IONIC HOMEOSTASIS IN RETICULO-RUMEN OF MOOSE AS A FACTOR OF ALIMENTARY ADAPTATION

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ABSTRACT: In this study the results obtained on the daily mineral intake by moose and on the concentration of mineral elements in the reticulo-rumen liquid of moose in summer and in winter months are compared to previous data on reindeer. In both species a great difference in seasonal nutrient consumption is not followed by variation in total mineral elements of the reticulo-rumen liquid. Nutrient homeostasis, particularly of sodium, potassium, phosphates, and nitrogen in reticulo-rumen content in winter is maintained by intensive excretion with saliva and by transepithelial shortchain fatty acid-bicarbonate exchange. The stable level of mineral elements in the reticulo-rumen liquid in winter is necessary for microorganisms and for enteric ion homeostasis. Na^+/H^+ and K^+/H^+ exchange through the rumen wall is a function of alkalinization of the epithelium. It is concluded that the reticulo-rumen in winter is a significant organ for nutrient deposition and its metabolism is a factor in wild ruminant seasonal adaptability.

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One of the problems faced by northern wild ruminants is the way they adapt to food extremely poor in minerals and protein in winter. The degree to which compartments of the ruminant gastrointestinal tract take part in compensating for seasonal change in imbalance of nutrients is the subject of many investigations (Staaland *et al.* 1984, White *et al.* 1984, Staaland and Thing 1991, Chalyshev and Weber 1994). The site of intensive nutrient absorption, particularly sodium and chloride, found on slaughtering animals, appeared to be the caecum-colon in reindeer (Staaland *et al.* 1984, White *et al.* 1984) as well as in moose and muskoxen (Staaland and Thing 1991). It has also been shown using the fistula method on the duodenum and ileum, and with a rough measure of saliva secretion, that the complex stomach is an important organ that allows ruminants to assimilate forage efficiently. In sheep, mineral utilization efficiencies, except chloride, in a complex stomach, as compared with the whole digestive tract, are 50% of the overall average in winter. Among other minerals, the utilization for sodium and potassium is approximately 50% and 60% respectively (Chalyshev and Weber 1994). In a complex stomach, the reticulo-rumen (RR) can be considered as an organ, where the processes of absorption occur most intensively. In sheep, goats and reindeer the RR is an important absorption location for sodium, potassium, magnesium, and especially shortchain fatty acids (SCFA) (Chalyshev 1986, 1995; Martens *et al.* 1991; Rechkemmer *et al.* 1995).

However, the physiological mechanism of ion exchange in the RR of wild northern ruminants which helps them adapt to extreme environmental conditions is not completely clear. Therefore, it was the objective of this study to compare the mineral composition of the RR liquid with the consumption of mineral elements in different seasons in moose and to evaluate the significance of ionic homeostazing in the RR as it pertains to alimentary adaptation of northern wild ruminants.

METHODS

Consumption of mineral components was measured with mass-balance experiments using 2 captive yearling moose (males, average weight 130 kg) in June, 5 yearling moose (males, average weight 170 kg) in July, and 5 1.5-year-old moose (4 males and 1 female, average weight 240 kg) in October-February at the moose farm of the Pechora-Ilych biosphere reserve. In summer, animals were fed birch and willow branches with foliage (June and July), willow-herb (July) and water *ad libitum*. In winter they were given birch and pine branches and plenty of water. For 3 days, mass-balances were made in early June, 10 in July, and 10 in October, January, and February. Adjustment of animal intake to that of body weight (BW) has been expressed as an intake of nutrients per unit metabolic size (mM/kg $BW^{0.75}$). Samples of the RR content were collected on different days at 2, 6, 8, 10, 14, 18, and 22 h in early June, at 2, 6, 10, 14, 18, and 22 h in middle July, and at 9, 11, and 14 h in the middle of October from each of 2 moose males (1-1.5 years old) with rumen fistulas, which were inserted in the field. The RR liquid (following water removal) was analysed.

Sodium, potassium, calcium, and magnesium concentrations in moose food and the RR liquid were determined by means of the atomic-absorption technique; ammonium, bicarbonate, chloride, phosphates, and sulphate have been estimated respectively by the Conwey and Van-Slyke methods of potensiometric titration and colorimetry.

All the results are expressed as means ± standard error. The Student t-test has been used to compare measurements among groups, and statistical significance has been considered ($P<0.05$).

RESULTS

The nutrient content of seasonal moose diets has been published previously (Weber *et al.* 1992). The variations in matter and mineral daily intake by these animals in summer and in winter ranges in the Pechora taiga region are presented in Table 1. It demonstrates that the cation and anion supply for moose in winter was 3 to 6 times less than in mid-summer. The difference was statistically significant for all mineral elements ($P<0.01$), with the exception of cal-

Table 1. Dry matter (g) and mineral elements (mM) consumption per kg $BW^{0.75}$ per day by moose at the Moose farm, Pechora-Ilych biosphere reserve, Russia.

	June	July	October-February
Number of mass-balances (animals)	4(2)	10(5)	10(5)
Dry matter	91.9±0.5	132.7±7.5	76.4±2.8
Sodium	7.7±2.5	2.8±0.9	0.4±0.0
Potassium	30.5±0.2	45.9±2.8	5.9±0.2
Calcium	12.1±0.1	41.3±6.2	11.3±0.2
Magnesium	8.2±0.1	17.4±1.9	2.4±0.1
Chloride	7.9±2.5	3.8±0.9	0.3±0.0
Phosphate	11.1±0.1	11.9±0.3	3.9±0.2
Sulphate	1.2±0.1	1.3±0.1	0.2±0.0

cium intake in June and October. The winter diet (consumed mineral element / consumed dry matter x 1000) of moose was 2 to 4 times more deficient in mineral elements that in summer.

Mineral composition in moose RR liquid in summer and in late fall appeared to be relatively constant in the total amount of cations and anions: the quantity of potassium, calcium, and magnesium statistically decreased ($P<0.05$) while the concentration of sodium statistically increased ($P<0.05$) in equivalent volumes (Table 2). In early June, mid-July, and mid-October pH's of 6.3±0.1, 6.3±0.1, and 6.4±0.2 respectively were maintained in RR liquid. The major cation in the RR liquid in early June and in mid-October was sodium. Potassium predominated in the liquid in July. At the same time, the level of calcium and magnesium was also higher than in June and October.

DISCUSSION

All indications are that moose like many other northern ruminants inhabit ranges with highly contrasting seasonal nutritional conditions and different dietary quality and quantity (Kochanov *et al.* 1981, Schwartz *et al.* 1984, Weber *et al.* 1992). The problem of adaptation of such animals to extreme environments is important for understanding ruminant evolution. Because of very limited data on moose rumen physiology, the reindeer is especially helpful for discussion of digestion strategy in the north. Moose are a selective ruminant (Hofmann and Nygren 1992). Reindeer also display features of a selector and prefer a non-, or low-fiber diet with readily digestible cell contents. They inhabit areas with the most extreme winter conditions from a nutrition perspective compared with other wild ruminants (Kochanov *et al.* 1981) and therefore serve as a good model for alimentary adaptation.

Table 2. Concentration of ions (mM/L of liquid) in reticulo-rumen liquid of 2 male moose fed natural food at the Moose farm, Pechora-Ilych biosphere reserve, Russia.

	June	July	October
Number of samples	14	12	6
Sodium	103.2±3.7	74.4±6.0	107.1±7.7
Potassium	58.1±1.8	85.9±6.8	59.3±5.6
Calcium	15.9±1.5	26.3±1.9	10.7±1.8
Magnesium	5.5±0.3	9.1±0.4	4.2±0.3
Ammonium	15.9±3.2	5.3±0.5	6.4±0.9
Bicarbonate	5.1±1.3	11.0±1.8	9.7±2.4
Chloride	6.6±0.6	8.7±0.6	7.8±0.2
Phosphate	71.1±1.5	72.7±2.3	76.7±2.7
Sulphate	0.3±0.1	1.6±0.2	0.8±0.1

The sites of net nutrient absorption in the digestive tract of ruminants can be evaluated on slaughtered animals or with a combination of fistulas in the rumen, abomasum, or duodenum and ileum. The first method shows that the caecum-colon is an important compartment of the alimentary tract for mineral absorption in reindeer, especially sodium and chloride (Staaland *et al.* 1984, White *et al.* 1984), as well as in muskoxen (Staaland and Thing 1991) and moose. However, mineral excretion with saliva cannot be correctly accounted for using this technique, and does not afford an opportunity for mineral exchange assessment in the stomach compartments, particularly in the RR.

Secretion of nitrogen and mineral substances by salivary glands is one of the main factors that makes possible intensive nutrient assimilation in the RR of ruminants. Usually, salivary glands in these animals are characterized by the production of the final saliva that is close to isotonic at all flow rates, is rich in sodium, phosphate, and bicarbonate and is produced continuously (Cook 1995). It was demonstrated (Kochanov *et al.* 1981, Chalyshev 1986), that reindeer saliva in winter is abundantly supplied with phosphate (23 - 25 mM/L wet weight) in relation to sheep (8 - 10 mM/L wet weight) and is deficient in bicarbonate (28 - 30 and 53 - 56 mM/L wet weight respectively). Salivary glands in selective ruminants are distinguished by high development. In moose their weight varies up to 0.18% of the body weight in winter and to 0.30% in summer (Hofmann and Nygren 1992). For comparison, the weight of salivary glands in cattle is only 0.06% of the body weight (Hofmann 1989). It seems surprising that the winter level of saliva secretion (0.66 kg / kg $BW^{0.75}$) in northern wild ruminants (reindeer) is greater than that for summer (0.5 kg / kg $BW^{0.75}$). The measured salivary supply (average) of ions (data from one parotid gland x 4) for the RR in reindeer (M/day per kg $BW^{0.75}$) is given in Table 3.

An intensive secretion of mineral and nitrogen substances with saliva in wild northern ruminants in winter maintains ionic homeostasis in the RR. Reindeer range is characterised by highly different nutritional conditions in different seasons. However, there is not much variation in the quantity of total mineral substances in the forestomach of reindeer and moose. The concentration of mineral elements in the RR of reindeer (mM/L of liquid) is given in Table 4. The RR liquid of reindeer has a pH of 7.0 in summer and 6.4 in winter (Chalyshev and Weber 1994).

Table 3. Salivary supply (average) of ions (M/day per kg $BW^{0.75}$) for the reticulo-rumen in reindeer (from Chalyshev and Weber 1994).

	Summer	Winter
Sodium	53.2	71.2
Potassium	22.8	17.7
Calcium	0.9	0.5
Magnesium	0.4	0.4
Bicarbonate	28.5	22.5
Chloride	9.7	5.4
Phosphate	19.8	24.8

It was demonstrated in reindeer using fistulas in the duodenum (before the biliary and pancreatic duct) and ileum with a rough measure of saliva secretion that mass and mineral matter (except chlorides) utilisation efficiencies in the complex stomach, as compared with the whole digestive tract, are approximately 70% and 40% (among other minerals, about 30% for sodium, and 55% for potassium) in summer and 80% and 70% (among other minerals, near 70% for sodium and 80% for potassium) in winter and are approximately half as much again in sheep. Mineral matter utilisation efficiency in the caecum-colon compartment in reindeer in winter when measured using this technique is near 12% (in particular, about 15% for sodium and 1% for potassium) (Chalyshev and Weber 1994). As for sodium, the efficiency of its reabsorption at first in the forestomach, then in the small

Table 4. Concentration of mineral elements (mM/L of liquid) reticulo-rumen of reindeer (from Chalyshev and Weber 1994).

	July	February -March
Sodium	54.6±2.5	109.3±4.5
Potassium	68.4±4.7	39.8±2.2
Calcium	25.3±1.8	6.8±0.3
Magnesium	7.5±0.4	4.2±0.1
Ammonium	13.7±0.6	7.9±0.5
Bicarbonate	18.8±3.6	12.7±0.6
Chloride	17.6±1.0	16.7±0.5
Phosphate	45.6±2.2	62.2±1.5

intestine and finally in the caecum-colon support its balance in diets which are deficient. It seems that the processes occurring in the RR help wild northern ruminants to maintain digestion, absorption, and metabolism under the conditions of nutrient deficiency in winter diets. Besides, relative selectors (reindeer) appear to be more effective assimilators of nutrients in the complex stomach compared to nonselective roughage grazers (sheep). This fact is in conflict with Hofmann's theory (1989) which states that ruminant feeders have an evolutionary tendency toward an increased fiber digestion. It seems that the highly functional role and specialization of the RR as the organ for digestion and absorption of many nutrients allows selectors as a separate evolutionary branch of ruminants to adapt to extreme nutritional conditions.

The saliva of reindeer in winter is slightly hypotonic, being high in potassium and low in sodium compared to sheep (Kochanov *et al.* 1981). However, the main ion of reindeer saliva is sodium and it is of vital importance in the RR functions. Sodium is the most common osmoregulating cation in the RR liquid and electrogenic extracellular cation. It is actively absorbed across the rumen epithelium immediately after intake. In reindeer roughly 40% of the sodium secreted with the saliva can be reabsorbed in the forestomach in summer and up to 80% in winter (Chalyshev and Weber 1994). As for sheep (Chalyshev 1990, Martens *et al.* 1991) and reindeer (Chalyshev 1995), a great deal of apical sodium uptake (up to 90%) into the rumen epithelium is characterised as Na^+/H^+ exchange. It was also shown that up to 60% of the potassium in the summer and 80% in winter can be reabsorbed in the forestomach of reindeer from the saliva (Chalyshev and Weber 1994). The K^+/H^+ exchange that provides the overall potassium absorption appears to be present in the RR wall of sheep and reindeer (Chalyshev 1995).

It was shown by Kochanov *et al.* (1981) that a mineral consumption increase in summer forms an acid-base balance with alkali characteristics whereas a low level of mineral elements in the diet of reindeer and moose in winter leads to acidosis. In a metabolic acidosis state in winter it is important for wild northern ruminants to have a mechanism for regulation of the acid-base balance. Excretion of hydrogen ions through the RR epithelium is brought about by Na^+/H^+ and K^+/H^+ exchange which presumably serves as an acid-base balance regulator throughout the gastrointestinal tract of wild ruminants. The fact that the alimentary tract assists in acid-base regulation is that faeces in moose and in reindeer have a pH of 7.4 and 8.2 respectively in summer, and 6.2 and 6.4 in winter (Kochanov *et al.* 1981). In addition, the acid-base homeostasis in epithelial cells is necessary for their proliferation. The rumen epithelium consists of 15 to 20 layers of cells, that need to be regenerated because of constant shedding of dead cells. One of the earliest events in rumen epithelial cell proliferation appears to be the activation of the plasma membrane Na^+/H^+ exchange (Gäbel *et al.* 1996). It follows that Na^+/H^+ and K^+/H^+ exchanges have great significance for RR functions with the hydrogen ions being neutralised by bicarbonates, phosphates, and ammonia.

In addition to secretion of mineral and nitrogen substances with saliva, transepithelial passing of SCFA is also a factor of homeostasis in the RR. In the forestomach, SCFA are produced by intraluminal microorganisms and more than 80% of them are directly absorbed. With ruminants, up to 90% of the energy requirements can be met by these fatty acids (Bergman 1990). It has been well documented that SCFA in sheep are absorbed across ruminal epithelium both as anions and as undissociated acids (Kramer *et al.* 1996). Absorption of SCFA as well as buffer inflow with the saliva are important factors in the maintenance of pH in the RR in winter within narrow limits (6.3 to 6.4). In addition to these processes, alkalising of intraruminal content may be achieved by bicarbonate secretion across the RR wall as shown by Ash and Dobson (1963). The ratio of bicarbonates to absorbed SCFA is 1:2. This mechanism is the reason why metabolic acidosis in both sheep and in reindeer adversely affects SCFA absorption and spontaneously limits energetic substratum supply for organisms at a low fermentation level in the RR (Chalyshev 1995). So, the RR wall has a combination of epithelial acidiferous and alkaliferous mechanisms which function in nutrient absorption. Regulation of a compromise warrants future investigations.

Nitrogen substances, mainly urea as well as phosphates which are secreted to the RR of reindeer or moose with saliva, provide microorganisms inhabiting the RR with nutrition (Kochanov *et al.* 1981). Since the intensity of rumen fermentation affects body temperature (Berman 1971), the maintenance of relatively constant conditions for fermentation in the RR of wild northern ruminants maintains the heat generation required in winter.

It seems reasonable to conclude that the RR of wild northern ruminants, especially moose and reindeer, is an important organ which assists in adaptation to extreme nutritional stresses and, to some extent, temperature conditions. Among the physiological processes providing the RR functions, the intensive recycling of mineral and nitrogen substances with saliva and transepithelial exchange of ions (sodium, potassium, proton, bicarbonate) and SCFA should be recognized.

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