oh, S., Shanmugaratnam, J., Donowitz, M., Yun, C. C. (2003)
Two histidine residues in the juxta-membrane cytoplasmic domain of Na^+/H^+ exchanger isoform 3 (NHE3) determine the set point
J. Membr. Biol. 191 (1): 49-58
http://www.ncbi.nlm.nih.gov/pubmed/?term=cha+nhe3+histidine

Chandrasekar, V. (1992)
Comparative gross anatomy of the omasum of sheep and goats
URL:
http://www.smallruminantresearch.com/article/0921-4488%2893%2990015-A/abstractref

Cheyron, D., Chalumeau, C., Defontaine, N., Klein, C., Kellermann, O., Paillard, M., Poggioli, J. (2003)
Angiotensin II stimulates NHE3 activity by exocytic insertion of the transporter: role of PI 3-kinase
Kidney Int. 64 (3): 939-49
http://www.ncbi.nlm.nih.gov/pubmed/?term=Cheyron+PI+3-kinase

Chien, W. J., Stevens, C. E. (1972)
Coupled active transport of Na and Cl across forestomach epithelium
Am. J. Physiol. 223 (4): 997-1003
http://www.ncbi.nlm.nih.gov/pubmed/5075173

Cordat, E.; Casey, R. (2009)
Bicarbonate transport in cell physiology and disease
Biochem. J. 417: 423-439
http://www.ncbi.nlm.nih.gov/pubmed/19099540

Counill, L.; Poyssegur, J. (2000)
The expanding family of eucaryotic Na(+)/H(+) exchangers
J. Biol. Chem. 275 (1): 1-4
http://www.ncbi.nlm.nih.gov/pubmed/?term=Counillon+and+Pouyss%C3%A9gur%2C+2000

D

De Silva, M. D., Elliot, K., Dahl, H.H., Fitzpatrick, E., Wilcox, S., Delatycki, M., Williamson, R., Efron, D., Lynch, M., Forrest, S. (2003)
Disruption of a novel member of a sodium/hydrogen exchanger family and DOCK3 is associated with an attention deficit hyperactivity disorder-like phenotype
J. Med. Genet. 40 (10): 783-40
http://www.ncbi.nlm.nih.gov/pubmed/14569117

Dobson, A. (1959)
Active transport through the epithelium of the reticulo-rumen sac
J Physiol 146(2): 235-51
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=13655229.

Donowitz, M., Cha, B., Zachos, N. C., Brett, C. L., Sharma, A., Tse, C. M., Li, X. (2005)
NHERF family and NHE3 regulation
J. Physiol. 567 (Pt 1): 3-11
http://www.ncbi.nlm.nih.gov/pubmed/15905209

Dorwart, M. R., Shcheynikov, N., Yang, D., Muallem, S. (2008)
The solute carrier 26 family of proteins in epithelial ion transport
Physiology (Bethesda) 23: 104-114
http://www.ncbi.nlm.nih.gov/pubmed/18400693

Dölle, M. (2008)
Charakterisierung des Na+-Transportes am isolierten Psalterepithel des Schafes
Berlin, Freie Universität Berlin. S.

E

Edrise, B. M., Smith, R. H. (1979)
Absorption and secretion in the omasum of the young steer
Ann Rech Vet 10(2-3): 354-5

Edrise, B. M., Smith, R. H. (1986a)
Exchanges of magnesium and phosphorus at different sites in the ruminant stomach
Arch Tierernahr 36(11): 1019-27
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3813874.

Edrise, B. M., Smith, R. H., Hewitt, D. (1986b)
Exchanges of water and certain water-soluble minerals during passage of digesta through the stomach compartments of young ruminating bovines
Br J Nutr 55(1): 157-68
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3663572.

Ehrlein, H.-J., Hill, H. (1968)
Motorik und Nahrungtransport des Psalters (Omasum) der Ziege
Zbl. Vet. Med., A, 16: 573-596
http://www.ncbi.nlm.nih.gov/pubmed/4981873

Ekman, J., Sperber, I. (1953)
The distribution of concentrations of bicarbonate (including carbon dioxide) and cloride in the omasum of cows
Kungl. Lantbrukshögskolans Annaler 19. S. 227 - 231

Engelhardt, W. v., Hauffe, R. (1975)
Role of the omasum in absorption an secretion of water and electrolytes in sheep and goats
In: Digestion and Metabolism in the Ruminant. / I. W. McDonald, and A. C. I. Warner (Hrsg.).
Armidale: University of New England Publishing Unit.: 216 - 230

Engelhardt, W. v., Hauffe, R. (1975a)
Functions of Omasum in Small Domestic Ruminants .4. Absorption and Secretion of Electrolytes
Zentralblatt Fur Veterinarmedizin - Reihe A 22(5): 363-375.
URL: <Go to ISI>://A1975AJ71000002.

Engelhardt, W. v., Hauffe, R. (1975b)
Role of the omasum in absorption an secretion of water and electrolytes in sheep and goats
In: Digestion and Metabolism in the Ruminant. / I. W. McDonald, and A. C. I. Warner (Hrsg.).
Armidale: University of New England Publishing Unit.:216 - 230.

Etschmann B, Suplie A, and Martens H. (2009) Change of ruminal sodium transport in sheep during dietary adaptation. Arch Anim Nutr 63, 26-38.

Etschmann, B., Heipertz, K. S., von der Schulenburg, A., Schweigel, M. (2006):
A vH+-ATPase is present in cultured sheep ruminal epithelial cells
Am J Physiol Gastrointest Liver Physiol 291(6). S. G1171-9.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16825706.

F

Favilli, N. (1937)
Struktur und Tätigkeit des dritten Magens (Blättermagen oder Psalter) bei den Hauswiederkäuern
Deutsche Tierarztliche Wochenschrift: 592 - 594

Ferreira, H. G., Harrison, F. A., Keynes, R. D. (1966)
Potential and Short-Circuit Current across Isolated Rumen Epithelium of Sheep
Journal of Physiology-London 187(3): 631-&
URL: <Go to ISI>://A19668782200011.

G

Gäbel, G., Aschenbach, J. R., Muller, F. (2002)
Transfer of energy substrates across the ruminal epithelium: implications and limitations
Anim Health Res Rev 3(1). S. 15-30.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12400867.

Georgi, M., Rosendhal, J., Ernst, F., Günzel, D., Aschenbach, J., Stumpf, F. (2013)
Epithelia of the ovine and bovine forestomach express basolateral maxi-anion channels permeable to the anions of short-chain fatty acids
Pfugers Arch - Eur J Physiol DOI 10.1007/s00424-013-1386-x (2013).
http://www.ncbi.nlm.nih.gov/pubmed/24240698

Goyal, S., Vanden Heuvel, G., Aronson, P. S. (2003)
Renal expression of novel Na^+/H^+ exchanger isoform NHE8
Am. J. Physiol. Renal. Physiol. 248 (3): F467-73
http://www.ncbi.nlm.nih.gov/pubmed/12409279

H

Harrison, F. A. (1971)
Ion Transport across Rumen and Omasum Epithelium
Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 262(842): 301-&
URL: <Go to ISI>://A1971K070300017.

Hauffe, R., Engelhardt, W. v. (1975)
Functions of Omasum of Small Ruminants
3. Absorption of Water
Zentralblatt Fur Veterinarmedizin - Reihe A 22(4): 283-295.
URL: <Go to ISI>://A1975AE99200003.

Höfelmeier, G. (1991)
Untersuchungen über die Wirkung von Lasalocid auf Transportvorgänge isolierter Vormagenepithelien von Schafen
Hannover, Tierärztl. Hochsch. 145 S.

Höglund, P. (2006)
SLC26A3 and congenital chloride diarrhoea
Novartis Found. Symposium 273: 74-86
http://www.ncbi.nlm.nih.gov/pubmed/17120762

J

Janecki, A. J., Montrose, M.H., Zimniak ,P., Zweibaum, A., Tse, C. M., Khurana, S., Donowitz, M. (1998)
Subcellular redistribution is involved in acute regulation of the brush border Na^+/H^+ exchanger isoform 3 in human colon adenocarcinoma cell line Caco-2. Protein kinase C-mediated inhibition of the exchanger
J. Biol. Chem. 273 (15): 8790-8
http://www.ncbi.nlm.nih.gov/pubmed/9535857

Jennings, M. L. (1976)
Proton fluxes associated with erythrocyte membrane anion exchange
J. Membr. Biology 28 (2-3): 187-205.
http://www.ncbi.nlm.nih.gov/pubmed/9514

K

Kaske, M. (1987)
Die retention von Partikeln unterschiedlicher Dichte und Grösse im Retikulorumen von Schafe
Inaugural Dissertation, Hannover.

Kaske, M, Engelhardt, W. v. (1990)
The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep
British J. of Nutrition 63: 457-465
http://www.ncbi.nlm.nih.gov/pubmed/2383525

Klanke, C. A., Su, Y. R., Callen, D. F., Wang, Z., Meneton, P., Baird, N., Kandasamy, R. A., Orlowski, J., Otterud, B. E., Leppert, M., et al. (1995)
Molecular cloning and physical and genetic mapping of a novel human Na^+/H^+ exchanger (NHE5/SLC9A5) to chromosome
Genomics 25 (3): 615-22
http://www.ncbi.nlm.nih.gov/pubmed/7759094

L

Lee-Kwon, W., Kim, J. H., Choi, J. W., Kawano, K., Cha, B., Dartt, D. A., Zoukhri, D., Donowitz, M. (2003)
Ca2+-dependent inhibition of NHE3 requires PKC alpha which binds to E3KARP to decrease surface NHE3 containing plasma membrane complexes
Am. J. Physiol. Cell. Physiol. 285 (6): C1527-36
http://www.ncbi.nlm.nih.gov/pubmed/12954600

Leniger, T., Thöne, J., Bonnet, U., Hufnagel, A., Bingmann, D., Wiemann, M. (2004)
Levetiracetam inhibits Na+-dependent Cl^-/ HCO_3^- exchange of adult hippocampal CA3 neurons from guinea-pigs
Br. J. Pharmacol. 142 (7): 1073-80
http://www.ncbi.nlm.nih.gov/pubmed/?term=Leniger+lev

Li, S., Sato, S., Yang, X., Preisig, P. A., Alpern, R. J. (2004)
Pyk2 activation is integral to acid stimulation of sodium/hydrogen exchanger 3
J. Clin. Invest. 114 (12): 1782-9
http://www.ncbi.nlm.nih.gov/pubmed/15599403

Lubis, D., O'Shea, J. D. (1978)
Development of the omasum in sheep
Acta Anat (Basel) 100(4): 400-10
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=636816.

M

H. Martens (1995) Gastrointestinale Transportmechanismen. In : Nutztierernährung. Herausgeber: HJ. Abel et al. 1995, Seite 219 - 246. Gustav Fischer Verlag Jena/Stuttgart

Martens, H. (1998)
Beziehungen zwischen Fütterung, Physiologie der Vormägen und der Pathogenese der Dislocatio abomasi
In: Ätiologie, Pathogenese, Diagnostik, Prognose, Therapie und Prophylaxe der Dislocatio abomasi
Leipzig, 14.Okt.1998. -
Proceeding Internationaler Workshop. S. 81 - 101.

Martens, H., Gäbel, G. (1988)
Transport of Na and Cl across the Epithelium of Ruminant Forestomachs - Rumen and Omasum - a Review
Comparative Biochemistry and Physiology a-Physiology 90(4): 569-575
URL: <Go to ISI>://A1988P849000009.

Martens, H., Gäbel, G., Strozyk, B. (1991)
Mechanism of electrically silent Na and Cl transport across the rumen epithelium of sheep
Exp Physiol 76(1): 103-14
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2015067.

Martens, H., Krützfeld, Th., and Wolf, K. (2004)
Sodium transport across the isolated epithelium of sheep omasum is influenced by luminal ammonia.J.
Vet. Med. A 51 (2004), 1-6

Martens H, Rabbani I, Zanming S, Stumpff F, Deiner D. (2012) Changes in rumen absorption processes during transition. Animal Feed Science and Technology (2012) 172, 95-102.

Masereel, B., Pochet, L., Laeckmann, D. (2003)
An overview of inhibitors of Na(+)/H(+) exchanger
Eur. J. Med. Chem. 38 (6): 547-54
http://www.ncbi.nlm.nih.gov/pubmed/12832126

McMurtrie, H. L.; Cleary, H. J.; Alvarez, B. V.; Loiselle, F. B., et al. (2004)
The bicarbonate transport metabolon
J Enzyme Inhib Med Chem 19(3): 231-6.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15499994.

McSweeney, C. (1986)
An omaso-abomasal cannula used to assess efflux from the omasum of sheep
Aust Vet J 63(10): 332-4.

McSweeney, C. (1988)
A Comparative-Study of the Anatomy of the Omasum in Domesticated Ruminants
Australian Veterinary Journal 65(7). S. 205-207.
URL: <Go to ISI>://A1988P812200004.

Musch, M. W., Bookstein, C., Xie, Y., Sellin, J. H., Chang, E. B. (2001)
SCFA increase intestinal Na absorption by induction of NHE3 in rat colon and human intestinal C2/bbe cells
Am. J. Physiol Gastrointest. Liver. Physiol. 280 (4): G687-93
http://www.ncbi.nlm.nih.gov/pubmed/11254495

Murer, H., Hopfer, U., Kinne, R. (1976)
Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney
J Am Soc Nephr. 9 (1): 143-50
http://www.ncbi.nlm.nih.gov/pubmed/9440099

N

Niebuhr, V. (2003)
In vitro Untersuchungen zum Bicarbonattransport des Blättermagenepithels von Schafen
Berlin, Freien Universität. Inaugural – Dissertation. 102 S.
URL: http://www.diss.fu-berlin.de/2003/283/.

Noel, J., Pouyssegur, J. (1995)
Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms
Am. J. Physiol. 268 (2 pt 1) C283-96
http://www.ncbi.nlm.nih.gov/pubmed/7864067

Numata, M., Orlowski, J. (2001)
Molecular cloning and characterization of a novel (Na^+,K^+)/ H^+ exchanger localized to the trans-Golgi network
J. Biol. Chem. 276 (20): 17387-94
http://www.ncbi.nlm.nih.gov/pubmed/?term=Numata+and+Orlowski+2001

O

Orlowski, J., Kandasamy, R. A., Shull, G. E. (1992)
Molecular cloning of putative members of the Na/H exchanger gene family. cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins
J. Biol. Chem. 267 (13): 9331-9
http://www.ncbi.nlm.nih.gov/pubmed/1577762

Orlowski, J., Grinstein, S. (1997)
Na^+/H^+ exchangers of mammalian cells
J. Biol. Chem. 272 (36): 22373-6
http://www.ncbi.nlm.nih.gov/pubmed/9278382

Oyaert, W., Bouckaert, J. H. (1961)
A study of passage of fluid through the sheep´s omasum
Res. vet. Sci. 2: 41 - 52

P

Pfeffer, E., Bertzbach, J., Lenkeit, W. (1966)
Untersuchungen über das Verhalten der mineralischen Mengenelemente im Verdauungskanal von Schafen bei Zufütterung von NaCl oder KCl
Z Tierphysiol Tierernahr Futtermittelkd 22:115-124.

Pizzonia, J. H, Biemesderfer, D., Abu-Alfa, A. K., Wu, M. S., Exner, M., Isenring, P., Igarashi, P., Aronson, P. S. (1998)
Immunochemical characterization of Na^+/H^+ exchanger isoform NHE4
Am. J. Physiol. 275 (4 pt 2): 510-7
http://www.ncbi.nlm.nih.gov/pubmed/9755122

D. W. Powell (1981) Barrier function of epithelia. Am J Physiol. 241: G275-88. Regulation of water and ion movement in intestine. Ciba Foundation Symposium 112, (1985), 14-23

Pushkin, A., Kurtz, I. (2006):
SLC4 base (HCO3 -, CO3 2-) transporters: classification, function, structure, genetic diseases, and knockout models
Am. J. Physiol. Renal Physiol. 290 (3): 580-599
http://www.ncbi.nlm.nih.gov/pubmed/16461757

R

Rabbani I., Siegling-Vlitakis, C., Noci, B., Martens, H. (2011)
Evidence for NHE3-mediated Na transport in sheep and bovine forestomach
Am. J. Physiol. Regul. Integr. Comp Physiol. 301 (2): R313-9
http://www.ncbi.nlm.nih.gov/pubmed/?term=rabbani+2011+nhe

Romero, M. F., Fulton, C. M., Boron, W. F. (2004)
The SLC4 family of HCO 3 - transporters
Pflugers Arch 447(5): 495-509.

Romero, M. F., Chen, A., Parker, M. D., Boron, W. F. (2013)
The SLC4 family of HCO 3 - transporters
Mol. Aspects Med 34 (2-3): 159-82
http://www.ncbi.nlm.nih.gov/pubmed/23506864

S

Sardet, C., Franchi, A., Pouyssegur, J. (1989)
Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter
Cell. 56 (2): 271-80
http://www.ncbi.nlm.nih.gov/pubmed/2536298

Schaechinger, T. J., Oliver D. (2007)
Nonmammalian orthologs of prestin (SLC26A5) are electrogenic divalent/chloride anion exchangers
Proc. Natl. Acad. Sci. U.S.A. 104 (18): 7693-7698
http://www.ncbi.nlm.nih.gov/pubmed/17442754

Schultheiss, G. (1995)
Calcium-sensitiver,elektrogener Natriumtransport des Psalterepithels von Schafen
Berlin, Freie Universität. 149 S.

Schultheiss, G., Martens, H. (1999)
Ca-sensitive Na transport in sheep omasum
Am J Physiol 276(6 Pt 1): G1331-44.

Sellin, J. H., Desoignie, R. (1989)
Regulation of bicarbonate transport in rabbit ileum: pH stat studies
Am J Physiol 257(4 Pt 1). S. G607-15.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2801943.

Siciliano-Jones, J., Murphy, M. R. (1989)
Production of volatile fatty acids in the rumen and cecum-colon of steers as affected by forage:concentrate and forage physical form
J Dairy Sci 72(2): 485-92.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2703570.

Stevens, C. E., Sellers, A. F., Spurell, F. A. (1960)
Function of the bovine omasum in ingesta transfer
Am. J. Physiol. 198(2): 449-455.
http://www.ncbi.nlm.nih.gov/pubmed/13834530

Stevens, C. E. (1964)
Transport of Sodium and Chloride by the Isolated Rumen Epithelium
Am J Physiol 206. S. 1099-105.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14208949.

Szaszi, K., Paulsen, A., Szabo, E. Z., Numata, M., Grinstein, S., Orlowski, J. (2002)
Clathrin-mediated endocytosis and recycling of the neuron-specific Na^+/H^+ exchanger NHE5 isoform. Regulation by phosphatidylinositol 3'-kinase and the actin cytoskeleton
J. Biol. Chem. 277 (45): 42623-32
http://www.ncbi.nlm.nih.gov/pubmed/12205089

T

Tamminga, S., van Vuuren, A. M. (1988)
Formation and utilization of end products of lignocellulose degradation in ruminants
Anim. Feed Sci. Techn. 21: 141-159
http://www.sciencedirect.com/science/article/pii/037784018890096X

Tiling, C. (1997)
In vitro Untersuchungen zum Cloridtransport des Blättermgenepithels von Schafen
Berlin, Freie Universität. Inaugural-Dissertation. 134 S.

Tse C. M., Ma, A. I., Yang V. W., Watso, A. J., Levine, S., Montrose, M. H., Potter, J., Sardet, C., Pouyssegur, J., Donowitz, M. (1991)
Molecular cloning and expression of a cDNA encoding the rabbit ileal villus cell basolateral membrane Na^+/H^+ exchanger
EMBO. J. 10 (8): 1957-67
http://www.ncbi.nlm.nih.gov/pubmed/1712287

Tse C. M., Brant, S. R., Walker, M. S., Pouyssegur, J., Donowitz, M. (1992)
Cloning and sequencing of a rabbit cDNA encoding an intestinal and kidney-specific Na^+/H^+ exchanger isoform (NHE-3)
J. Biol. Chem. 267 (13): 9340-6
http://www.ncbi.nlm.nih.gov/pubmed/1374392

Tse C. M. , Levine, S., Yun, C. H., Brant, S. R., Pouyssegur, J., Montrose, M. H., Donowitz, M. (1991)
Functional characteristics of a cloned epithelial Na^+/H^+ exchanger (NHE3): resistance to amiloride and inhibition by protein kinase C
Proct. Natl. Acad. Sci. U.S.A. 90 (19): 9110-4
http://www.ncbi.nlm.nih.gov/pubmed/8415663

Tyagi. S, Venugopalakrishnan. J, Ramaswamy. K. Dudeja, P. K. (2002)
Mechanism of n-butyrate uptake in the human proximal colonic basolateral membranes
Am. J. Physiol. Gastrointest. Liver. Physiol. 282 (4): G676-82
http://www.ncbi.nlm.nih.gov/pubmed/11897627

U

Ussing, H. H. (1949)
Transport of ions across cellular membranes
Physiol Rev 29(2):127-55
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18144413.

Ussing, H. H.; Zerahn, K. (1951)
Active transport of sodium as the source of electric current in the short-circuited isolated frog skin
Acta Physiol Scand 23(2-3):110-27
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14868510.

V

W

Wakabayashi, S., Fafournoux, P., Sardet, C., Pouysségur J. (1992)
The Na^+/H^+ antiporter cytoplasmic domain mediates growth factor signals and controls "$H(^+)$-sensing"
Proc. Natl. Acad. Sci. U. S. A. 89 (6):2424-8
http://www.ncbi.nlm.nih.gov/pubmed/1372444

Wakabayashi, S., Bertrand, B., Ikeda, T., Pouysségur, J., Shigekawa, M. (1994)
Mutation of calmodulin-binding site renders the Na^+/H^+ exchanger (NHE1) highly H(+)-sensitive and Ca2+ regulation-defective
J. Biol. Chem. (269 (18): 13710-5
http://www.ncbi.nlm.nih.gov/pubmed/8175807

Warner, R.G. and Flatt, W.P. (1965)
Anatomical development of the ruminant stomach
Physiology of Digestion of the Ruminant, Butterworth, Inc. Washington. P 24.

Wegeler, C. (2008)
Transport von HCO_3^- am isolierten Psalterepithel des Schafes (Charakterisierung und Einfluss der Fütterung)
Berlin, Freie Universität Berlin. S.

Weinman, E. J., Cunningham, R., Shenolikar, S. (2005)
NHERF and regulation of the renal sodium-hydrogen exchanger NHE3
Pflugers Arch. 450 (3): 137-44
http://www.ncbi.nlm.nih.gov/pubmed/15742180

Y

Yamamoto, Y., Kitamura, N., Yamada, J., Andren, A., et al. (1994a)
Morphological study of the surface structure of the omasal laminae in cattle, sheep and goats
Anat Histol Embryol 23(2): 166-76
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7978351.

Yamamoto, Y.; Kitamura, N.; Yamada, J.; Yamashita, T. (1991a)
Scanning electron microscopy of vascular architecture in the mucosa of sheep omasal laminae
J Vet Med Sci 53(5): 833-8.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1836370.

Yamamoto, Y., Kitamura, N., Yamada, J., Yamashita, T. (1991b)
Muscular architecture in the omasal laminae of cattle and sheep
Vet Res Commun 15(4): 249-56.
URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1949596.

Yun, C. H.; Tse, C. M.; Nath, S.; Levine, S. H.; Donowitz, M. (1995)
Structure/function studies of mammalian Na-H exchangers--an update
J Physiol 482: 1S-6S
http://www.ncbi.nlm.nih.gov/pubmed/7730970

Z

Zachos, N. C.; Tse, M.; Donowitz, M. (2005)
Molecular physiology of intestinal Na^+/H^+ exchange
Annu. Rev. Physiol. 67: 411-43
http://www.ncbi.nlm.nih.gov/pubmed/15709964

8. APPENDIX

8.1. Buffer solutions

8.1.1. Standard buffer solutions

Substance	Mucosal control buffer in $mmol \cdot l^{-1}$
Sodium Chloride (NaCl)	15,2
Na-Gluconate ($C_6H_{11}O_7Na$)	74,6
Sodium Bicarbonate ($NaHCO_3$)	50
Na-dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$)	0,4
di-Na-hydrogenphosphate (Na_2HPO_4 2 H_2O)	2,4
Potasium Chloride (KCl)	5
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calcium Chloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesium Chloride ($MgCl_2 \cdot 6\ H_2O$)	1,2

Substance	Serosal control buffer in $mmol \cdot l^{-1}$
Sodium Chloride (NaCl)	90,2
Sodium Sulfate (Na_2SO_4)	27,4
Potasium Chloride (KCl)	5
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calcium Chloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesium Chloride ($MgCl_2 \cdot 6\ H_2O$)	1,2

8.1.2. Buffer solutions used for Na^+ reduction experiments

Substance	Buffer Na = 50 $mmol \cdot l^{-1}$
Sodium Chloride (NaCl)	15,2
Na-Gluconate ($C_6H_{11}O_7Na$)	0
Sodium Bicarbonate ($NaHCO_3$)	29,6
Na-dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$)	0,4
di-Na-hydrogenphosphate (Na_2HPO_4 2 H_2O)	2,4
Potasium Chlorid (KCl)	5
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calcium Chloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesium Chloride ($MgCl_2 \cdot 6\ H_2O$)	1,2
Potasium Bicarbonate ($KHCO_3$)	20,4
Potasium Gluconate ($C_6H_{11}KO_7$)	74,6

Substance	Buffer Na = 20 mmol·l^{-1}
Sodium Chloride (NaCl)	14,8
Na-Gluconate ($C_6H_{11}O_7Na$)	0
Sodium Bicarbonate ($NaHCO_3$)	29,6
Na-dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$)	0,4
di-Na-hydrogenphosphate (Na_2HPO_4 2 H_2O)	2,4
Potasium Chloride (KCl)	5,4
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calcium Chloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesium Chloride ($MgCl_2 \cdot 6\ H_2O$)	1,2
Potasium Bicarbonate ($KHCO_3$)	50
Potasium Gluconate ($C_6H_{11}KO_7$)	74,6

8.1.3. Buffer solutions used to investigate the role of SCFA concentrations on $HCO3^-$ transport

Substance	SCFA = 64 mmol·l^{-1}
Sodium Chloride (NaCl)	11
Na-Gluconate ($C_6H_{11}O_7Na$)	
Potassiumgluconate ($C_6H_{11}O_7K$)	
Sodiumhydrogencarbonate ($NaHCO_3$)	50
Potassiumhydrogencarbonate ($KHCO_3$)	
Na-dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$)	0,4
di-Na-hydrogenphosphate (Na_2HPO_4 2 H_2O)	2,4
Potassiumchloride (KCl)	5
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calciumchloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesiumchloride ($MgCl_2 \cdot 6\ H_2O$)	1,2
Sodium acetate ($C_2H_3NaO_2 \cdot 3\ H_2O$)	40
Sodium propionate ($C_4H_7NaO_2$ $3H_2O$)	16
Sodium butyrate ($C_4H_7NaO_2$ $3H_2O$)	8
Na	130,2
K	5
Cl	20,8
HCO	50
Gluconat	

Substance	SCFA = 100 mmol·l^{-1}
Sodium Chloride (NaCl)	
Na-Gluconate ($C_6H_{11}O_7Na$)	
Potassiumgluconate ($C_6H_{11}O_7K$)	
Sodiumhydrogencarbonate ($NaHCO_3$)	25
Potassiumhydrogencarbonate ($KHCO_3$)	25
Na-dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$)	0,4
di-Na-hydrogenphosphate (Na_2HPO_4 2 H_2O)	2,4
Potassiumchloride (KCl)	5
Glucose ($C_6H_{12}O_6 \cdot H_2O$)	5
Calciumchloride ($CaCl_2 \cdot 2\ H_2O$)	1,2
Magnesiumchloride ($MgCl_2 \cdot 6\ H_2O$)	1,2
Sodium acetate ($C_2H_3NaO_2 \cdot 3\ H_2O$)	62,5
Sodium propionate ($C_4H_7NaO_2$ $3H_2O$)	25
Sodium butyrate ($C_4H_7NaO_2$ $3H_2O$)	12,5
Na	130,2
K	5
Cl	20,8
HCO	50
Gluconat	

8.2. Acknowledgements

First and foremost, I would like to express my deepest gratitude to my mentor, Professor Dr. Holger Martens, for his valuable support and encouragement throughout my research. Without his guidance and persistent help this dissertation would not have been possible.

I acknowledge my sincere indebtedness and gratitude to Ms Katharina Wolf for her friendship, support and unlimited technical assistance during my work. Special thanks are also conveyed to Prof. Dr. Kurtesh Sherifi, without whose support this work would not have been initiated. Gratitude is extended to Dr. Isabel Dittmann for her assistance during the preparation of this thesis.

My sincere appreciation goes to members of the Institute of Veterinary Physiology, FU Berlin for providing a warm and friendly working environment.

I would also like to convey my thanks to German Academic Exchange Service and Margrete-Markus-Charity. Their generous and indispensable support is greatly acknowledged.

Finally, I wish to thank my brother and mother for their love and firm support throughout these years.

8.3. Eidesstattliche Erklärung

Hiermit erkläre ich, Driton Çaushi, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe. Ferner erkläre ich, dass die Arbeit bisher in keinem anderen Promotionsverfahren angenommen oder abgelehnt worden ist.

Berlin, den 03.02.2014

Driton Çaushi