Rukhsana Amin Runa

Adaptation capacity to saline drinking water in goats (*Capra hircus*)

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Rukhsana Amin Runa

born in Netrakona, Bangladesh

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1. Name of supervisor: Prof. Dr. Martina Gerken

2. Name of co-supervisor: Prof. Dr. Jürgen Hummel

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SUMMARY

Fresh water availability is considered a pivotal factor for animal health and production. Salinity intrusion in surface and ground water due to sea level rises, may affect natural water sources, especially in the coastal areas and some arid islands. It is anticipated, that increasing contamination of natural fresh water sources with salinity will threaten farm animal production in many regions across the globe. Therefore, it is important to know the animals' physiological and adaptation capacity to tolerate saline water without impairing their health.

The first Chapter reviews the effects of saline drinking water on farm animals. Salt (NaCl) is an essential element in animal diets. The biological role of sodium (Na^+) in regulating body fluid homeostasis, water metabolism, nerve function and osmotic pressure is extensively reviewed. It is pointed out, that different definitions and measurement of salinity in drinking water hamper comparisons between studies. Aspects of salt tolerance, sensory thresholds and the effect of high salt intake are addressed for different species with an emphasis on ruminants.

The detailed objectives of this thesis were:

1. To evaluate goats' capacity to differentiate saline water in a free choice system and to determine whether goats are able to adjust their choice in managing salt intake and to avoid excess salt ingestion by their self-selection (Chapter 2).
2. To investigate the individual adaptation capacity of goats to salted drinking water, and to test the differences in salt sensitivity after habituation (Chapter 3).
3. To measure changes in blood metabolites and body temperature in goats during their adaptation to saline drinking water (Chapter 4).

The experiments were carried out at the Department of Animal Sciences, University of Göttingen. The focus of the first study (Chapter 2) was to investigate the capacity of Boer goats to adjust their salt intake from saline drinking water in a free choice system. Twelve non-pregnant Boer goats aged between 1 to 8 years with an average body weight of 46.4 ± 8.3 kg were kept in individual pens for 4 weeks. In the control phase (1 week), only fresh water was supplied in five identical buckets for each pen. During the subsequent treatment phase (3 weeks), fresh water and four different concentrations (0.75, 1.0, 1.25, and 1.5% NaCl) of saline water were offered simultaneously in a free choice system. The positions of the concentrations were changed daily at random. Cut hay and water were provided *ad libitum* and a mineral supplement was allocated. Feed and water intake, mineral supplement intake, ambient temperature and relative humidity were recorded daily, while body weight and body condition score were measured weekly. Dry matter intake, total water intake and total sodium intake were significantly (*P<0.001*) higher during the treatment phase. Body weight and body condition were not affected by saline water intake. Across the treatment phase, saline water consumption was significantly (*P<0.001*) lower in young (19.6 ± 27.1 g/kg $BW^{0.82}$ per day) than in adult goats (27.9 ± 31.5 g/kg $BW^{0.82}$ per day), indicating that young goats were more sensitive towards saline water. All goats had a significant preference for fresh water (0% salt) over saline water. At the first offering of the simultaneous choice situation (week 2), animals did not differentiate between salt concentration of 0.75% and 1.0%. However, with successive treatment (week 3 and 4), animals distinguished between saline water concentrations and preferred the 0.75% salt concentration. Salt concentrations of 1% to 1.5% were avoided. The total sodium intake of the goats ranged between 0.37 – 0.55 g /kg $BW^{0.75}$ per day during the treatment phase, being 8 to 11 fold higher than the daily requirements of sodium for body maintenance. The results suggest that goats are able to differentiate between saline water concentrations and to adjust their sodium intake by quick adjustments in self-selection in a free choice system.

The second study (Chapter 3) was designed to investigate the adaptation capacity of goats towards sodium chloride (NaCl) in drinking water and their sensitivity responses after habituation to salted water. In this study, twelve non-pregnant Boer goats aged between 1.5 to 7.5 years with an average body weight of 50.5 ± 9.0 kg were kept in individual experimental pens under controlled stable conditions. A two choice preference test was used as the principal method. The study was conducted in four phases. After the control phase (phase 1), when only fresh water was supplied in two containers, water with different salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5%) was offered in one container and tap water in the other (phase 2; sensitivity test). During the third phase (adaptation), goats were stepwise habituated to saline water by offering only saline water with different concentrations (between 0% and 1.5% NaCl) in both containers. Subsequently, in phase 4 (sensitivity re-test) the same treatment as of phase 2 was repeated. Goats had access to hay and water *ad libitum,* and a mineral licking block was provided throughout the experimental period. Individual water and feed intake were recorded daily, while body weight and body condition score were measured bi-weekly. Body weight was not affected by saline water intake, whereas dry matter intake and body condition scores decreased significantly during the experiment. Water intake was significantly (*P<0.001*) higher in phase 2 (sensitivity test) and 3 (adaptation), while it approached control phase values in the sensitivity re-test (phase 4). Total sodium intake (TNaI) followed the same pattern. In phase 2, when goats had the choice between fresh and saline water for the first time, they preferred higher salt concentrations and consumed significantly (*P<0.001*) higher amounts of saline water (75.4 ± 53.2 g/kg $BW^{0.82}$ per day) than in the re-test (40.4 ± 34.0 g/kg $BW^{0.82}$ per day) after the habituation period. In tandem, salt discrimination rejection thresholds were lowered to 1.25% in phase 4, compared to 1.5% in phase 2. During the adaptation phase, initially, goats preferred saline water of 0.25% over fresh water and did not differentiate between concentrations of 0.25 and 0.5% when offered simultaneously. However, with increasing salt concentrations, they showed strong preferences for the lower concentrations. The results suggest that a stepwise adaptation to saline drinking water in goats is an effective method to habituate the animals to saline water intake when concentrations where below 1.5%.

The aim of third study (Chapter 4) was to determine the effects of saline water consumption on physiological parameters and the health status of goats during stepwise adaptation to saline water. The animals and experimental design were the same as in the second study (Chapter 3). Blood parameters were monitored on a weekly basis during phase 3, and at the end of phases 1, 2 and 4. Daily water and feed intake, respiration rate and rumen temperature (T_r) were measured throughout the experiment. Body weight (BW) and body condition score (BCS) were recorded bi-weekly. Across the experimental period, BW was not affected by saline drinking water, whereas BCS decreased during phase 3 and 4. Young goats (below two years, N=4) had lower (*P<0.001*) water and feed intakes compared to older animals (N=8) throughout the experiment. Total sodium intake (g/kg $BW^{0.75}$ per day) was higher during phases 2 (*P<0.05*) and 3 (*P<0.001*) in adult goats than in young goats (0.41 ± 0.03, 0.58 ± 0.02 *versus* 0.28 ± 0.05, 0.40 ± 0.04, respectively), while it approached control phase values in phase 4 with no difference between ages. The T_r significantly (*P<0.01*) decreased during the treatment (39.13 ± 0.15°C) compared to the control phase (39.18 ± 0.19°C) and the lowest T_r was recorded in phase 3 (adaptation) when goats consumed only saline water. However, in young goats T_r significantly increased with prolonged saline water intake, while it decreased in adult goats, suggesting

a higher metabolic impact due to salt excretion in young goats. Drinking saline water had no effect on the concentrations of ALT, AST, glucose, urea, calcium, sodium and osmolality. Serum magnesium decreased (*P<0.001*), while potassium concentration increased from phase 2 when saline water was first introduced and remained elevated until the end of the experiment (*P<0.001*). All measured blood parameters of our study remained within reference ranges, indicating remarkable adaptation capacities of Boer goats to saline water without damage to liver and kidney functions. Our results suggest that the applied scheme of a stepwise adaptation with salt concentrations below 1.5% is a suitable method for goats in regulating excess salt intake from drinking water without health impairment. However, the differences between young and old animals among all phases of the study underline that young animals are less resistant to salt toxicity compared to older ones.

In Chapter 5, the present results are compared with those from other livestock species. Taking into consideration other published results, the tolerance order of sodium chloride in drinking water in livestock species is suggested as follows: camels > goats = sheep > cattle > deer > poultry. The present studies suggest that Boer goats are able to differentiate between saline water concentrations in different designs of choice tests, and to adjust their sodium intake by quick adjustments in self-selection in a free choice system. Compared to two choice preference tests, the free choice situation allows evaluating changes in saline water acceptance with prolonged exposure. The stepwise adaptation to saline drinking water is an effective method to habituate the exposed animals to increasing water salinity. The animals reacted more sensitively when offered the choice between fresh water and different concentrations of saline water after the adaptation period. Accordingly, choice discrimination and rejection thresholds were not constant but depended on the individual sensitivity to salt, learning capacity, total sodium balance, and age of the animal. Changes in physiological parameters suggest that goats have a good adaptation capacity towards saline water and can regulate salt intake without health impairment for short durations.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Water and soil are important natural resources for the survival of living beings. Secured availability of sufficient fresh water is essential for animal production. In many tropical coastal regions, both underground and surface water are being contaminated with increased salinity due to sea level rises resulting from global climate changes (IPCC, 2014; WWAP, 2015). In Europe, nowadays, more moderate coastal habitats are also increasingly confronted with this phenomenon (IPCC, 2014). The problem of increased soil and water salinity is not bounded only within coastal areas as inside water bodies in many arid and pacific areas are also frequently getting contaminated by salinity (Cherfane, 2015; Berthe *et al*., 2015).

The change in climate and its variability may affect natural water balance and fresh water availability by increasing temperature that causes higher water evaporation from soil and open surfaces, and increased transpiration by vegetation (Buytaert *et al*., 2015). Over longer duration, climate induced changes will create huge challenges and threats for local and regional water resources (Buytaert *et al*., 2015). This may also foster greater extremes in weather, including higher temperature, heavy precipitation, droughts, violent storms, sea level rises and ocean acidification (Dessler, 2012; WWAP, 2015). Changes in sea level can affect coastal groundwater, threaten coastal aquifers with potential effects on drinking water quality (Hipsey and Arheimer, 2013) and coastal ecosystems by increasing risk of flood and degradation of essential ecosystem services (World Bank, 2010; Hallegatte *et al.,* 2013). However, it is almost impossible to avoid or abandon these salinity affected areas as they are required for food production. Therefore, it is important to adopt proper adaptation measures or mechanisms for farming practices, such as livestock management in these areas. In doing so, it is a pre-requisite to know the animals' normal physiological capacity of salinity tolerance without impairing their health.

Definition and measurement of salinity

Salinity is the saltiness or quantity of dissolved salt in a water body. Salts are compounds like sodium chloride, magnesium sulfate, potassium nitrate and sodium bicarbonate which dissolve into ions. Saline water (more commonly known as salt water) is defined as water containing a significant concentration of mainly sodium chloride salt. Based upon salinity, water bodies are divided into two classes (Por, 1972), defined as homoiohaline (salinity remains constant over time) and poikilohaline (salinity varies biologically ranging from 0.5 to >300 g/kg). According to the United States Geological Survey, saline water is classified into three categories; in slightly saline water, salt concentration is 1–3 g salt/kg water (0.1–0.3%), in moderately saline water 3–10 g/kg water (0.3–1%), and 10–35 g/kg water (1–3.5%) in highly saline water. Seawater has an average salt content of approximately 35 g/kg water (3.5%) and the saturation level depends on temperature and solubility of salt. However, seawater is not uniformly saline throughout the world. Lower values are found near coasts where rivers enter the ocean compared to salinity in the deep sea. The salinity level of different water sources and areas is presented in Table 1.1.

In the 1980s, a 'Practical Salinity' scale was developed to estimate the salinity of sea water where salinity was measured in 'Practical Salinity Units' (PSU) (Millero, 1993). The current standard for salinity is the 'Reference Salinity' scale (Millero et al., 2008) with the salinity expressed in units of g/kg. It is also expressed in parts per thousand (ppt, ‰) or parts per million (ppm). There are two main methods of determining the salt content of water; Total Dissolved Salts or Solids (TDS) and Electrical Conductivity (EC). EC measurement is much quicker, simpler and is very useful for field measurement compared to TDS (Agriculturevictoria, 2017). Total Dissolved Salts (TDS) is measured by evaporating a known volume of water to dryness, then weighing the solid residue remaining. TDS is recorded in milligrams of dissolved salt or solid in one liter of water (mg/L). Parts per million (ppm) is equivalent to mg/L but it is not a favored unit. Electrical conductivity (EC) is measured by passing an electric current between two metal plates (electrodes) in the water sample and measuring how readily current flows (i.e. is conducted) between the plates. The more dissolved salt is in the water, the stronger the current flow and the higher the EC. The standard EC unit is microSiemens per centimeter (µS/cm). EC measurement can also be used to estimate the TDS by converting with the relationship: TDS (mg/L) = EC (µS/cm at 25^{o}C) x 0.6 (Agriculturevictoria, 2017). Other units are used by some scientist to express the salinity of sea water. However the relationships between them need to be taken into account and µS/cm relates to other units as follows:

1000 µS/cm = 1 milliSiemens/centimeter (mS/cm)

10 µS/cm = 1 milliSiemens/metre (mS/m)

Table 1.1 Water salinity of different water sources and areas (Eilers *et al*., 1990; Anati, 1999)

Water sources	Salinity	
	(TDS, mg/kg)	(%)
Fresh water	< 500	< 0.05
Brackish water	500 – 30,000	0.05 – 3
Saline water	30,000 – 35,000	3 – 3.5
Brine	> 50,000	> 5
Rivers and lakes	< 10 – 500	< 0.001 – 0.05
Average sea water	35,000	3.5
Dead sea	200,000	20

Physiology

Biological role of Na+ (sodium)

Salt (NaCl) is an essential element in animal diets (Suttle, 2010). Animals are often fed sodium (Na^+) and chloride (Cl^-) in the form of salt (NaCl), either salt granule mixed with feed and water or in saltlicks. Salt consists of 39% sodium and 61% chlorine (Hollum, 1998; National Research Council (NRC), 2007). Because of their close ionic interactions, related functions, metabolism and requirements in the animal body, sodium and chlorine are considered as compound salt with Na^+ being the major cation in the interstitial fluids (Suttle, 2010). Sodium plays a dominant role in regulating body fluid homeostasis, control of water metabolism, nerve function and the absorption of chlorine and water (Agricultural Research Council (ARC), 1980; NRC, 2007; Suttle, 2010). It is also involved in transport systems for maintaining body temperature, glucose and amino acids absorption and neuromuscular activities (NRC, 2007). Together with potassium (K^+), sodium is one of the most important ions to regulate osmotic pressure and pH of the body by providing an 'osmotic skeleton' that is 'clothed' with an appropriate volume of water (Michell, 1995; Suttle, 2010).

In cattle and sheep, sodium is mostly absorbed in rumen, omasum and particularly the large intestines (ARC, 1980; Khorasani *et al*., 1997). Sodium uptake from the intestinal lumen is achieved by adhering to glucose and amino acid uptake via co-transporters (Harper *et al*., 1997). Sodium is transported across the cell membrane by ATP-dependent Na^+-K^+ pumps, electroneutral Na-K-Cl co-transporters, Na-HCO_3-Cl co-transporters and Na^+ Channels (NRC, 2007; Suttle, 2010). Metabolism of sodium is regulated hormonally by activating renin-angiotensin mechanisms. Aldosterone is the responsible hormone, which is excreted from the adrenal cortex when sodium levels decrease in the blood (Hollum, 1998), changing blood pressure and extra cellular fluids through adjusting thirst and water balance (Michell, 1995; Burnier, 2007). Sodium is excreted from the body via urine, skin secretions, and milk (Suttle, 2010).

Sensory thresholds

Saltiness is a unique taste among the five basic taste qualities (sweet, sour, bitter, salty and umami) perceived and detected by human and animals (Sugita, 2006). Sweet, salt and umami flavors are innately appetizing, whereas sour and bitter ones are instinctively unappetizing (Yoshida *et al*., 2006). However, salt taste can trigger two divergent behavioral responses; low concentrations of salt are attractive to animals, while high concentration of NaCl is rejected, even when salt deprived (Chandrashekar *et al.,* 2010). The anatomical units of this taste detection are taste receptor cells, which are located in taste buds and distributed across the different taste papillae of the tongue as well as the respiratory and upper gastrointestinal tracts (Sugita 2006; Yoshida *et al*., 2006). These taste receptor cells activate different gustatory nerves, which transmit the taste information to the brain (Yoshida *et al*., 2006). Response properties of taste receptor cells for different taste qualities depend on the receptors and transduction mechanisms (Lindemann, 2001). To activate salt receptor cells, sodium (Na^+) and chloride (Cl^-) ions are required (van der Klaauw and Smith 1995) and these receptor cells function through the Na^+ ion channels of the tongue (Chandrashekar *et al.,* 2010).

Variation in salt taste sensitivity was found to be related to many factors such as individual differences, exposure to foods and water with high sodium contents, age, sex and species (Goatcher and Church, 1970b; Bertino *et al*., 1986). Recent studies revealed that specific genes have also been associated with salt taste perception in humans (Dias *et al*., 2013). Taste function evaluation can be conducted by the estimation of detection and recognition thresholds using different methods. Studies have been carried out investigating the taste responses and thresholds to saline drinking water in sheep (Goatcher and Church, 1970a, b), goats (Bell, 1959) and cattle (Bell and Williams, 1959) by using an electrophysiological method (Bernard, 1964) and two choice preference test (Bell, 1959; Bell and Williams, 1959; Goatcher and Church, 1970a).

Feeding recommendations

Feeding recommendations refer to the minimum intake of nutrients required for production and good health. The recommended levels vary among different animals. Based on NRC (1981 and 1985), Berger (2006) recommended 5,000 mg/kg salt required to the total diet or 10,000 mg/kg salt to the concentrate portion of the diet in goats and sheep. However, sodium requirements for sheep and goats are estimated using a factorial approach considering maintenance, growth, pregnancy and lactation (NRC 2007). Endogenous sodium losses also need to be considered when estimating total daily sodium requirements for small ruminants. Sodium requirements for maintenance and lactation are added to the true absorption rate of 0.91 (NRC, 2007). The absorption rate of sodium in goats is around 80%, which is slightly lower than in sheep (NRC, 2007). The recommended daily sodium intake for sheep and goats are presented in Table 1.2.

Table 1.2 Recommended sodium intake for feed in sheep and goats (NRC, 2007)

	Sodium (g/day)	
	Sheep	Goats
Maintenance	(0.0108 x BW)/0.91	(0.015 x BW)/0.80
Growing	(1.1 x average DWG)/0.91	(1.6 x average DWG)/0.80
Pregnancy (105–133 days)	(0.021 x LBW)/0.91	(0.034 x LBW)/0.80
Lactating	(0.4 x MY)/0.91	(0.4 x MY)/0.80

BW = Body weight; DWG = Daily weight gain; LBW = Lamb born weight; MY = Milk yield

It is important to know the amount of salts present in the drinking water for the calculation of sodium requirements in a feeding plan. The amount of salt in drinking water is largely determined by the total dissolved salt content of the water. There are no distinct requirements of salt in drinking water. However, the following points need to be considered when using saline water for livestock and poultry (Table 1.3). The upper limits of soluble salts in water for livestock are listed in Table 1.4.

Table 1.3 Guidelines for the use of saline water by livestock and poultry (NRC, 1974; Greg, 2014)

Salinity (TDS in mg/L)	%	Effects for livestock
Less than 1,000	0.1	Relatively low level of salinity. Should not present any serious burden to livestock.
1,000 – 2,999	0.1–0.3	Should be satisfactory but may cause temporary and mild diarrhea in unaccustomed livestock. Should have no other effects on health or performance.
3,000 – 4,999	0.3–0.5	Should be satisfactory for livestock, although might cause temporary diarrhea or be refused at first. Poor waters for poultry, often causing watery feces, increased mortality and decreased growth, especially in turkeys.
5,000 – 6,999	0.5–0.7	Can be used with reasonable safety for dairy and beef cattle, sheep, pigs and horses. For pregnant or lactating animals, or horses in work, avoid using water with salinity at higher levels in this range. Unacceptable for poultry.
7,000 – 10,000	0.7–1.0	Considerable risk in using water for pregnant or lactating stock, young animals or any animals subjected to heavy heat stress or water loss. Unacceptable for poultry. Unsuitable for pigs and horses. In general, should be avoided in livestock, although older livestock may subsist on these waters in conditions of low stress.
10,000 – 15,000	1.0–1.5	Risky. Cannot be used for stock other than adult, dry sheep.
Around 19,000	1.9	Toxic: effects will vary depending on the type of salts present.

Table 1.4 Safe upper limit of total salt in water for livestock (Glauert, 2007)

Livestock	mg/L	mS/m	%
Poultry	3,000	545	0.3
Pigs	3,000	545	0.3
Dairy cattle (lactating)	3,500	600	0.4
Beef cattle	5,000	900	0.5
Lambs, weaners, breeder ewes, dry dairy cattle	5,000	900	0.5
Horses	6,000	1,100	0.6
Adult sheep (dry)	10,000	1,820	1.0

Effect of high salt intake

Feed

Ingestion of high salt in feed has an impact on feed and water intake, salt-water balance and hormonal control of energy (Digby *et al*., 2011). Excess sodium intake is associated with water retention, diarrhea, dehydration, muscle cramps and neurological problems (Peirce, 1957; Marai *et al.*, 1995; Kii and Dryden, 2005). Excessive dietary salt intake depresses feed intake (Masters *et al*., 2005; Blache *et al.*, 2007) and increases water intake which is associated with increased rate of nutrient passage, lowered protein degradation in rumen (Digby *et al*., 2011), damage kidneys (ARC, 1980) and reduced energy allocation for production (Arieli *et al*., 1989). High salt diets also affect meat quality by reducing intramuscular fat content (Pearce *et al*., 2008). Moreover, high dietary salt intake has significant consequences on reproductive capacity of rams (Leung and Sernia, 2003). Meyer and Weir (1954) reported increased weight losses during lactation in ewes receiving 13.5% sodium chloride in the diet compared to sheep consuming 9.1% or less. However, excess salt intake does not create any problem as long as animals have free access to fresh water. When animals ingest excess salt, they increase their water consumption, probably in an attempt to increase the urinary excretion of sodium to inhibit its surplus (Wilson and Dudzinski, 1973; Hamilton and Webster, 1987). If salt intake approaches poisoning levels, it has been associated with rapid breathing, diarrhoea, excessive thirst, head pressing, hydropericardium and death (Hungerford, 1990).

Drinking water

Animals can compensate higher salt intake in feed by increasing fresh water consumption. However, when the drinking water has a high salt content, this mechanism does not work and animals cannot get out of the surplus which leads to increased risk of toxicity. There are specific problems for livestock animals associated with the consumption of excess salt, particularly sodium, potassium and chloride in drinking water. Sea water intake leads to higher salt concentrations on the outside of the cell membrane compared to the inside. This difference in salt concentration impairs osmosis and causes cell shrinkage (Giuggio, 2018). To regain an isotonic state, the body attempts to eliminate the excess Na^+ from extracellular fluids through increased urination resulting in dehydration, muscle cramps, thirst, dry mouth and eyes (Giuggio, 2018). The body further tries to compensate this fluid loss by increasing heart rate and constricting blood vessels, which consequently leads to a reduced blood supply to the brain and other organs causing coma and eventually death (Giuggio, 2018).

It was found that 13,000 to 15,000 mg/kg salt in the drinking water only had small effects on production traits in sheep, while long term intake of water containing 20,000 mg/kg or more salt led to severe reduction in food intake, loss of body weight, emaciation and possibly death in sheep, goats and cattle (Peirce, 1957 and 1959; Weeth and Haverland, 1961; Wilson and Dudzinski, 1973; Hamilton and Webster, 1987; McGregor, 2004). High salt exposure in drinking water also has a dramatic effect on the reproductive capacity of ewes (Digby *et al*., 2011). Ewes exposed to 9,000 mg/kg sodium chloride in water occasionally failed to conceive (Peirce, 1968b) and water containing 13,000 mg/kg salt caused distress at parturition, a decrease in lambing percentage and an increase in lamb mortality (Peirce, 1968a, b; Potter and McIntosh, 1974).

Moreover, increased salinity concentration was found to decrease blood volume, plasma volume, urea concentrations as well as extracellular and interstitial fluids in ewes (Assad and El-Sherif, 2002) and German Merino sheep (Meintjes and Engelbrecht, 2004). Increased sodium intake can also alter ion absorption mechanisms resulting in hypertension (Elliott *et al*., 1996) and increased blood pressure associated with renal and cardiovascular diseases (Tuomilehto *et al*., 2001). Godwin and Williams (1986) reported increased urinary volume, electrolyte excretion and glomerular filtration rate in sheep and goats receiving high concentrations of saline water. On the contrary, drinking saline water had no effect on the urea concentration in urine of goats but caused a decrease in sheep and camels (Abou Hussien *et al*., 1994).

Salt tolerance and adaptation

In the coastal areas and some arid islands where natural freshwater is not available, animals are forced to drink saline water throughout their lives. This has helped them to develop certain physiological and behavioral adaptation mechanisms against increased body salinity which have enabled them to survive (Dunson, 1974; Gould, 1990). It has been observed that goats apparently drink seawater on beaches and islands. An increased renal salt excretion through their urine was measured in these animals probably as an adaptation to their living conditions (Dunson, 1974).

Salt tolerance varies between animal species and relates to available fresh water consumption. The salt tolerance in drinking water for livestock is summarized in Table 1.5.

Compared to other mammals, ruminants are more capable to tolerate higher salt in drinking water (Goatcher and Church, 1970b). Some factors affect salt tolerance in animals such as salt ingestion period (Peirce 1957 and 1959), age (Wilson and Dudzinski, 1973), environmental condition (Weeth and Haverland, 1961), physiological adaptation, and even foetal programming (Digby *et al*., 2011). Animals start to show sensitivity against salinity when they are supplied with saline water over longer durations. Sensitivity responses to salt ingestion differ based on food or drinking water (Masters *et al*., 2005). It was found that sheep can tolerate high sodium chloride (NaCl) concentrations ranging from 5% to 20% in their diet (Digby *et al*., 2011) while deer can tolerate at least 6% (Ru *et al*., 2004) and chickens only around 3% (Kare and Biely, 1948).

Excess sodium intake is primarily regulated by the kidneys through controlling sodium reabsorption, mediating changes in the active transport and membrane permeability (Suttle, 2010), and increasing renal sodium excretion through urine (Suttle 2010). It seems that the kidneys' capacity to concentrate urine and its ability to reduce urinary water loss during dehydration is directly related to the relative medullary thickness (RMT) of the kidneys. The greater the RMT, the longer the loop of Henle and therefore the larger the kidney's ability to reabsorb water. Increased sodium and chloride ions in plasma following consumption of high saline water can be excreted by (i) increased glomerular filtration rate and changes in renal plasma flow (Potter, 1968), (ii) reduced sodium chloride reabsorption in individual nephrons and (iii) increased water retention (Digby *et al*., 2011). Another powerful adaptive mechanism is the NaK ATPase enzyme, induced in the ilium, liver and kidney after exposure to saline water (Macfarlane, 1982). In its function, it increases the pumping of sodium out of cells and in return the pumping of potassium into the intracellular space.

The renin- angiotensin system (RAS) is responsible to maintain salt and water balance by the secretion of aldosterone, which controls sodium retention and arginine vasopressin (AVP), thereby stimulating water reabsorption by the kidneys (Digby *et al.*, 2011). Ingestion of high amounts of salt leads to increased plasma volume and osmolality, to which the animals body can adapt by exerting negative feedback on aldosterone, thereby reducing sodium reabsorption and promoting sodium excretion (Digby *et al.*, 2011). However, high salt intake from both feed and water does not induce any changes in plasma AVP when the animal consumes sufficient fresh water (Cowley *et al*., 1986).

Table 1.5 Published salt tolerance in drinking water for livestock

Type of livestock	Salt in drinking water		References
	(mg/kg)	(%)	
Goat	15,000 up to 32,000	1.5 up to 3.2	Nassar and Moussa, 1981
	Safe 11,000; preferred 12,500	safe 1.1; preferred 1.25	McGregor, 2004
Pygmy goat	6,300	0.63	Goatcher and Church, 1970b
Normal goat	1,600	0.16	Goatcher and Church, 1970b
Young goat	7,000	0.7	Scarlett, 2002
Dry adult goat	14,000	1.4	Scarlett, 2002
Lactating goat	10,000	1.0	Scarlett, 2002
Sheep	10,000 – 15,000; safe 10,000 – 13,000	1.0 – 1.5; safe 1.0 – 1.3	Peirce,1957and 1959
	Could be tolerated 24,000	Could be tolerated 2.4	
Young sheep	5,000	0.5	Court, 2002
Dry adult sheep	10,000	1.0	Court, 2002
Lactating sheep	5,000	0.5	Court, 2002
Heifer	10,000	1.0	Weeth *et al.,* 1960
Growing cattle	10,000 – 20,000; preferred 12,000	1.0– 2.0; preferred 1.2	Weeth and Haverland, 1961
Red weaner deer	8,000	0.8	Ru *et al.*, 2005
Fallow weaner deer	12,000	1.2	Ru *et al.*, 2005
Poultry	3,000 – 9,000	0.3 – 0.9	Kare and Biely, 1948

Scope of thesis

The aim of this study was to investigate preferences and adaptation capacities of goats to sodium chloride in drinking water as well as the physiological mechanisms underlying the adaptation to increased salinity. This was carried out using two different methods (two choice and free choice tests). Another focus was to determine whether body temperature is influenced by saline water intake.

Three experiments were conducted in female Boer goats. In one experiment, a free choice test was used. This design is similar to natural range conditions, where choices between different water sources may be required to explore whether goats can differentiate fresh water and different saline concentrations. Two experiments were performed using a two choice preference test to evaluate individual preferences and habituation capacity towards saline drinking water. The effects of saline drinking water on rumen temperature and blood parameters as well as feed and water intake were determined. The changes in the parameters can be used to draw conclusions on the adaptation capacity of these animals.

The detailed objectives of this thesis were:

1. To evaluate goats' capacity to differentiate saline water in a free choice system, to determine whether goats are able to adjust their choice in managing salt intake and to avoid excess salt ingestion by their self-selection (Chapter 2).
2. To investigate individual adaptation capacity of goats to salted drinking water, and to test the differences in salt sensitivity after habituation (Chapter 3).
3. To measure changes in blood metabolites and body temperature in goats during the adaptation to saline drinking water (Chapter 4).

Finally, the results are discussed in a broader context and the knowledge on other species is included for comparison (Chapter 5).

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CHAPTER 2

Reactions to saline drinking water in Boer goats in a free choice system

R. A. Runa, L. Brinkmann, A. Riek, J. Hummel and M. Gerken

Department of Animal Sciences, University of Göttingen, Albrecht-Thaer-Weg 3, 37075 Göttingen, Germany

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Reactions to saline drinking water in Boer goats in a free choice system

R. A. Runa, L. Brinkmann, A. Riek, J. Hummel and M. Gerken

Abstract

Salinization of groundwater and soil is a prevalent global issue with serious consequences on animal health and production. The present study was conducted to investigate the capacity of Boer goats to adjust their salt intake from saline drinking water in a free choice system. Twelve non-pregnant Boer goats aged between 1 to 8 years with an average body weight of 46.4 ± 8.3 kg were kept in individual pens for 4 weeks. In the control phase (1 week), only fresh water was supplied in five identical buckets for each pen. During the subsequent treatment phase (3 weeks), fresh water and four different concentrations (0.75, 1.0, 1.25, and 1.5% NaCl) of saline water were offered simultaneously in a free choice system. The positions of the concentrations were changed daily at random. Cut hay and water were provided *ad libitum* and a mineral supplement was allocated. Feed and water intake, mineral supplement intake, ambient temperature and relative humidity were recorded daily, while body weight and body condition score were measured weekly. Dry matter intake, total water intake and total sodium intake were significantly ($P<0.001$) higher during the treatment phase. Body weight and body condition were not affected by saline water intake. Across the treatment phase, saline water consumption was significantly ($P<0.001$) lower in young (19.6 ± 27.1 g/kg $BW^{0.82}$ per day) than in adult goats (27.9 ± 31.5 g/kg $BW^{0.82}$ per day), indicating that young goats were more sensitive towards saline water. All goats had a significant preference for fresh water (0% salt) over saline water. At the first offering of the simultaneous choice situation (week 2), animals did not differentiate between salt concentration of 0.75% and 1.0%. However, with successive treatment (week 3 and 4), animals distinguished between saline water concentrations and preferred the 0.75% salt concentration. Salt concentrations of 1% to 1.5% were avoided. The total sodium intake of the goats ranged between 0.37 – 0.55 g /kg $BW^{0.75}$ per day during the treatment phase, being 8 to 11 fold higher than the daily requirements of sodium for body maintenance. The results suggest that goats are able to differentiate between saline water concentrations and to adjust their sodium intake by quick

adjustments in self-selection in a free choice system. Compared to two choice preference tests, the present free choice situation allows evaluating changes in saline water acceptance with prolonged exposure.

Keywords: salt tolerance, water intake, adaptation, sodium intake, ruminant

Introduction

Salt (NaCl) is an essential element in animal diets (Suttle, 2010). Sodium (Na+) is the main cation in interstitial fluids and plays a dominant role in regulating body fluid homeostasis, nerve function, body temperature maintenance, absorption of chlorine, amino acids, glucose and water (Suttle, 2010; National Research Council (NRC), 2007). In drinking water, the amount of sodium chloride can vary enormously depending on its source, from virtually none in some streams to high concentrations from deep wells or boreholes (Underwood, 1981). However, prolonged ingestion of salt concentrations of 1.5–2% and greater from saline drinking water causes a reduction in food intake and increases mortality in sheep (Peirce, 1957; Wilson, 1966b; Wilson and Dudzinski, 1973). Salt tolerance has been found to be related to many factors such as mineral concentrations in the diet, physiological adaptation, thermal stress and even foetal programming (e.g. review by Digby *et al*., 2011). Studies also revealed a different sensitivity of salt intake according to the source of intake, with higher salt tolerance from food than from drinking water, indicating that different physiological responses are involved (Wilson, 1966b; Mdletshe *et al*., 2017). This observation may be partly explained by the fact that nutrients dissolved in water are absorbed much faster than nutrients from solid food (Sherwood *et al*., 2005). In order to avoid high salt concentration in body fluids one major adaptive response is to consume more water and to increase salt excretion through urine (Wilson and Dudzinski, 1973). However, this adaptive response is compromised when only saline water is available because drinking more water would add to the salt load.

It is postulated that animals possess innate regulation mechanisms that allow them to select a balanced diet according to their nutrient requirements (Fedele *et al.*, 2002). There is evidence based on choice experiments that this occurs in sheep (Kyriazakis and Oldham, 1993), goats (Fedele *et al.,* 2002) or laying hens (Emmans, 1977). So far there are few studies describing taste responses and thresholds to saline drinking water in goats (Bell, 1959), sheep (Goatcher and Church, 1970a and 1970b) and cattle (Bell and Williams, 1959) using a two choice preference test (Patton and Ruch, 1944). However, this situation is not comparable with natural range conditions where choices between different water sources may be required. Zhang *et al.* (2015) described that equids adapt their drinking behaviour in desert areas where water points of differing salinity levels (5–11‰) were available. While Przewalski horses mainly used the low salinity water resources, the khulan consumed water from all water points.

In our study, we designed a free-choice situation close to natural conditions where goats had free access to fresh water along with four different concentrations of salted water. The aim of the study was to evaluate the capacity of goats to choose when different sources of saline or fresh water are offered simultaneously. To our knowledge, comparable free-choice studies on saline water concentrations have not been reported. Properties of the water may stimulate the senses (e.g. smell and taste) and enable the animal to anticipate possible post-ingestive effects of the saline water (Fedele *et al*., 2002). We focused on how quickly the animals were able to adjust their choice to manage their salt intake and whether they were able to avoid excessive salt ingestion by timely adjustments in their selection.

Material and methods

Ethics statement

Procedures performed in our study were in accordance with the German animal ethics regulations and approved by the State Office of Lower Saxony, Germany for Consumer Protection and Food Safety (ref. no.: 33.9-42502-04-16/2310).

Animals and management

The experiment was conducted in two test runs at the Department of Animal Sciences, University of Göttingen, Germany in spring 2017. In each test run, the same experimental procedure was followed with six Boer goats for 4 weeks. In total, twelve non-pregnant female Boer goats aged between 1 and 8 years with an average body weight (BW) of 46.4 ± 8.3 kg were involved. Animals originated from the breeding herd of the Department. Animals were transferred to the experimental pens one week before the start of the trial for acclimatization. Three identical rooms, subdivided into two individual pens (2.85 x 2 m) with a controlled environment were used. Straw was provided as bedding material. Each pen was equipped with one feed trough (diameter 53 cm) and five identical water buckets (diameter 28 cm, 10 l capacity), which were placed side by side along the wall numbered from 1 (closest to the feed trough) to 5 (Figure 2.1).

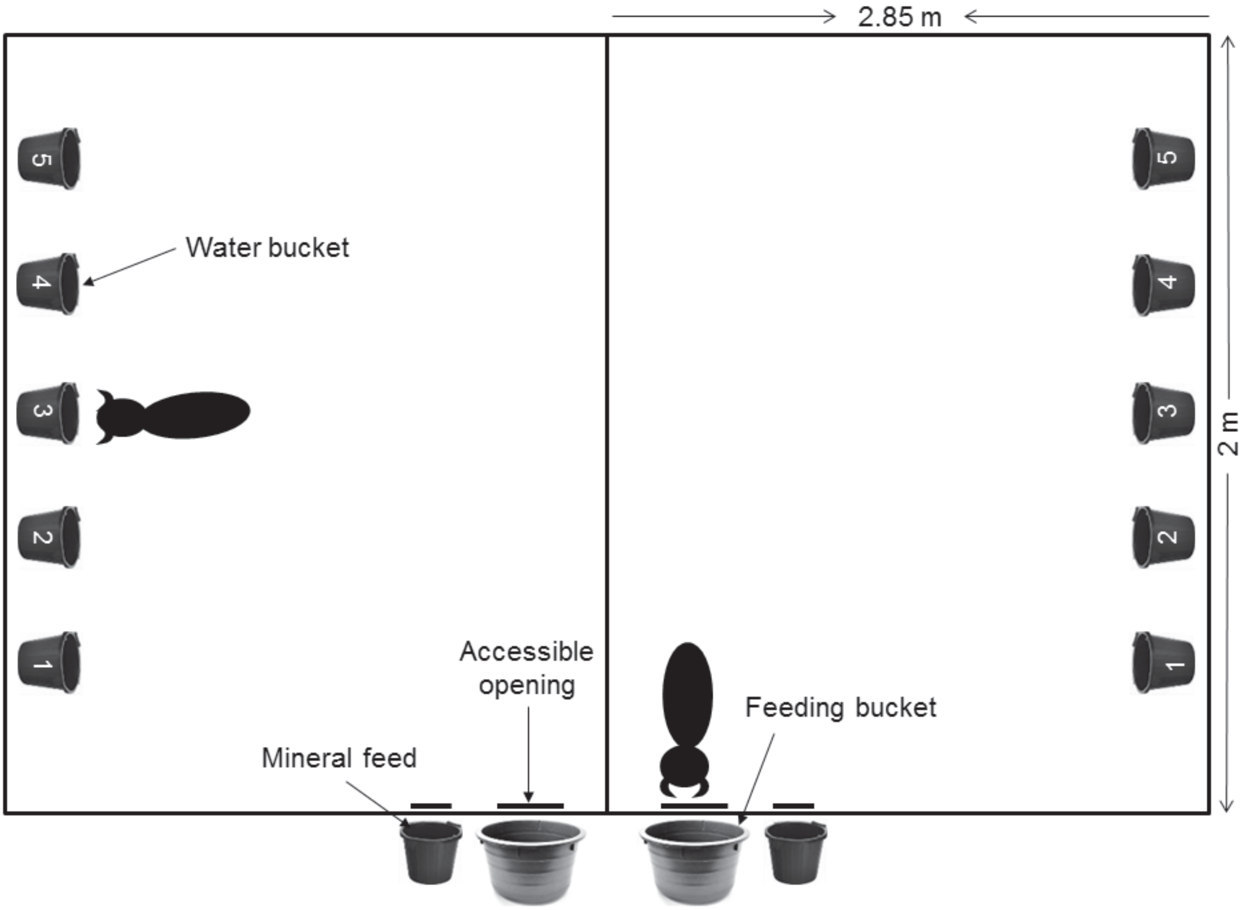

Figure 2.1 Positions of feeding (big black bucket for hay and small bucket for mineral feed at the outside of pens accessible by openings) and drinking water buckets (5 small black buckets at the inside of the pens) in the experimental pens for Boer goats.

Throughout the experimental period, goats had access to cut hay and water *ad libitum*. Additionally, a mineral supplement (Solan 178 Mineralfutter für Schafe and Zeigen®, Solan Kraftfutter Schmalwieser GesmbH, Bachmanning, Austria; ingredients per kg: Calcium 25.00%, Phosphorus 5.00%, Sodium 5.60%, Magnesium 2.30%, Vitamin A 800 000 I.E., Vitamin D3 85 000 I.E., Vitamin E 3 000 mg, Manganese as manganese oxide 1.850 mg, Zinc as zinc oxide 5 900 mg, Iodine as calcium iodate 55 mg, Selenium as sodium selenite 40 mg and Cobalt as cobalt carbonate 30 mg) was offered in a separate bucket at the amount of 25 and 30 g/day in young and old goats, respectively. The ambient temperature (°C) and relative humidity (%) in each room were recorded continuously at 20 min intervals with miniature data loggers (iButton DS1923L-F5#, resolution: 0.0625°C, Maxim Integrated Products, Sunnyvale, CA, USA) placed at a height of 1.0 meter from the floor. The daily average ambient temperature (T_a) and relative humidity (RH) were 19.3 ± 0.9°C and 41.0 ± 7.9% in test run 1 and 20.0 ± 1.1°C and 40.6 ± 6.5% in run 2, respectively. The stables were equipped with windows to provide natural light. The light schedule was maintained constant at 14 hours light: 10 hours dark, with artificial lighting from 0630 h until 2030 h.

The study was conducted in two phases. In the control phase, only tap water was supplied for one week and water intake from each bucket was measured. Each animal received the same amount of water in its five buckets in order to habituate to the following choice situation. In the subsequent treatment phase of three weeks, fresh water and four different concentrations (0.75, 1.0, 1.25, and 1.5% NaCl) of saline water were offered simultaneously. Goats could choose freely among all buckets in their pen. The average sodium content of tap water was 7.55 mg/l. For the preparation of saline water, salt (Esco Siede-Speisesalz, Hannover, Germany) with 99.8% NaCl purity was used and added at defined amounts to tap water. The water buckets were replenished with test solutions daily and the positions of the concentrations were changed daily at random in order to avoid a bias due to position effects.

Body weight, body condition score, water and feed intakes

Individual body weight was recorded on a weekly basis in the morning before feeding with a mobile scale (Salter Brecknell LS300, capacities: 300 kg, resolution: 0.2 kg, Salter Brecknell, Smethwick, West Midlands, UK). The body condition score (BCS, scale: 1=emaciated, 5=obese with 0.5 increments) was assessed weekly as described by Villaquiran *et al*. (2007). Individual water intake (WI) from each bucket was recorded daily at 0830 h using an electronic scale (Sartorius model CP 34 000, Sartorius AG, Göttingen, Germany) as well as the corresponding NaCl concentration and the position of the bucket. The daily amount of test solution consumed was expressed as a percentage of the total fluid intake from all water buckets. A control bucket was placed in the stable and re-weighed daily to estimate the water loss by evaporation and the total daily drinking water intake (TDWI) was corrected accordingly. The total water intake (TWI) was determined as the sum of TDWI and the water content in consumed food. The cut hay was offered at

0800 h and the feed buckets were refilled twice per day. Feed consumption was determined daily by reweighing. Feed intake was also expressed as intake per kg $BW^{0.75}$ and water intake as intake per kg $BW^{0.82}$ since water use by animals is related to the live weight of animals to the power of 0.82, as water is used in the body for intermediary metabolism and also for evaporative cooling (Wilson, 1989).

Hay samples were collected weekly. Ground samples (1-mm screen) were analyzed using standard methods (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 2012; all values summarized in table 2.1) for dry matter (VDLUFA method 3.1), ash (method 8.1), crude protein (method 4.1.2 – Dumas; Elementar VarioMAX CN, Langenselbold, Germany), ether extracts (method 5.1.1), neutral detergent fiber and acid detergent fiber (expressed without residual ash – NDFom and ADFom, methods 6.5.1 and 6.5.2; Ankom Fiber Analyzer 220, Macedon, USA). The metabolizable energy (ME) was estimated using prediction equations for ruminants (Gesellschaft für Ernährungsphysiologie/ Society of Nutrition Physiology, 2009), including values from Hohenheim gas test (Menke *et al*., 1979; VDLUFA method 25.1). For the feed and water, Na and Cl were determined from aqueous extract using ion chromatography with conductivity detection (LUFA 10.5.2) (Dionex DX-100; Sunnyvale, USA). For Ca, Mg, P and K analyses, samples were ashed and dissolved in HCl. Ca, Mg and K were measured via atomic absorption spectroscopy (Varian SpectrAA-300, Palo Alto, USA) and P was measured photometrically. Individual mineral feed intake was determined by re-weighing the residual of the mineral supplement at the end of the week with a scale (Sartorius Practum 5101-1S, Sartorius Lab Instruments GmbH, Göttingen, Germany). Total sodium (Na^+) intake was determined as the sum of the amount of sodium consumed from hay, mineral supplement, fresh and saline drinking water.

Table 2.1 Chemical composition of hay supplied (means ± SD) to Boer goats during the experiment

Composition	Means ± SD
Dry matter (%)	89.8 ± 0.2
Metabolizable energy (MJ/kg DM)	8.9 ± 0.2
Hohenheimer feed value test (HFT) (ml/200mg DM)	45.9 ± 0.6
Nutrients in dry matter (g/kg DM)	
Neutral Detergent Fiber (NDF)	610.9 ± 10.9
Acid Detergent Fiber (ADF)	349.9 ± 11.9
Crude fat	14.1 ± 1.2
Raw ash	55.7 ± 2.9
Crude protein	75.8 ± 4.0
Mineral contents (mg/100g DM)	
Sodium (Na^{+})	5.8 ± 2.4
Chloride (Cl^{-})	197.6 ± 0.4
Magnesium (Mg^{2+})	114.0 ± 13.4
Calcium (Ca^{2+})	329.5 ± 32.5
Phosphorus (P)	109.3 ± 5.5
Potassium (K^{+})	1 427.7 ± 21.2

Statistical Analysis

The results from earlier studies showed a higher salt sensitivity in young compared to adult sheep (Wilson and Dudzinski, 1973). In order to take into account a possible age effect, goats were classified as young (N=8) or adult (N=4) when younger or older than two years, respectively. Statistical analyses of all recorded data were performed using the MIXED procedure of the computer software package Statistical Analysis System (SAS version 9.3). Weekly averages were calculated for dry matter intake, TDWI, TWI and saline water intake. In addition, the amount of each test solution consumed (experimental week 2 to 4) was expressed as a percentage of the total fluid intake from all containers. For all traits, with the exception of WI during the experimental phase when different salt concentrations were offered, the model included the fixed effects of test-run, week, age, their interactions and the random effect of the animals.

Model (1) was:

$$Y_{ijklm=} \mu + R_i + W_j + A_k + (R*W)_{ij} + (W*A)_{ik} + (R*A)_{ik} + G_l + e_{ijklm}$$

Where: Y_{ijklm}: observation value; μ: overall mean; R_i: run (i = 1, 2); W_j: week (j = week 1 to 4); A_k: age (k = young, old); interactions (R*W), (W*A), (R*A); G_l: the random effect of the goats; e_{ijklm}: the random error.

The following model (2) was used for sodium intake throughout the experiment:

$$Y_{ijklm=} \mu + R_i + P_j + A_k + (R*P)_{ij} + (P*A)_{ik} + G_l + e_{ijklm}$$

Where: Y_{ijklm}: observation value; μ: overall mean; R_i: run (i = 1, 2); P_j: phase (j = control, treatment); A_k: age (k = young, old); interactions (R*P), (P*A); G_l: the random effect of the goats; e_{ijklm}: the random error.

For WI during the experimental phase, the following model (3) was used:

$$Y_{ijklmno=} \mu + R_i + C_j + A_k + W_l + B_m + (W*A)_{kl} + (C*W)_{jl} + (C*A)_{jk} + (C*B)_{jm} + (R*C)_{ij} + (R*A)_{ik} + (R*B)_{im} + (C*W*A)_{jlk} + G_n + e_{ijklmno}$$

Where: $Y_{ijklmno}$: observation value; μ: overall mean; R_i: run (i = 1, 2); C_j: concentration (1 to 5); A_k: age (k = young, old); W_l: week (l = week 2 to 4); B_m: bucket position (m = 1 to 5); interactions (W*A), (C*W), (C*A), (C*B), (R*C), (R*A), (R*B), (C*W*A); G_n: the random effect of the goats; $e_{ijklmno}$: the random error. All values were presented as least squares means ± SEM. The significance level was set at *P<0.05*.

Results

Body weight, body condition score, feed and water intakes

Body weight remained constant during the entire experiment (*P=0.99*), while there was a tendency for a decrease in body condition score (BCS) (*P=0.13*) (Table 2.2). Older goats showed significantly (*P<0.001*) higher BW and lower BCS (52.8 ± 10.9 kg and 2.5 ± 0.2 points) than young goats (42.7 ± 2.9 kg and 3.7 ± 0.3 points). Dry matter intake (DMI, g/kg $BW^{0.75}$ per day) increased significantly during the experiment (*P<0.001*). Food consumption was higher (*P=0.012*) in old goats compared to young animals (52.3 ± 12.8 g/kg $BW^{0.75}$ per day *vs.* 49.2 ± 9.1 g/kg $BW^{0.75}$ per day). Similarly, older goats had a significantly (*P<0.001*) higher TWI (129.3 ± 41.0 g/kg $BW^{0.82}$ per day) compared to young goats (93.3 ± 23.6 g/kg $BW^{0.82}$ per day). In the control phase (week 1) when only fresh water was offered, total drinking water intake (TDWI, g/kg $BW^{0.82}$ per day) and total water intake (TWI, g/kg $BW^{0.82}$ per day) were significantly (*P<0.001*) lower than in the successive weeks. Both TWI and TDWI increased in week 2 when goats first received different concentrations of saline water along with fresh water (Table 2.2).

The responses to saline drinking water in a simultaneous choice situation are given in figure 2.2. Across the treatment phase, salt water consumption was significantly (*P<0.001*) higher in adult (27.9 ± 31.5 g/kg $BW^{0.82}$ per day) than in young goats (19.6 ± 27.1 g/kg $BW^{0.82}$ per day). Young and old goats consumed similar amounts of fresh water (58.7 ± 27.1 g/kg $BW^{0.82}$ per day and 54.8 ± 33.6 g/kg $BW^{0.82}$ per day, respectively). However, young goats avoided the intake of saline water more strictly than older animals. Across both age groups, saline water intake was continuously reduced for increasing salt concentrations. Figure 2.3 depicts the development of daily saline water intake, expressed as a percentage of total drinking water intakes. Throughout the treatment phase, all goats had a significant preference for fresh water (0% salt) over saline water. At the first confrontation with the simultaneous choice situation (week 2), animals did not differentiate between salt concentration of 0.75% and 1.0%. However, with proceeding treatment (week 3 and 4), animals clearly distinguished saline water concentrations and preferred the 0.75% salt concentration.

Table 2.2 Average body weight (BW), body condition score (BCS), daily feed, water and sodium intake for control (week 1) and treatment (week 2–4) phases in Boer goats (N=12) corrected by body mass

Traits	Experimental phase				Significance of effects			
	Control phase	Treatment phase			Week		Age	
	Week 1	Week 2	Week 3	Week 4	SEM	*P*-value	SEM	*P*-value
BW (kg)	48.31	47.36	47.39	47.84	2.23	0.989	1.56	<0.001
BCS (points)	3.28	3.09	3.09	3.03	0.08	0.129	0.05	<0.001
DMI (kg/day)	0.85^{a}	0.87^{ab}	0.94^{b}	1.04^{c}	0.03	<0.001	0.02	<0.001
DMI (g/kg $BW^{0.75}$/day)	46.05^{a}	47.79^{a}	51.83^{b}	57.30^{c}	1.16	<0.001	0.83	0.012
TDWI (kg/day)	2.02^{a}	2.89^{b}	2.49^{c}	2.81^{b}	0.07	<0.001	0.05	<0.001
TDWI (g/kg $BW^{0.82}$/day)	84.74^{a}	122.86^{b}	104.58^{c}	118.26^{b}	3.39	<0.001	2.36	<0.001
TWI (kg/day)	2.10^{a}	2.98^{b}	2.58^{c}	2.90^{b}	0.08	<0.001	0.05	<0.001
TWI (g/kg $BW^{0.82}$/day)	88.12^{a}	126.48^{b}	108.37^{c}	122.27^{b}	3.40	<0.001	2.37	<0.001
TWI/DMI	2.55^{a}	3.72^{b}	2.93^{c}	2.80^{ac}	0.12	<0.001	0.08	<0.001
NaIF (g/day)	0.049^{a}	0.051^{ab}	0.055^{b}	0.060^{c}	0.002	<0.001	0.001	<0.001
NaIF (g/kg $BW^{0.75}$/day)	0.0027^{a}	0.0028^{a}	0.0030^{b}	0.0033^{c}	0.0001	<0.001	0.0001	0.111
NaIW (g/day)	0.02^{a}	9.66^{b}	6.56^{c}	7.96^{d}	0.35	<0.001	0.25	<0.001
NaIW (g/kg $BW^{0.75}$/day)	0.001^{a}	0.54^{b}	0.36^{c}	0.43^{d}	0.02	<0.001	0.02	<0.001
NaIMF (g/day)	0.74^{a}	0.22^{b}	0.17^{b}	0.12^{b}	0.04	<0.001	0.04	<0.001

NaIMF (g/kg $BW^{0.75}$/day)	0.04^{a}	0.01^{b}	0.01^{b}	0.01^{b}	0.003	<0.001	0.002	<0.001
TNaI (g/day)	0.80^{a}	9.93^{b}	6.77^{c}	8.14^{d}	0.35	<0.001	0.25	<0.001
TNaI (g/kg $BW^{0.75}$/day)	0.05^{a}	0.55^{b}	0.37^{c}	0.44^{d}	0.02	<0.001	0.02	<0.001

DMI = dry matter intake; TDWI = total drinking water intake; TWI = total water intake; TNaI = total sodium intake; NaIF, NaIW and NaIMF = sodium intake from feed, water and mineral feed respectively.

^{a,b,c,d} Means within the same row with different superscripts differ significantly by *P<0.05*.

Table 2.3 Influence of age of Boer goats on daily sodium (Na^+) intake in control (only fresh water) and treatment (different concentrations of saline water) phases

	Control phase			Treatment phase			Effects for phase	
Traits	Young goats	Old goats	All goats	Young goats	Old goats	All goats	SEM	*P*-value
NaIF (g/day)	0.04^{aA}	0.06^{b}	0.05	0.05^{aB}	0.06^{b}	0.06	0.002	0.007
NaIF (g/kg $BW^{0.75}$/day)	0.002^{A}	0.003	0.003	0.003^{B}	0.003	0.003	0.0001	0.001
NaIW (g/day)	0.01^{A}	0.02^{A}	0.02	5.82^{aB}	10.30^{bB}	8.06	0.311	<0.001
NaIW (g/kg $BW^{0.75}$/day)	0.001^{A}	0.001^{A}	0.001	0.34^{aB}	0.54^{bB}	0.44	0.019	<0.001
NaIMF (g/day)	0.88^{aA}	0.60^{bA}	0.74	0.24^{aB}	0.09^{bB}	0.17	0.036	<0.001
NaIMF (g/kg $BW^{0.75}$/day)	0.05^{aA}	0.03^{bA}	0.04	0.015^{aB}	0.005^{bB}	0.01	0.002	<0.001
TNaI (g/day)	0.93^{A}	0.68^{A}	0.80	6.11^{aB}	10.45^{bB}	8.28	0.316	<0.001
TNaI (g/kg $BW^{0.75}$/day)	0.06^{A}	0.03^{A}	0.05	0.36^{aB}	0.55^{bB}	0.46	0.019	<0.001

NaIF, NaIW and NaIMF = sodium intake from feed, water and mineral feed respectively; TNaI = total sodium intake.

^{ab} significant differences between ages within one row P<0.05

^{AB} significant differences between treatment within one row P<0.05

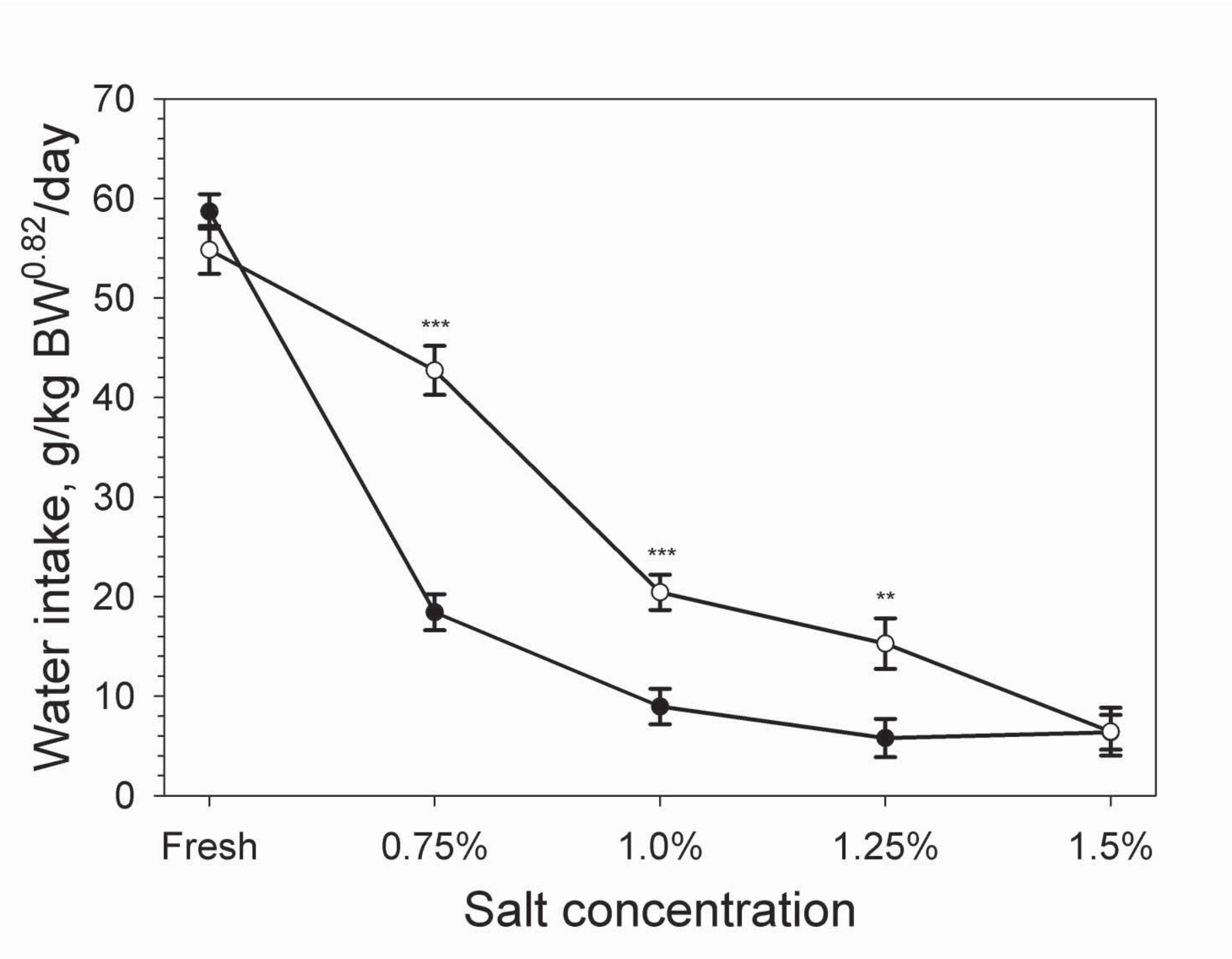

Figure 2.2 Daily fresh and saline water intake in young (N= 8, 1–2 years, filled circles) and old (N=4, 2–8 years, open circles) Boer goats in a free choice system across treatment phases (3 weeks; see text for details). Goats had the choice between fresh and four different concentrations of saline water. ** *P<0.01;* *** *P<0.001* for comparison between ages for respective salt concentration.

Sodium (Na^+) intake

Total sodium (Na^+) intake from feed, water and mineral feed differed significantly (*P<0.001*) across the whole experiment. Due to the exclusive provision of fresh water in week one (control phase), Na^+ intake in this week was significantly lower than in weeks 2–4 when different concentrations of saline water were provided (Table 2.2, 2.3). Across the entire experiment, young goats had a significantly (*P<0.001*) lower total sodium intake (4.8 ± 4.1 g/day) compared to older goats (8.0 ± 6.7 g/day).

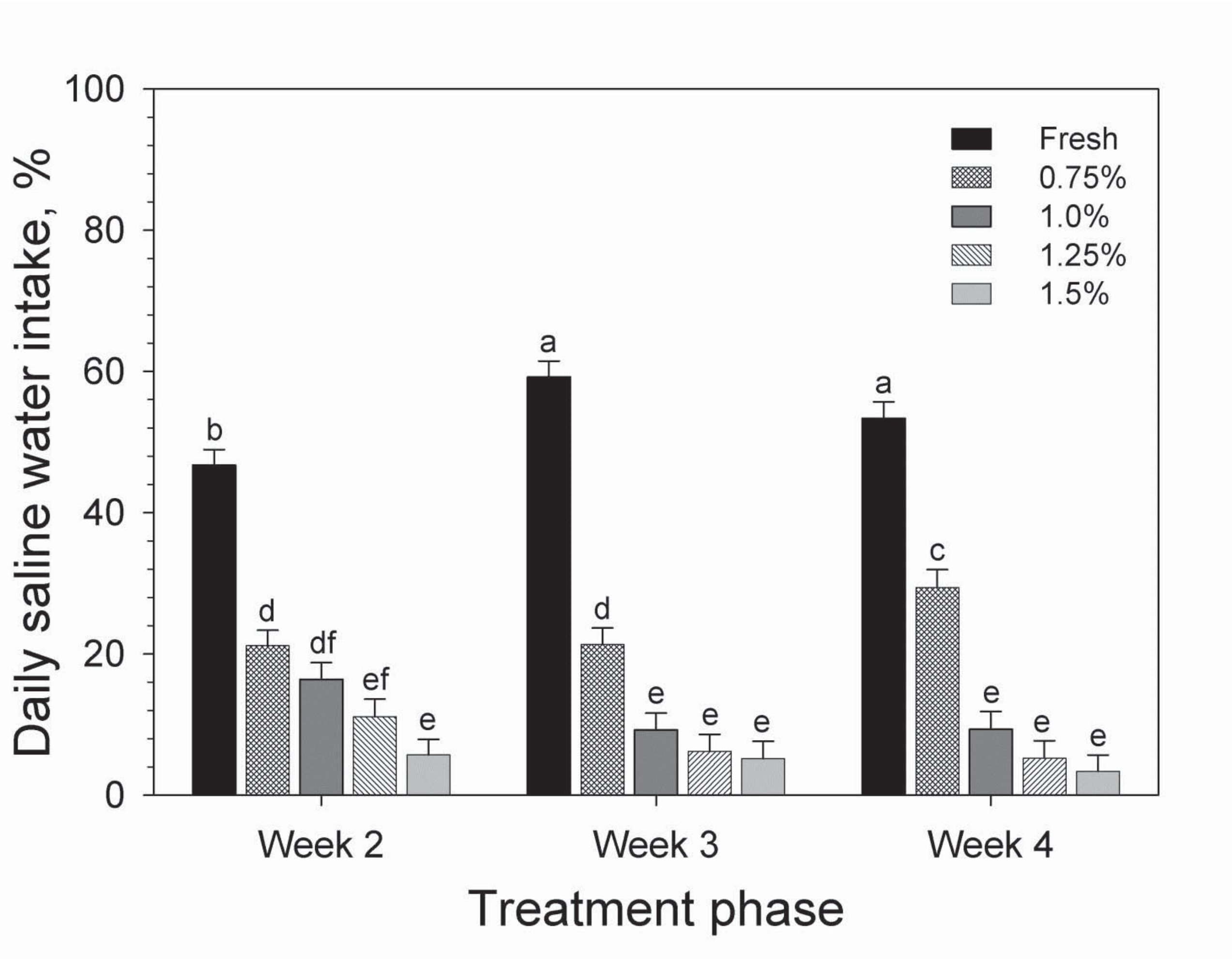

Figure 2.3 Daily fresh and saline water intake (% of total drinking water intake) of Boer goats (N=12) in a free choice system during the treatment phase (week 2–4). Animals could choose between five buckets; one with fresh water and four with increasing concentrations of NaCl in drinking water (see legend). [a,b,c,d e,f] significant differences between intake of salt concentrations across weeks, *P<0.05.*

Discussion

In our study, Boer goats quickly adjusted their salt ingestion in a free-choice design when fresh water and different concentrations of salinated water were offered simultaneously. Our results suggest that Boer goats can balance salt intake from saline water without health impairment when they have free access to fresh water.

Sodium (Na^+) intake

In the control phase when only fresh water was offered, the total sodium intake of the goats ranged between 0.68 ± 0.34 and 0.93 ± 0.40 g/day and compares well with the maintenance recommendation of 0.75 – 0.95 g/day (40 – 50 kg BW goats) given by NRC (2007). During the treatment phase, however, the sodium intake of the goats sharply increased to values between 6.77 ± 4.53 and 9.93 ± 5.90 g/day being 8 to 11 fold higher than the daily requirement. It has been shown that herbivores possess a specific appetite for sodium (Denton and Sabine, 1961) and if sodium is provided *ad libitum*, animals tend to consume more than they need. The highest Na^+ ingestion was observed in the first week of the free choice situation and decreased in the subsequent weeks. These observations suggest a learning effect in the regulation of Na^+ intake, similar to results from a free choice system of diets in goats (Fedele *et al.*, 2002). Interestingly, goats decreased their Na^+ intake significantly via the mineral feed in the free choice phase. Similarly, higher sodium intake from water decreased the intake of sodium from mineral supplement in dairy cows (Smith *et al.,* 1953) and sheep (Langbein *et al*., 1998). Our results indicate the animals' capacity to balance their sodium intake from different sources and confirm the observation that salt intake from feed and fluids involve different physiological responses in the animal (Wilson 1966b; Masters *et al*., 2005; Digby *et al*., 2011). This difference in ingesting salt in feed or drinking water may be partly explained by the fact that nutrients dissolved in water are absorbed much faster than nutrients from solid food (Sherwood *et al*., 2005). It seems that the capacity to self-select drinking water with different sodium contents was not perfect because total salt ingestion was higher in the treatment phase than in the control phase. However, present duration of three weeks might have been too short for the adaptive behavioural and physiological responses to be expressed fully.

Water intake

Total water consumption increased with saline water ingestion, which is in agreement with previous findings for saline water intake in sheep (Peirce, 1957; Wilson and Dudzinski, 1973), rusa deer stags (Kii and Dryden, 2005) as well as fallow and red deer (Ru *et al*., 2004). Similarly, excess sodium in the diet increased water consumption in all aforementioned species and also in poultry (Mongin, 1981) resulting in higher volume and moisture contents of excreta. In the present free choice design, Boer goats also had the opportunity to consume fresh water, which might have balanced the intake of sodium from saline drinking water and avoided an increase in water content of body fluids. Several studies showed that excess sodium intake is principally regulated in the kidneys by control of reabsorption of sodium, changes in the active transport and the membrane permeability (Suttle, 2010) and by increasing renal sodium excretion through urine and skin (Wilson, 1966b; Suttle 2010). A key physiological response to high salt ingestion is to increase water intake. However, this adaptive reaction is compromised when only saline drinking water is available, as it increases salt load in the body fluids. In such cases, the animals have limited other adaptive responses to draw upon, beyond the excretion of increased amounts of salt via urine and a reduction of feed and water intake. We did not observe any clinical signs such as diarrhoea, general weakness or any nervous symptoms that may accompany excessive salt intake in drinking water (Peirce, 1957; Kii and Dryden, 2005). Accordingly, Boer goats appear to be able to balance their excess sodium intake by preferring fresh water or saline water with the lowest salt concentration and remained within a physiologically acceptable range.

Salt sensitivity

Fresh water intake was significantly higher compared to saline water intake underlining the ability of goats to distinguish between fresh and different concentrations of saline water. The choice of goats for saline water was variable with preference for lower concentrations, whereas salt concentrations of 1% to 1.5% were clearly rejected. Pygmy goats and cattle showed strong preferences for 1.25% saline water, while sheep and normal goats remained indifferent when sufficient sodium was provided in their diet (Goatcher and Church 1970b). In our experiment, the lowest saline water intake (less than 20% of total water intake) was recorded with 1.25% and 1.5% of salt concentration, indicating a rejection threshold (Goatcher and Church, 1970a). Similar preferences for low salt concentrations of 0.85% and 1.25% were found for goats and sheep by Goatcher and Church (1970b), while their estimated rejection threshold of 3.12 – 3.50% was higher than in our study. However, the approach used by Goatcher and Church (1970a and 1970b) is not fully comparable with our study as their two choice preference tests evaluate the short term taste discrimination whereas the present free choice system allows insight into long term preferences for saline drinking water.

Influence of age on sodium (Na^+) intake

In our free choice system, sensitivity responses of goats to saline drinking water varied with their ages. Young goats showed a higher sensitivity to saline water with a lower rejection threshold at 0.75% salt concentration (Figure 2.2). A similar lower salt tolerance was found in growing sheep ingesting salt from both food and drinking water (Wilson and Dudzinski, 1973). Our results might be explained by a better learning capacity of young goats to find the fresh water in a choice situation. However, physiological explanations appear more plausible. Salt taste receptors are more sensitive in younger animals (Grzegorczyk *et al*., 1979) and children (Fukunaga *et al*., 2005) and allow a more differentiated choice between saline water concentrations. On the other hand, younger animals contain a higher percentage of body water (Riek and Gerken, 2010), which may enhance the transport of Na^+ to cell tissues and activate respective regulation mechanisms at lower salt ingestion than in older animals.

Dry matter intake, body weight and body condition score

Dry matter intake increased gradually when goats had access to both saline and fresh water. This increase may be caused by increasing palatability of feed (Grovum and Chapman, 1988) and enhanced digestion due to stimulated microbial activities in the rumen when drinking water supplemented with small amounts of salt is ingested. Similarly, Kattnig *et al*. (1992) found that food intake tended to be higher in Holstein steers when 0.23% saline water was supplied. Studies in sheep and rusa deer (Wilson and Dudzinski, 1973; Kii and Dryden, 2005) also support these results. Feed intake was not affected by 1.5% salt water concentrations in sheep (Peirce, 1957) and in deer (Ru *et al*., 2004). However, water containing 2% or more salt leads to a severe reduction in food intake (Peirce, 1957; Wilson, 1966b). Similarly, high salt concentrations (20%) in diets resulted in reduced feed intake in sheep (Wilson, 1966b; Masters *et al*., 2005). On the other hand, the intake of halophytic plants such as *Atriplex* (saltbush) and *Kochia* (bluebush) (Wilson, 1966a) was improved with availability of fresh water. Saline drinking water had little effect on BW and BCS of our experimental goats. Similar results were found by Peirce (1957) in sheep receiving salt concentration up to 1% in drinking water. The tendency for lower body condition score found in the treatment phase could indicate that water retention may have contributed to constant body weight (Masters *et al*., 2005).

Free choice system

Earlier studies applied a two-choice preference test for saline water (Goatcher and Church, 1970a) with increasing or decreasing concentrations (Patton and Ruch, 1944). To our knowledge, the present study is the first report of a free choice system for salt concentrations in drinking water in ruminants. We observed a marked shift in preferences of saline water during the course of the experiment with the rejection threshold becoming lower with prolonged exposure. Apparently, there are different behavioral and physiological mechanisms between salt ingestion in feed and drinking water influencing the animals' choices. In the first week, goats had to learn that the buckets contained different saline water concentrations. In experiments of Langbein *et al*. (2007) dwarf goats showed a remarkable learning capacity to solve complex learning tasks. Occasional video observations in our study revealed that goats tested the solutions from the different buckets before they made a final decision.

It is obvious that Na+ intake needs to be regulated by the animal because it is the major extracellular element influencing osmolarity of body fluids. Its important role may also explain the fact that it can be quantified sensorically by the animal. Despite this sensory capacity, the additional offer of sodium in water led to a considerably higher (app. 10x) sodium intake in this trial, and it poses the question why goats did not down regulate their sodium intake via reducing the intake of water containing sodium. An explanation may be that while goats were accustomed to regulate Na^+ intake from solid feeds, they were far less trained to regulate Na^+ intake from water. Against this background, some adaptation time seems comprehensible even for a nutrient that can be detected by the animal directly. In addition, any regulation via sensory detection must not be considered perfect, but has rather been developed to prevent excessive ingestion of amounts of osmotically highly effective amounts of sodium. The fine adjusting of daily sodium ingestion will still involve learning and physiological feedback from ingested Na^+. Despite its significance for osmolarity of body fluids, ruminants are considered very tolerant to high sodium intakes as long as free access to fresh water is available. In fact, animals in this trial were always well beyond the limits communicated as having the potential to influence the performance of an animal negatively, e.g. 9% sodium in DM (Jeroch *et al*., 2007) or 4% sodium in DM (NRC, 2007).

Conclusions

The results of our study suggest that goats are able to balance their sodium intake through water in a free choice system. Compared to two choice preference tests, the present free choice protocol allows the evaluation of how quickly animals are able to adjust their salt ingestion by self-selection as well as the change in their acceptance of saline water over time. After only one week of exposure, goats had developed a stable preference. However, it remains open to question, whether the salt intake was regulated by post ingestive mechanisms only as suggested by Fedele *et al*. (2002) in their choice system of diets. The higher rejection of saline water ingestion in young goats suggests at least some involvement of intake regulation via taste sensitivity.

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CHAPTER 3

Adaptation capacity of Boer goats to saline drinking water

R. A. Runa, L. Brinkmann, M. Gerken and A. Riek

Department of Animal Sciences, University of Göttingen, Albrecht-Thaer-Weg 3, 37075 Göttingen, Germany

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Adaptation capacity of Boer goats to saline drinking water

R. A. Runa, L. Brinkmann, M. Gerken and A. Riek

Abstract

Due to global climatic changes, water and soil salinization is an increasing worldwide phenomenon, thus creating new threats for farm animal production. The present study was designed to investigate the adaptation capacity of goats towards sodium chloride (NaCl) in drinking water. Twelve non-pregnant Boer goats with an average body weight of 50.5 ± 9.0 kg were kept in individual pens. The study was conducted in four phases applying a two choice preference test. In the control phase (phase 1) only fresh water was supplied in two containers. In phase 2, water with different salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5%) was offered in one container and tap water in the other (sensitivity test). During the third phase (adaptation), goats were stepwise habituated to saline water by offering only saline water with different increasing concentrations (between 0% and 1.5% NaCl) in both containers. Subsequently, in phase 4 (sensitivity re-test) the same treatment as in phase 2 was repeated. Goats had *ad libitum* access to hay, water and a mineral licking block. Individual water and feed intake were recorded daily, while body weight and body condition score were measured biweekly. Body weight was not affected by saline water intake, whereas dry matter intake and body condition scores decreased significantly during the experiment. Water intake was significantly ($P<0.001$) higher in phases 2 (sensitivity test) and 3 (adaptation), compared to phase 1 (control) and 4 (sensitivity re-test). Total sodium intake followed the same pattern. In phase 2 when goats had the choice between fresh and saline water for the first time they preferred higher salt concentrations and consumed significantly ($P<0.001$) higher amounts of saline water (75.4 ± 53.2 g/kg $BW^{0.82}$ per day) than in the re-test (40.4 ± 34.0 g/kg $BW^{0.82}$ per day) after the habituation period. Thus, salt discrimination rejection thresholds were lowered to 1.25% in phase 4 compared to 1.5% in phase 2. During the adaptation phase, initially, goats preferred saline water of 0.25% over fresh water and did not differentiate between concentrations of 0.25% and 0.5% when offered simultaneously. The results suggest that a stepwise adaptation to saline drinking water in goats is an effective method

to habituate the animals to saline water intake when concentrations are below 1.5%. Goats reacted more sensitively to the salinity of drinking water after prolonged exposure to saline water indicating flexible regulation mechanisms depending on the total sodium balance of the animal.

Key words: adaptability, climate change, drinking water, ruminants, salt tolerance

Introduction

Domestic farm animals have demonstrated remarkable adaptation capacities to a broad range of environments. Apart from climatic challenges due to global changes, salinization of groundwater and soil is an increasing threat for livestock, in particular in coastal areas (Hallegatte *et al*., 2013). Compared to other mammals, ruminants are able to tolerate more salt in drinking water (Goatcher and Church, 1970b), depending on the duration of salt ingestion (Peirce, 1957), age (Wilson and Dudzinski, 1973), physiological status and environmental factors. Several studies show that among ruminants, goats are well adapted to dry environments and water scarcity (Silanikove, 2000), and can even survive on seawater (Dunson, 1974).

Ruminants are particularly sensitive to sudden and drastic changes in feed composition due to their rumen physiology (Grubb and Dehority, 1975; Mackie *et al*., 1978) and a stepwise adaptation is recommended for changes in diets (Mackie *et al*., 1978). Similarly, a stepwise habituation to saline water might help exposed animals to cope with higher concentrations of saline water without health impairment. So far, some studies have been conducted on the sensory perception and perception thresholds of salt in drinking water in goats (Bell, 1959; Runa *et al*. 2018) and sheep (Goatcher and Church, 1970a and 1970b). However, there is a lack of experimental studies on how to adapt ruminants to saline water ingestion. There are only practical recommendations available (Department of Agriculture and Food, 2014) to mix fresh and saline water for a few days as an adaptation period. Therefore, the aim of our study was to investigate the individual adaptation capacity of Boer goats towards sodium chloride (NaCl) in drinking water. The focus was on the possibility to adapt the animals to increased saline water concentrations via a stepwise habituation. We hypothesized that Boer goats would tolerate saline water after an adaptation period.

Materials and methods

Procedures performed in this study were in accordance with the German animal ethics regulations and approved by the State Office of Lower Saxony, Germany for Consumer Protection and Food Safety, Germany (Ref. no.: 33.9-42502-04-15/1946).

Animals, management and experimental treatments

The study was carried out at the Department of Animal Sciences, University of Göttingen, Germany between November 2015 and January 2016. Twelve non-pregnant Boer goats aged between 1.5 and 7.5 years with an average body weight of 50.5 ± 9.0 kg were involved. Animals originated from the breeding herd of the Department. Animals were transferred to the experimental pens two weeks before the start of the trial for acclimatization. Three identical rooms, subdivided into four individual straw pens each (each pen 3.0 m^2) were used. Each pen was equipped with an individual feed trough (diameter 53 cm, approximate capacity: 3 kg hay) and two water buckets (diameter 28 cm, 10 liter capacity per bucket), which were placed at each side of the feed trough to allow free choice between contents. Animals had access to cut hay, salt lick and water *ad libitum* throughout the experimental period. The rooms were equipped with windows to provide natural light. The lighting schedule was kept constant at 14 h light: 10 h dark, with additional artificial lighting from 0630 h until 2030 h.

Throughout the study, a two choice preference test (Goatcher and Church, 1970a) was used. The study was conducted in four phases (Figure 3.1). In the control phase (phase 1), only fresh tap water was supplied in two identical buckets for one week. In phase 2 (sensitivity test, 2 weeks), water with ascending salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25, and 1.5% NaCl) was offered in one container and unsalted tap water in the other. Each salt concentration was tested for two days. This procedure allowed to determine the individual salt sensitivity. During the third phase (adaptation, 4 weeks), goats were stepwise habituated to saline water by offering only saline water in both buckets. The saline water of the lower concentration was offered in one container and the salt water with higher concentration in the other (Figure 3.1). The concentrations were gradually increased between 0% and 1.5% NaCl. The highest concentration was close to the rejection threshold described by Bell (1959) and Goatcher and Church (1970a). Subsequently, in phase 4 (sensitivity re-test, 2 weeks) the same treatment as in phase 2 was repeated to test for differences in discrimination thresholds after the habituation period. The water buckets were replenished with test solutions daily and the positions of the concentrations were changed daily at random in order to avoid a bias due to position effects. The average Na^+ content of tap water was 7.55 mg/l. Saline water was prepared by adding a defined amount of salt (Esco Siede-Speisesalz, Hannover, Germany) with 99.8% NaCl purity to tap water. The accurate salt concentration provided was measured by using a refractometer (HI96821 refractometer, Hanna Instruments Inc, Woonsocket, RI 02895 USA).

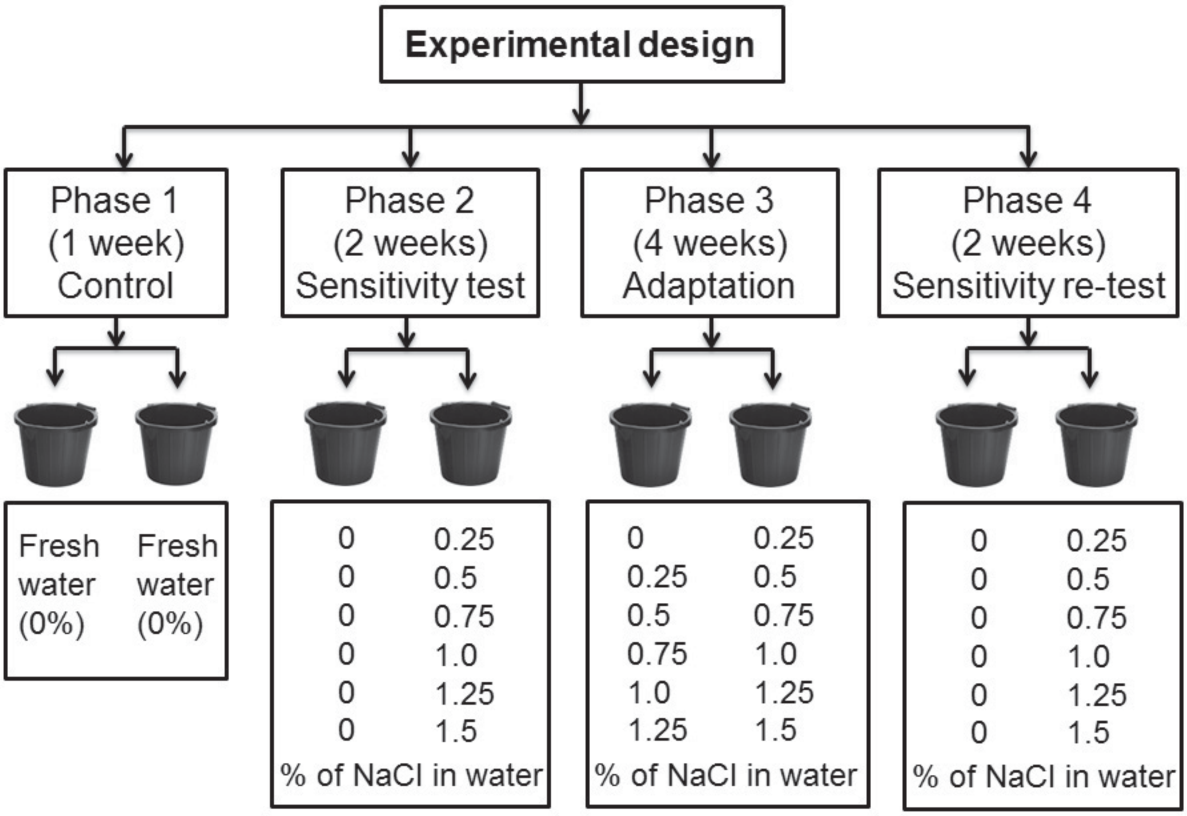

Figure 3.1 Experimental design based on a two choice preference test: provision of different concentrations of salt in drinking water during the different phases of the experiment. Two water containers were placed at each side of the feed trough for *ad libitum* intake. The positions of the concentrations were changed daily at random in order to avoid a bias due to position effects.

Body weight, body condition score, water and feed intakes

Individual body weight was recorded in bi-weekly intervals with a mobile scale (Salter Brecknell LS300, capacities: 300 kg, resolution: 0.2 kg, Salter Brecknell, Smethwick, West Midlands, UK). The body condition score (BCS scale: 1=emaciated, 5=obese with 0.5 increments), a palpable and visual assessment of the degree of fatness and muscle over and around the lumbar vertebrae, sternum, ribs and intercostal (between ribs) spaces was assessed bi-weekly according to Villaquiran *et al*. (2007).

Individual water intake from each bucket was recorded daily by weighing and re-weighing water buckets before and after water administration with an electronic scale (Sartorius model CP 34000, Sartorius AG, Göttingen, Germany). Water refusals were discarded and the buckets refilled after cleaning. The daily amount of test solution consumed was expressed as a percentage of the total fluid taken from both containers. A separate bucket (10 liter) containing water was placed in an adjacent area and re-weighted daily to estimate the amount of water lost by evaporation and the total daily drinking water intake (TDWI) was corrected accordingly. The total water intake (TWI) was determined as the sum of TDWI from both containers and the water content in consumed hay. Water intake was also expressed as intake per kg $BW^{0.82}$ since water use by animals is related to the live weight of animals to the power of 0.82, as water is used in the body for intermediary metabolism and also for evaporative cooling (Wilson, 1989).

The cut hay with an average dry matter content of 90.3 ± 0.2% and energy content of 8.8 ± 0.1 MJ/kg DM was offered *ad libitum* daily and individual feed consumption was measured by weighing the remaining feed on the next day. Hay samples of approximately 150 g were collected weekly and analyzed for dry matter content. Collected hay samples were ground through a 1-mm screen and analyzed using standard methods for dry matter (VDLUFA method 3.1), ash (method 8.1), crude protein (method 4.1.2 – Dumas; Elementar VarioMAX CN, Langenselbold, Germany), ether extracts (method 5.1.1), neutral detergent fiber and acid detergent fiber (expressed without residual ash – NDFom and ADFom, methods 6.5.1 and 6.5.2; Ankom Fiber Analyzer 220, Macedon, USA). The metabolizable energy was estimated using prediction equations for ruminants, including values from Hohenheim gas test (Menke *et al*., 1979; VDLUFA method 25.1). For the feed and water, Na and Cl were determined from aqueous extract using ion chromatography with conductivity detection (LUFA 10.5.2) (Dionex DX-100; Sunnyvale, USA). For Ca, Mg, P and K analyses, samples were ashed and dissolved in HCl. Ca, Mg and K were measured via atomic absorption spectroscopy (Varian SpectrAA-300, Palo Alto, USA) and P was measured photometrically. A mineral licking block (Solsel®, esco-european salt company, Hannover, Germany) with 37% sodium was supplied for each animal throughout the experimental period. Consumption of the mineral lick was determined by weekly re-weighing the salt block with a scale (Sartorius model CP 34000, Sartorius AG, Göttingen, Germany) to the nearest 1 g. Total sodium (Na^+) intake was determined as the sum of the amount of sodium consumed from hay, licking mineral block, fresh and saline drinking water. Feed and sodium intakes were also expressed as intake per kg $BW^{0.75}$.

Statistical analysis

The results from earlier studies showed a higher salt sensitivity in young compared to adult sheep (Wilson and Dudzinski, 1973). In order to take into account a possible age effect, goats were classified as young (N=4) or adult (N=8) when younger or older than two years, respectively. Analyses of variance were performed using the PROC MIXED procedure of the software package Statistical Analysis Systems version 9.3 (SAS). For all traits, the model included the fixed effect of the room, the experimental phase, age of the animal, their interactions, and the random effect of the goats. The model (1) was:

$$Y_{ijklm} = \mu + R_i + P_j + A_k + (R*P)_{ij} + (P*A)_{jk} + (R*A)_{ik} + (R*P*A)_{ijk} + G_l + e_{ijklm}$$

Where: Y_{ijklm}: observation value; μ: overall mean; R_i: room (i = room 1 to 3); P_j: phase (j = control, phases 2, 3 and 4); A_k: age (k = young, old); interactions: (R*P), (P*A), (R*A), (R*P*A); G_l: random effect of the goat and e_{ijklm}: random error.

Data related to different salt concentrations during experimental phases 2, 3 and 4 were analyzed by using the following model (2):

$$Y_{ijklmno} = \mu + R_i + P_j + A_k + C_l + B_m + (P*A)_{jk} + (P*C)_{jl} + (P*B)_{jm} + (A*C)_{kl} + (C*B)_{lm} + (P*A*C)_{jkl} + G_n + e_{ijklmno}$$

Where: $Y_{ijklmno}$: observation value; μ: overall mean; R_i: room (i = room 1 to 3); P_j: phase (j = phases 2, 3 and 4); A_k: age (k = young, old); C_l: concentration (l = 1 to 7); B_m: bucket position (m = right, left); interactions: (P*A), (P*C), (P*B), (A*C), (C*B), (P*A*C); G_n: random effect of the goat and $e_{ijklmno}$: random error.

An integrated Tukey test was used to detect the differences between means at a 5% significance level. All values are presented as least squares means ± SEM. Theoretically, 50% of the total water intake was expected to be consumed from each bucket. A 95% confidence interval was then placed on the theoretical mean. The 40% level of intake referred to as lower discrimination threshold (LDT) and the 60% intake as upper discrimination threshold (UDT). A rejection threshold (RET) was set at 20% intake (Bell, 1959; Goatcher and Church, 1970a).

Results

Ambient temperature, body weight and body condition score

Ambient temperature (T_a) varied during the different phases of the experiment and ranged from 17.9 °C to 26.2 °C. Daily mean T_a was 21.1 ± 1.2 °C in the control phase and phase 2, and 19.7 ± 0.7 °C during phases 3 and 4 of the study. Body condition score significantly (P=0.002) decreased during the experiment, while there was no effect of treatment on body weight (Table 3.1). However, older goats had a significantly (P<0.001) higher body weight and lower body condition score (54.56 ± 7.52 kg and 2.89 ± 0.45 points) compared to young goats (41.60 ± 3.57 kg and 3.41 ± 0.35 points).

Feed and sodium (Na+) intakes

Dry matter intake (DMI) decreased significantly during the experiment (Table 3.1). Total sodium intake was higher in phases 2 (sensitivity test) and 3 (adaptation), while it approached control phase values in the sensitivity re-test (phase 4; Table 3.1). In phases 2 and 3 most sodium intake originated from the drinking water. Due to the experimental design, sodium intake was highest in the adaptation period (phase 3) when only saline water was offered. Sodium ingestion from the salt lick was strongly reduced during phases 2, 3 and 4 compared to the control phase (Table 3.1). Significant age effects were found for all traits recorded with higher values in older than younger goats. The age x phase interactions was significant for total sodium intake, sodium intake from water and sodium intake from salt lick. However, when corrected for body weight, these interactions became non-significant, with the exception of sodium intake from salt lick in phase 1, where old goats had a higher consumption (Table 3.2).

Water intake and sensitivity responses

Both TWI and TDWI were significantly (*P<0.001*) higher in phases 2 (sensitivity test) and 3 (adaptation), compared to control phase and sensitivity re-test (phase 4; Table 3.1). When goats had the choice between fresh water and saline water for the first time (phase 2), they preferred higher salt concentrations and consumed higher amounts of saline water than in the re-test (phase 4) after prolonged exposure to saline water (Figure 3.2). Thus, daily saline water intake was significantly (*P<0.001*) higher (75.4 ± 53.2 g/kg $BW^{0.82}$ per day) in phase 2 (sensitivity test) than in phase 4 (sensitivity re-test) (40.4 ± 34.0 g/kg $BW^{0.82}$ per day). Thus, salt discrimination rejection thresholds were lowered to 1.25% in phase 4 compared to 1.5% in phase 2 (Figure 3.3). In both phases (2 and 4), saline water intake was reduced with increasing further salt concentrations and the lowest (10–20% of total water intake) salt water intake was recorded at a concentration of 1.5% (Figure 3.3).

Figure 3.4A and 3.4B depict the stepwise adaptation to saline drinking water during the third (adaptation) phase. Across the entire adaptation phase, the total water (g/kg $BW^{0.82}$ per day) consumption increased with higher salt concentrations in drinking water (Figure 3.4A). Initially, goats preferred saline water of 0.25% over fresh water (Figure 3.4B). Goats did not differentiate between concentrations of 0.25 and 0.5% when offered simultaneously. However, with increasing concentrations, the preference for the lower concentration of saline water became more pronounced.

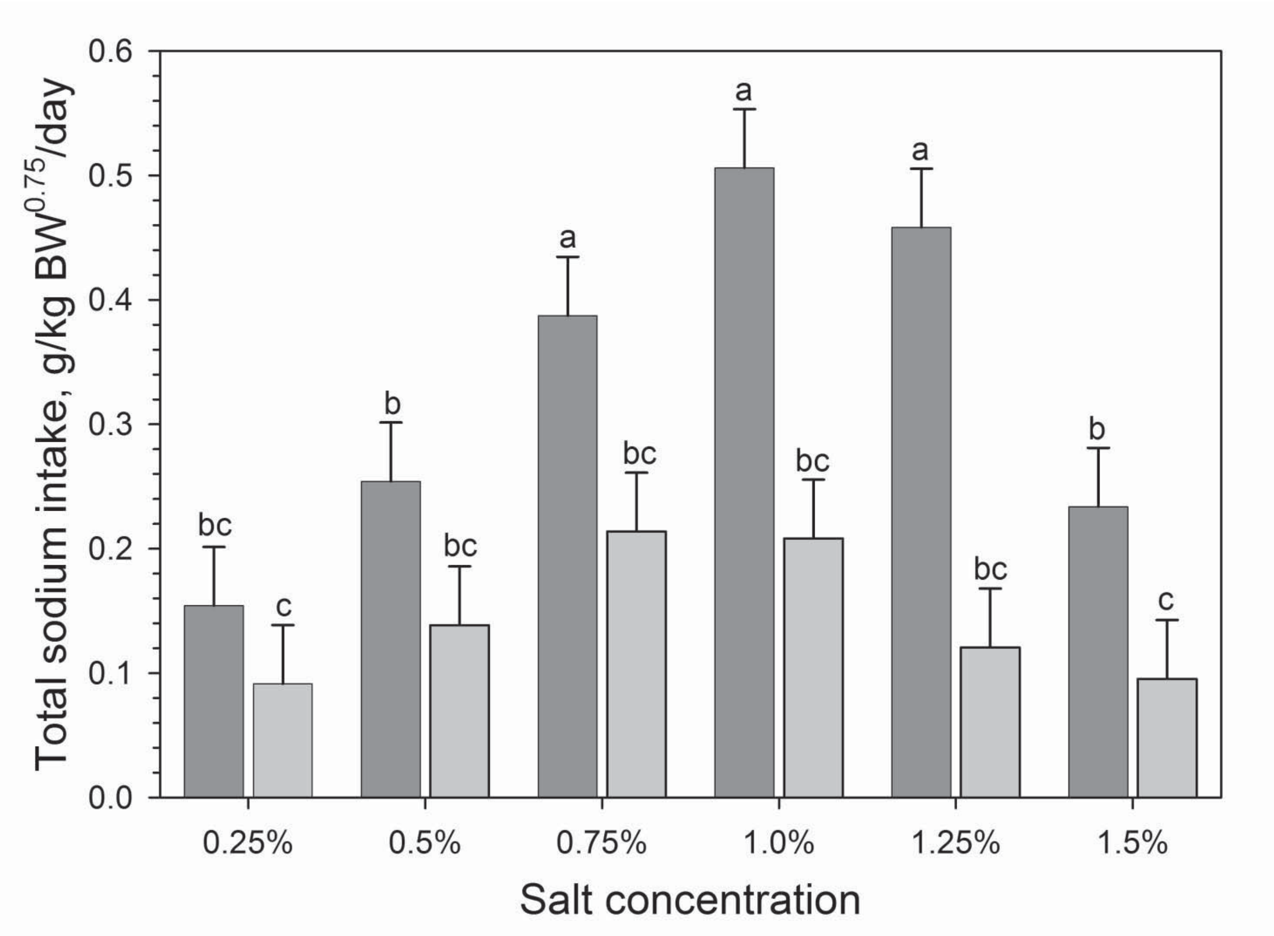

Figure 3.2 Daily average total sodium intake (g/kg BW0.75/day) for the sensitivity test in phase 2 (dark grey bar) and the sensitivity re-test in phase 4 (grey bar) of Boer goats (N=12) exposed to a two choice preference test. One bucket contained fresh water while the second was filled with different salt concentrations. Values are presented as means ± SE. abc Means with different superscripts differ significantly by $P<0.05$.

Table 3.1 Average body weight, body condition score, daily feed, water and sodium intakes for control (phase 1: 1 week) and treatment phases (phase 2: 2 weeks; phase 3: 4 weeks and phase 4: 2 weeks) in Boer goats (N=12), corrected for body weight

Item	Experimental phase[1]				Significance of effects					
	Phase 1 (C)	Phase 2 (ST)	Phase 3 (A)	Phase 4 (SRT)	Phase		Age		Phase x Age	
					SEM	*P*-value	SEM	*P*-value	SEM	*P*-value
BW (kg)	49.48	47.82	47.50	47.51	2.04	0.835	1.44	<0.001	2.83	0.973
$BW^{0.75}$ (kg)	18.61	18.14	18.03	18.04	0.57	0.818	0.40	<0.001	0.79	0.966
BCS (points)	3.33[a]	3.33[a]	3.08[b]	2.85[b]	0.09	0.002	0.06	<0.001	0.13	0.889
DMI (kg/day)	1.14[ab]	1.18[a]	1.09[bc]	1.05[c]	0.02	<0.001	0.02	<0.001	0.03	0.449
DMI (g/kg $BW^{0.75}$/day)	59.86[a]	63.85[b]	59.67[a]	57.91[a]	0.96	<0.001	0.69	<0.001	1.33	0.124
TDWI (kg/day)	2.33[a]	3.05[b]	3.04[b]	2.33[a]	0.10	<0.001	0.07	<0.001	0.14	0.036
TDWI (g/kg $BW^{0.82}$/day)	93.08[a]	126.61[b]	125.99[b]	98.02[a]	3.47	<0.001	2.49	<0.001	4.81	0.074
TWI (kg/day)	2.47[a]	3.19[b]	3.15[b]	2.43[a]	0.10	<0.001	0.07	<0.001	0.14	0.038
TWI (g/kg $BW^{0.82}$/day)	98.56[a]	131.80[b]	130.49[b]	102.54[a]	3.49	<0.001	2.51	<0.001	4.85	0.071
TNaI (g/day)	2.39[a]	6.38[b]	9.13[c]	2.72[a]	0.62	<0.001	0.44	<0.001	0.86	0.023
TNaI (g/kg $BW^{0.75}$/day)	0.12[a]	0.34[b]	0.49[c]	0.15[a]	0.03	<0.001	0.02	0.001	0.04	0.123
NaIF (g/day)	0.085[ab]	0.088[a]	0.082[bc]	0.079[c]	0.002	<0.001	0.001	<0.001	0.002	0.448
NaIF (g/kg $BW^{0.75}$/day)	0.005[a]	0.005[a]	0.004[b]	0.004[b]	0.001	<0.001	0.001	<0.001	0.0001	0.244
NaIW (g/day)	0.02[a]	6.09[b]	8.95[c]	2.43[d]	0.62	<0.001	0.44	<0.001	0.86	0.006
NaIW (g/kg $BW^{0.75}$/day)	0.001[a]	0.329[b]	0.482[c]	0.133[d]	0.03	<0.001	0.02	0.013	0.04	0.074

NaISL (g/day)	2.28^{a}	0.20^{b}	0.10^{b}	0.21^{b}	0.06	<0.001	0.04	<0.001	0.08	<0.001
NaISL (g/kg $BW^{0.75}$/day)	0.113^{a}	0.011^{bc}	0.006^{b}	0.012^{c}	0.002	<0.001	0.002	<0.001	0.003	<0.001

BW = Body weight; BCS = Body condition score; DMI = dry matter intake; TDWI = total drinking water intake; TWI = total water intake; TNaI = total sodium intake; NaIF, NaIW and NaISL = sodium intake from feed, water and salt lick, respectively.

[1]Phase 1(C) = Control, only fresh (tap) water was supplied in two buckets, Phase 2 (ST) = Sensitivity test, water with ascending salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25, and 1.5% NaCl) was offered in one container and unsalted tap water in the other, Phase 3 (A) = Adaptation to saline water, only saline water (concentration between 0% and 1.5%) was provided in both buckets, Phase 4 (SRT) = Sensitivity re-test, the same treatment as in phase 2 was repeated.

[abc] Means within the same row with different superscripts differ significantly by $P<0.05$.

Table 3.2 Average total sodium intake, sodium intake from water and salt lick for young (N=4) and old (N=8) Boer goats during the control (phase 1: 1 week) and treatment phases (phase 2: 2 weeks; phase 3: 4 weeks and phase 4: 2 weeks), corrected for body weight

Item	Phase 1 (C)		Phase 2 (ST)		Phase 3 (A)		Phase 4 (SRT)		Effects (Phase x Age)	
	Young goats	Old goats	Young goats	Old goats	Young goats	Old goats	Young goats	Old goats	SEM	*P-value*
TNaI (g/day)	1.13	3.64	4.64[a]	8.12[b]	6.52[a]	11.75[b]	2.32	3.12	0.86	0.023
TNaI (g/kg $BW^{0.75}$/day)	0.07	0.17	0.28[a]	0.41[b]	0.40[a]	0.58[b]	0.14	0.15	0.04	0.123
NaIW (g/day)	0.02	0.02	4.43[a]	7.76[b]	6.36[a]	11.54[b]	2.00	2.86	0.86	0.006
NaIW (g/kg $BW^{0.75}$/day)	0.001	0.001	0.27[a]	0.39[b]	0.39[a]	0.57[b]	0.12	0.14	0.04	0.074
NaISL (g/day)	1.05[a]	3.52[b]	0.14	0.26	0.10	0.11	0.25	0.17	0.08	<0.001
NaISL (g/kg $BW^{0.75}$/day)	0.06[a]	0.17[b]	0.01	0.01	0.01	0.01	0.02	0.01	0.003	<0.001

TNaI = total sodium intake; NaIW and NaISL = sodium intake from water and salt lick, respectively.

Phase 1(C) = Control; Phase 2 (ST) = Sensitivity test; Phase 3 (A) = Adaptation to saline water; Phase 4 (SRT) = Sensitivity re-test.

[ab] Means within the same row and phase with different superscripts differ significantly by $P<0.05$

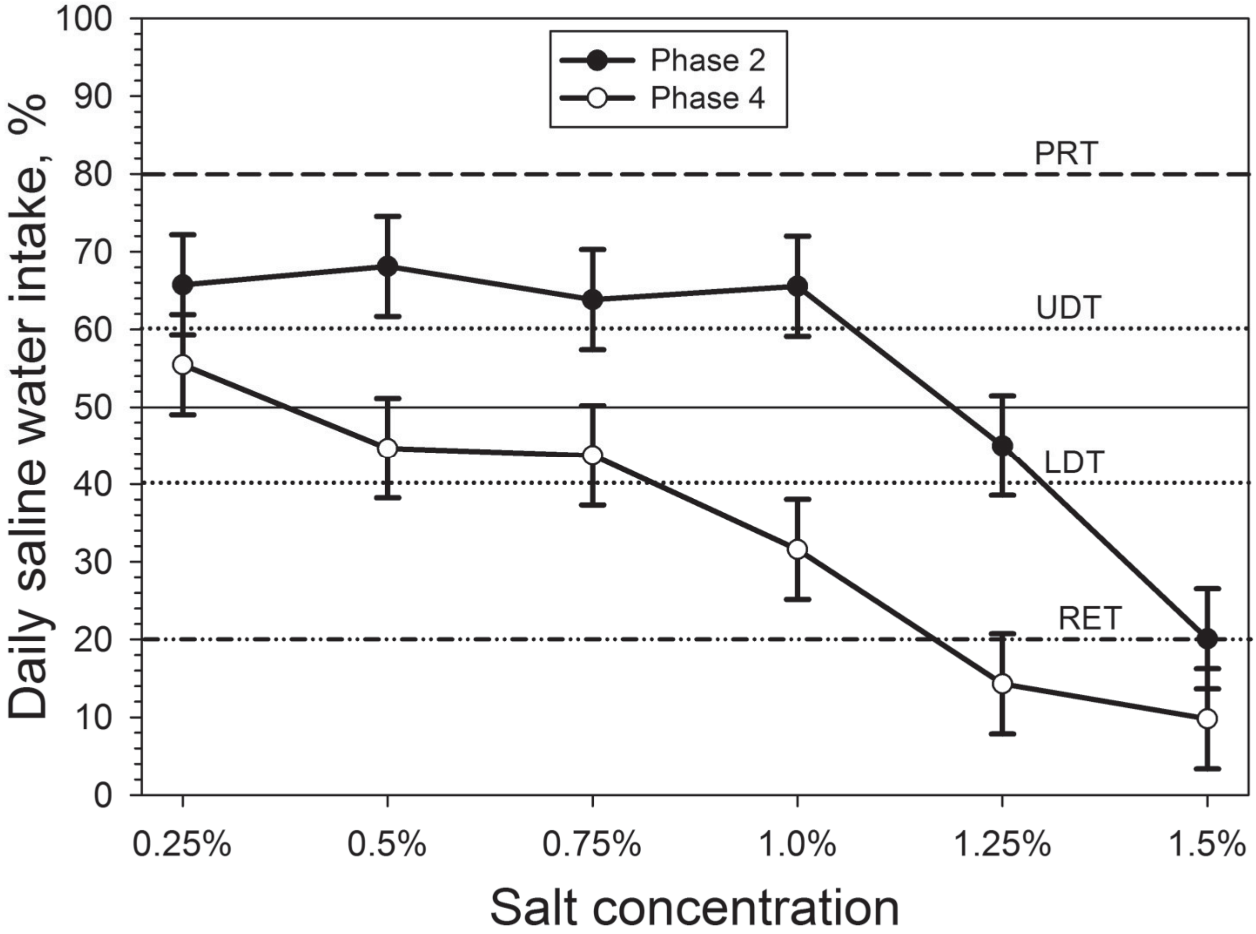

Figure 3.3 Responses of Boer goats (N=12) to saline drinking water in a two choice preference test during the sensitivity test (phase 2) and the sensitivity re-test (phase 4): daily amount of test solution consumed expressed as a percentage of the total fluid taken from both containers. The horizontal lines indicate threshold limits; dash-dotted line= rejection threshold (RET), dashed line= preference threshold (PRT), dotted lines indicate lower and upper discrimination threshold (LDT and UDT, respectively; see text for details).

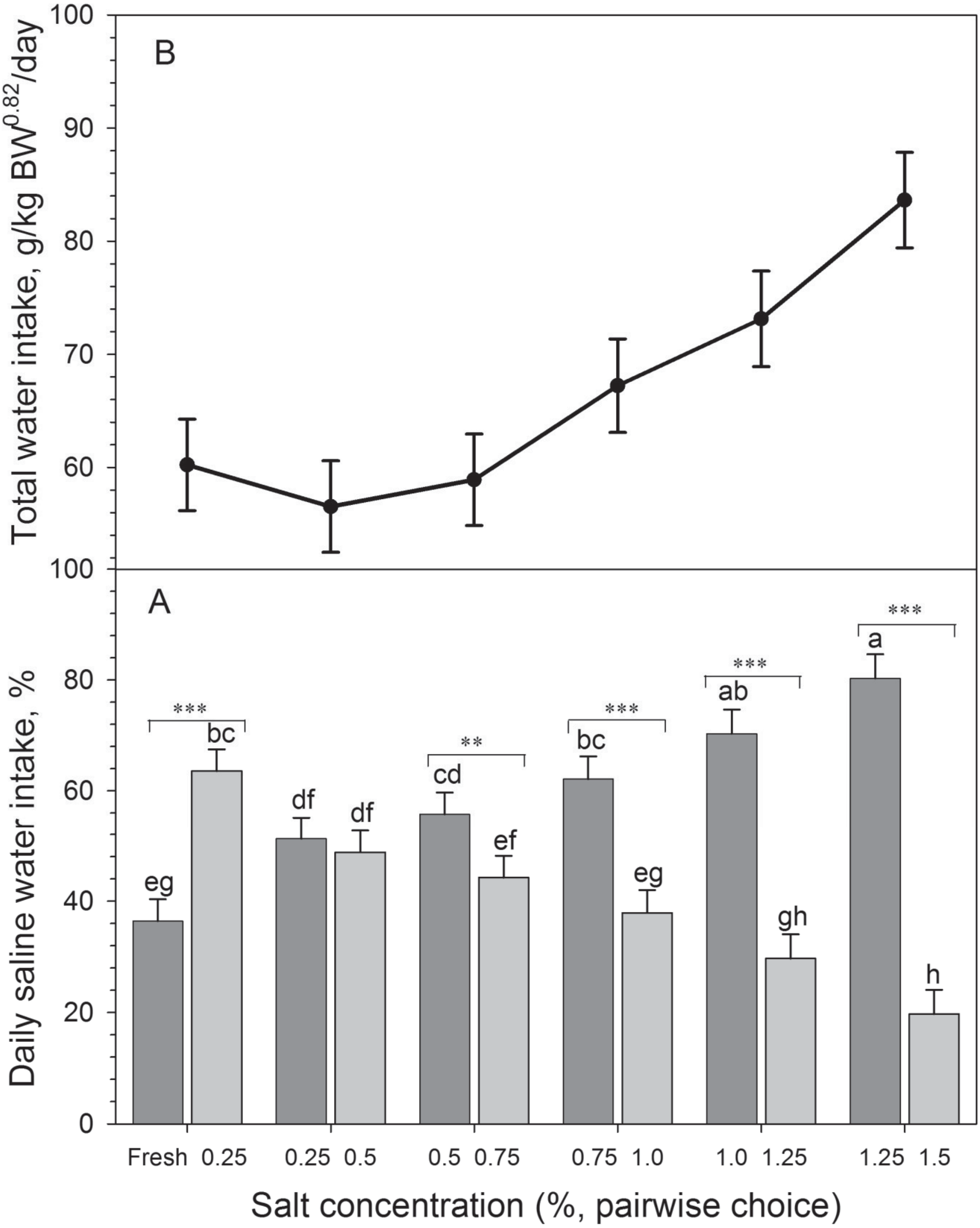

Figure 3.4 Stepwise adaptation of Boer goats (N=12) to saline drinking water during the adaptation to saline water (phase 3). A) Total daily saline water intake (g/kg $BW^{0.82}$/day); B) Daily saline water intake of different salt concentrations (% of total water intake). Dark grey and light grey color bars indicate lower and higher concentrations, respectively; means ± SE. [abcdefgh] significant differences (*P*<0.05) between salt concentrations (*** *P*<0.001; ***P*<0.05).

Discussion

To our knowledge this study is the first experimental adaptation trial for saline drinking water in goats. While body weight remained unchanged during the study, the changes in body condition scores of our experimental goats were directly associated with feed and water consumption. Continuous intake of high saline water depressed appetite, reduced feed intake and digestibility, resulting in body weight loss in sheep (Masters *et al*., 2005). The lower body condition score found in our treatment phases could indicate that water retention may have contributed to a constant body weight (Masters *et al*., 2005). Similarly, sheep increased their body water content in the intracellular space to excrete excess sodium from the body fluids (Assad and El-Sherif, 2002).

Feed and water intakes

Saline water intake induced only small changes in dry matter intake in our study. Animals initially ingested more feed when saline water was first introduced together with fresh water, most likely due to increasing feed palatability. Similar results have been reported by Ru *et al*. (2004) where the feed intake of red and fallow weaner deer was increased at the beginning of drinking saline water and then decreased when drinking water salinities increased to 1.2–2.4%. Studies in rusa deer stags (Kii and Dryden, 2005) reported similar results. Furthermore in a study on sheep, feed intake was not affected by drinking water containing 1.3–1.5% salt and a greater reduction of feed intake was only observed when animals were given 2% sodium chloride in water (Peirce, 1957).

In our experiment, the TDWI was increased with an increased salt concentration, which is in agreement with previous findings (Peirce, 1957; Kattnig *et al*., 1992). Abou Hussien *et al*. (1994) found that the total water intake of goats and sheep was increased by 59% and 99%, respectively, when drinking water salinity was increased to 1.7%. Similar results were recorded in rusa deer stags (Kii and Dryden, 2005) and in fallow and red deer (Ru *et al*., 2004). However, several studies with cattle found that water intakes were not increased with water salinity up to 1.1% (Bahman *et al*., 1993). It has been postulated that increased water consumption is a physiological response to an excess in salt in the body (Kattnig *et al*., 1992) in order to maintain systemic osmotic balance. This strategy, in turn, allows animals to adapt to increased quantities of ingested salt through renal adjustment (Potter, 1961). Macfarlane (1982) observed an induction of Na^+/K^+ ATPase enzymes in the kidney, liver and ileum in goats exposed to saline water. As these enzymes play a central role in the active transfer of sodium out of the cell and other ion-transporting mechanisms (Suttle, 2010), the higher tolerance to saline water found in goats compared to sheep may be attributed to their slightly more effective sodium pumps (Macfarlane, 1982).

Sensitivity responses

The comparison between salt acceptance thresholds before and after the habituation period revealed very interesting results. Contrary to our expectation, prolonged higher intake of sodium in phase 3 did not lead to a higher salt acceptance as there was a considerable shift towards lower thresholds in the sensitivity re-test (phase 4), when offered a choice between fresh and saline water. Accordingly, discrimination and rejection thresholds are not constant but depend on the total sodium balance of the animal indicating flexible regulation mechanisms.

Sodium is the only mineral that mammals recognize sensorial by taste. Variations in salt taste sensitivity have been associated with individual differences such as genetic variations, age, sex, and feed or water containing high sodium contents (Noh *et al*. 2013). In the adaptation phase of this study (phase 3), goats reacted more sensitive to higher concentrations of saline water which may be related to aversion behavioral responses (Chandrashekar *et al*., 2010). Thus, different salt taste pathways are involved in response to salt stimulation via drinking water, including prominent taste receptor cells in the salt sensing system and transfer mechanisms of the taste information to the brain (Yoshida *et al*., 2006; Chandrashekar *et al*., 2010). It is open to question, whether the prolonged exposure to saline water modified the sensitivity of the taste buds or the epithelial sodium ion channel of the salt receptor cells.

Adaptation to saline water

In ruminants, gradual changes in diets are recommended to enable the animals to adjust with the microbiological and chemical changes occurring in the rumen (Grubb and Dehority, 1975; Mackie *et al*., 1978). In previous studies on sheep, a stepwise adaptation was successfully achieved through diets containing different ratios of roughage and concentrates (Grubb and Dehority, 1975), or high concentrate diets containing 1% NaCl

(Mackie *et al*., 1978). To our knowledge, the current study is the first applying a stepwise adaptation towards saline drinking water in goats. We observed a remarkable shift of preferences during the adaptation period with indifferent choices up to 0.5%, followed by strong preferences for lower sodium concentrations, indicating the capabilities of goats to regulate their sodium intake even when they had only the choice between different concentrations of saline water.

Adaptation to saline water could be achieved in several ways. In our study, the goat breed was adapted to temperate climatic conditions and had no prior experience with saline water. Their higher sensitivity after the long term exposure to saline water may indicate a learning process. Taste cells may have been modified in their responsiveness thus causing taste alteration in the animal (Bernays and Singer, 2005). Our results support the view of Ginane *et al*. (2011) that the attractiveness of sodium greatly depends on the mineral status of the animal. Thus, calves deprived of sodium exhibited a marked preference for NaCl solutions over water (Bell and Sly, 1979). Similarly, cows without sodium supplement preferred to graze on a pasture with NaCl application (Chiy and Phillips, 1991).

Examples for a long-term morphological and behavioural adaptation to saline drinking water are given by reports, that goats on arid islands and beaches voluntarily drink sea water (Dunson, 1974). Interestingly, Dunson (1974) found that the kidneys of feral goats from arid islands had greater relative medullary thickness (RMT) than those from domestic goats. That in turn, allows these goats to adapt to increased salinity of water through a greater capacity of the kidneys to reabsorb water, concentrate urine and reduce urinary water loss during dehydration.

There is increasing evidence that maternal nutrition during gestation can induce epigenetic modifications that alter gene expression in the offspring and the study of imprinted genes has received increasing attention in livestock production. Lan *et al*. (2013) demonstrated in sheep the association between maternal diet during mid-to late-gestation and transcriptomic and epigenomic alterations of imprinted genes and DNA methyltransferase genes in the fetal tissues. Calves from cows that were supplemented with sodium during the last 2 months of pregnancy showed a higher appetite for sodium (Mohamed and Phillips, 2003). In sheep, lambs from ewes exposed to high salt during pregnancy had altered responses to sodium (Digby *et al*., 2010). The authors suggested two types of fetal programming by prenatal exposure to salt: modulation of the thirst threshold and of the regulatory systems controlling salt and water balance.

Conclusions

The present study shows that a stepwise adaptation to saline drinking water in goats is an effective method to habituate the animals to saline water intake. The adult goats tolerated prolonged choices between 1.25% and 1.5% saline without health impairment. However, the applied stepwise adaptation for 4 weeks did not result in an overall higher tolerance of saline water. After the adaptation period the animals reacted more sensitively when offered the choice between fresh water and different concentrations of saline water. Accordingly, the observed adaptation to saline water may be of short term duration only. In regions, where salinization of drinking water becomes an increasing challenge for livestock production, the controlled exposure to salt during gestation could be beneficial to the offspring because of fetal imprinting (Digby *et al*., 2010). In this context, it would be interesting to investigate, whether the high tolerance of saline water described for several species such as camels (Abou Hussien, 1994; Assad and El-Sherif, 2002) is also influenced by epigenetic factors.

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CHAPTER 4

Health status in Boer goats while adapting to saline drinking water

R. A. Runa, M. Gerken A. Riek and L. Brinkmann

Department of Animal Sciences, University of Göttingen, Albrecht-Thaer-Weg 3, 37075 Göttingen, Germany

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Health status in Boer goats while adapting to saline drinking water

R. A. Runa, M. Gerken, A. Riek and L. Brinkmann

Abstract

The effects of adaptation to saline water consumption on physiological parameters and the health status were investigated in goats. The study was conducted in four phases involving twelve female Boer goats. During the control phase (phase 1) only fresh water was supplied. In phase 2, water with different salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5% NaCl) and tap water were offered in a two choice test. During the third phase, goats were stepwise habituated to saline water by only offering saline water with different increasing concentrations (between 0% and 1.5% NaCl). In phase 4 the procedure of phase 2 was repeated. Blood parameters, water and feed intake, rumen temperature (T_r), body weight (BW) and body condition score (BCS) were measured throughout the experiment. BW was not affected by saline water intake, whereas BCS decreased. Total sodium intake (g/kg body weight$^{0.75}$/day) in adult goats was higher than in young goats during phases 2 ($P < 0.05$) and 3 ($P < 0.001$), while it approached control phase values in phase 4. In adult goats, T_r significantly decreased with prolonged saline water intake, while it increased ($P < 0.01$) in young goats. Drinking saline water had no effect on the concentrations of ALT, AST, glucose, urea, calcium, sodium and osmolality. Serum magnesium decreased ($P < 0.001$), while potassium concentration increased from phase 2 ($P < 0.001$). All measured blood parameters of our study remained within reference ranges, indicating remarkable adaptation capacities of Boer goats to saline water without health impairment.

Keywords: adaptation, blood parameters, goats, rumen temperature, saline water, sodium intake

Introduction

Water is an essential nutrient for all living beings. It plays important roles in maintaining body homeostasis, regulation of body temperature and cellular functions including metabolism and transportation of other nutrients (Kavouras and Anastasiou, 2010).

However, changing water quality by salinization of fresh water sources due to climate changes (World Bank 2010) creates a serious threat to the health of livestock populations. Excessive dietary salt intake leads to hypertension (Elliott *et al*., 1996), associated with renal and cardiovascular diseases (Tuomilehto *et al*., 2001; He and MacGregor, 2007; Reynolds *et al*., 2013). High saline water consumption also impairs osmosis by altering the intra-cellular sodium-potassium pumping mechanism (Giuggio, 2018). Acute excess of sodium has been associated with raised pulse and respiration rates, water retention, muscle cramps and neurological problems (Peirce, 1957; Marai *et al.*, 1995; Kii and Dryden, 2005). If salt intake approaches poisoning levels, it depresses appetite, increases thirst and causes vomiting, diarrhea, blindness, seizures, hydropericardium and finally death (Hungerford, 1990; Thompson, 2018). Serum concentrations of sodium above 160 mEq/L indicate salt poisoning (Thompson, 2018).

A number of studies investigated the effects of water salinity on health in humans (Khan *et al*., 2011; Vineis *et al*., 2011; Talukder *et al*, 2016a, 2016b). However, data on health risks of increased water salinity in livestock are limited. Blood and plasma volume, extracellular and interstitial fluids as well as urea concentrations decreased with increased water salinity in sheep (Assad and El-Sherif, 2002; Meintjes and Engelbrecht, 2004). Yousfi and Ben Salem (2017) found increased plasma glucose, creatinine, urea and gamma-glutamyl-transferase when sheep were exposed to 1.5% saline water for 3 weeks. Goats receiving 1.1% saline water for 4 weeks reacted with an increased pulse rate as well as a decreased rectal temperature and respiration rate (Mdletshe *et al*., 2017).

In arid areas or coastal regions with intrusion of sea water into the ground water after inundations (Hallegatte *et al.,* 2013), the availability of unsalinated water for livestock may be limited. A careful habituation to saline drinking water might help animals to adapt without health risks. In the present study, a stepwise adaptation to saline water was applied to Boer goats. To our knowledge, there are no reports on the health status of goats during such a habituation to saline water. The aim of our study was to determine the effects of prolonged saline water consumption on physiological parameters and health status of the goats. Blood parameters and rumen temperature were recorded to evaluate their capacities in maintaining sodium balance, to detect possible health problems and evaluate the suitability of the applied adaptation scheme.

Materials and Methods

Ethical approval

Procedures performed in this study were in accordance with the German animal ethics regulations and approved by the State Office for Consumer Protection and Food Safetyof Lower Saxony, Germany (Ref. no.: 33.9-42502-04-15/1946).

Animals and managements

The study was undertaken at the Department of Animal Sciences, University of Göttingen, Germany, from November 2015 until January 2016. The experiment involved 12 female non-pregnant Boer goats (age 1.5 to 7.5 years, average body weight 50.5 ± 9.0 kg). Goats were classified as young (N=4) or adult (N=8) when younger or older than two years, respectively. All animals originated from the breeding herd of the Department. Animals were transferred to the experimental stable two weeks before the start of the trial for acclimatization. Goats were kept individually under controlled stable conditions (room temperature 20.1± 1.2°C; relative humidity 46.7 ± 8.0 %; light schedule: 10 h dark and 14 h light) in one of 12 identical straw pens (3.0 m^2) allocated to three different rooms. Each pen was equipped with an individual feed trough (diameter 53 cm, approximate capacity: 3 kg hay) and two identical water buckets (diameter 28 cm, 10 liter capacity per bucket), which were placed at each side of the feed trough to allow free choice between contents.

Experimental design

The study was conducted in four phases (Figure 4.1) applying a two choice preference test design (Goatcher and Church, 1970). In the control phase (phase 1, 1 week), only fresh (tap) water was supplied in two identical buckets. In phase 2 (sensitivity test, 2 weeks), water with ascending salt concentrations (0.25, 0.5, 0.75, 1.0, 1.25, and 1.5% NaCl) was offered in one container and unsalted tap water in the other. Each concentration was tested for two days. During the third phase (adaptation, 4 weeks), goats were stepwise habituated to increasing saline water concentrations by offering only saline water in both buckets. The saline water of the lower concentration was offered in one bucket and the salt water with higher concentration in the other (Figure 4.1).The concentrations were gradually increased between 0% and 1.5% NaCl. Subsequently, in phase 4 (sensitivity re-test, 2 weeks) the procedure of phase 2 was repeated to test for differences in sensitivity thresholds before and after the adaptation period. The water buckets were replenished with test solutions daily and the positions of the concentrations were changed daily at random in order to avoid a bias due to position effects.

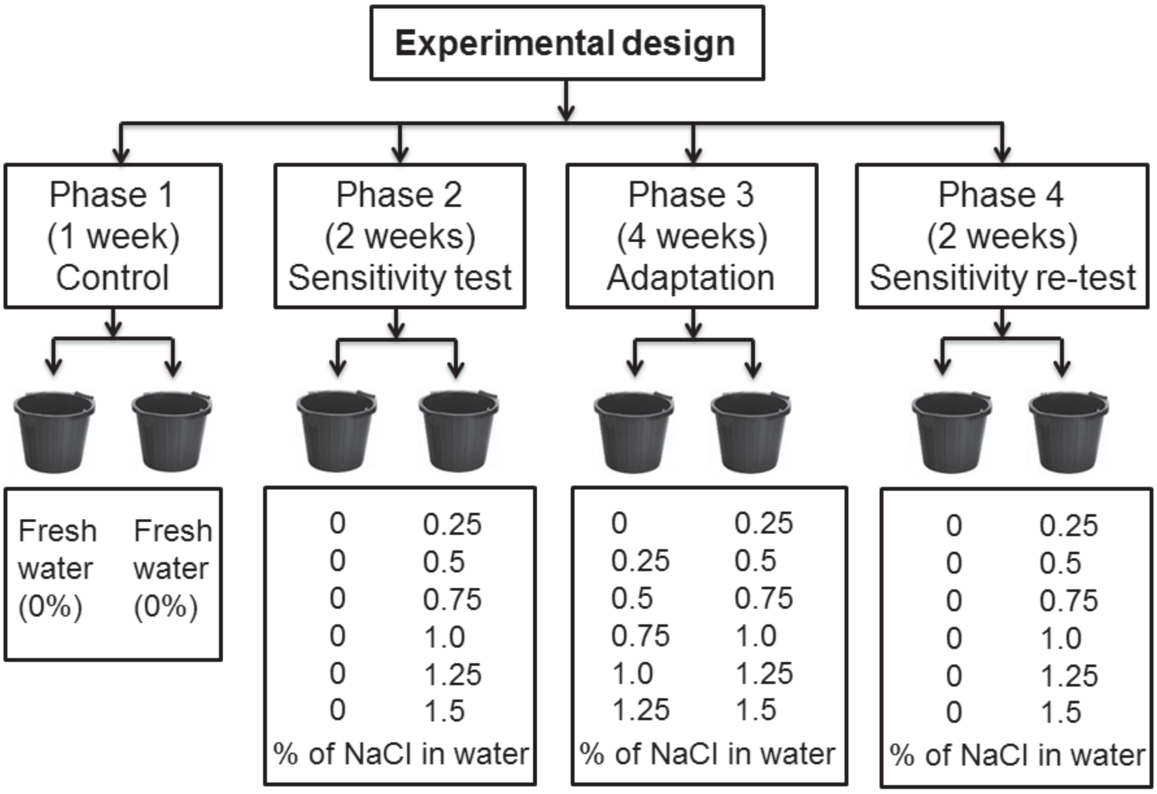

Figure 4.1 Experimental design based on a two choice preference test: provision of different concentrations of salt in drinking water during the different phases of the experiment. Two water containers were placed at each side of the feed trough for ad libitum intake. The positions of the concentrations were changed daily at random in order to avoid a bias due to position effects.

Feeding and drinking

Animals had access to chopped hay (dry matter: 90.3 ± 0.2%; energy: 8.8 ± 0.1 MJ/kg dry matter) and water *ad libitum* throughout the experimental period. An electronic scale (Sartorius model CP 34000, Sartorius AG, Göttingen, Germany) was used to determine daily individual feed and water intake from each bucket by weighing and re-weighing all buckets before and after administration of feed and water. Total drinking water intake (TDWI) was corrected by the evaporative loss from the buckets (measured by weighing and re-weighing a control bucket). The total water intake (TWI) was determined as the sum of TDWI from both buckets and the water content in consumed hay. A mineral salt lick (Solsel®, esco-european salt company, Hannover, Germany) with 37% sodium (Na^+) was available *ad libitum* throughout the study. Consumption of the salt lick was determined by weekly re-weighing with an electronic scale (Sartorius model CP 34000, Sartorius AG, Göttingen, Germany) to the nearest 1 g. Total sodium intake was determined as the sum of sodium consumed from hay, salt lick as well as fresh and saline drinking water. Feed and sodium intakes were also expressed as intake per kg body weight$^{0.75}$. Water intake was expressed as intake per kg body weight$^{0.82}$ since water use by animals is related to the live weight of animals to the power of 0.82, as water is used in the body for intermediary metabolism and also for evaporative cooling (Wilson, 1989).

Measurements

Individual body weight was recorded bi-weekly with a mobile scale (Salter Brecknell LS300, capacities: 300 kg, resolution: 0.2 kg, Salter Brecknell, Smethwick, West Midlands, UK). Likewise, the body condition score (BCS), a palpable and visual assessment of the degree of fatness and muscle mass over and around the lumbar vertebrae, sternum, ribs and intercostal (between ribs) spaces (BCS scale: 1=emaciated, 5=obese with 0.5 increments) was assessed bi-weekly according to Villaquiran *et al.* (2007). Respiration rate was measured twice per day (at 1100 h and 1500 h) by counting the (rate of) flank movement for one minute by using a stopwatch (898M, E.A. Combas Limited, London, UK).

The ambient temperature (°C) and relative humidity (%) were recorded every 30 min throughout the study with miniature data loggers in each experimental room (iButton DS1923L-F5#, temperature range from -20 to +85°C, resolution: 0.0625°C, humidity resolution: 0.04%, Maxim Integrated Products, Sunnyvale, CA, USA).

For measuring rumen temperature (T_r), a self-developed rumen bolus (diameter 22 mm, length 33 mm and weight ca. 100 g) was used. Temperature loggers (iButton DS1922L-F5#, temperature range from -40 to +85°C, accuracy = ±0.5°C, resolution: 0.0625°C, Maxim Integrated Products, Sunnyvale, CA, USA) were inserted into the bolus. Rumen temperature loggers were set to record temperature every 30 min. The bolus was administered *perorally* into the rumen with an applicator by a veterinarian. All animals were observed intensively for 24 hours to confirm the boluses remained in the rumen without any digestive impairment. At the end of the experiment, all animals were sacrificed and boluses were collected. Retrieved rumen boluses were washed, temperature loggers were collected and data were downloaded via a laptop.

Blood samples (10 ml) of each goat were taken at the end of the control phase, phase 2 and phase 4, and on a weekly basis during phase 3.The samples were drawn from the *vena jugularis* between 0800 h and 0900 h before provision of new hay and drinking water on the sampling day. Samples were transferred into serum tubes (12 ml KABE SE 95 PP, KABE Labortechnik GmbH, Nümbrecht-Elsenroth, Germany) and blood tubes containing EDTA (9ml SARSTEDT Monovette K3E, SARSTEDT AG & Co, Nümbrecht, Germany) for collecting serum and plasma, respectively. All samples were immediately cooled (8°C), centrifuged for 15 minutes at 3000 rpm and 20°C (centrifugation force: 1620g). The serum and plasma samples were pipetted into labelled glass vials and stored at -20°C until further analysis at LABOKLIN laboratory (Bad Kissingen, Germany). ALT, AST, glucose, urea, creatinine, calcium and magnesium were determined photometrically using Cobas c701 (Roche GmbH, Waiblingen, Germany). Potassium and sodium were measured potentiometrically using an ion-selective electrode, Cobas c701 (Roche GmbH, Waiblingen, Germany). Osmolality (mosmol/kg) was calculated using the formula: 2 * c (sodium) + c (glucose) + c (urea), with c presenting the analyzed values of respective solutes (Na, glucose and urea) in mmol/l.

Statistical analysis

All statistical analyses were performed using the software package Statistical Analysis Systems version 9.3 (SAS). For every phase daily averages of all recorded data were calculated. Differences of all measured physiological parameters between experimental phases were tested using a mixed model (PROC MIXED) to account for repeated measurements of the same individuals. The model was:

$$Y_{ijklm} = \mu + R_i + P_j + A_k + (R*P)_{ij} + (P*A)_{jk} + (R*A)_{ik} + (R*P*A)_{ijk} + G_l + e_{ijklm}$$

Where: Y_{ijklm}: observation value; μ: overall mean; R_i: fixed effect of room (i = room 1 to 3); P_j: fixed effect of phase (j = control, phases 2, 3 and 4); A_k: fixed effect of age (k = young and old); interactions: (R*P), (P*A), (R*A), (R*P*A); G_l: random effects of the goats and e_{ijklm}: random error.

For rumen temperature (T_r), the measurements contained non-physiological declines, probably due to ingestion of cooler water or food (Singer *et al*., 2010). These data points were removed by visually checking the raw data. Prior to analysis, hourly and daily means of rumen temperature and ambient temperature were calculated. Differences between experimental phases in T_r were calculated using the PROC MIXED procedure with animal as a random factor and phase, age and the phase x age interaction as fixed factors. An integrated Tukey test was used to detect the differences between means (with 5% significance level). All values are presented as least square means ± SEM.

Results

Daily mean T_a was 21.0 ± 0.4°C (control phase: maximum T_a= 21.8°C; minimum T_a = 19.5°C), 21.2 ± 0.3°C (phase 2: maximum T_a= 22.3°C; minimum T_a = 20.2°C), 19.7 ± 0.3°C (phase 3: maximum T_a= 20.7°C; minimum T_a = 18.9°C) and 19.6 ± 0.4°C (phase 4: maximum T_a= 24.4°C; minimum T_a = 18.7°C), respectively.

Body weight, body condition score and respiration rate

Throughout the experiment, there was no effect of phase on body weight (Table 4.1). However, young goats showed significantly (*P<0.001*) lower body weight and higher BCS than older goats (41.60 ± 3.57 kg and 3.41 ± 0.35 points vs. 54.56 ± 7.52 kg and 2.89 ± 0.45 points, respectively). Dry matter intake (DMI) and BCS decreased significantly (*P<0.001* and *P=0.002*, respectively) during the experiment. Feed and water intakes (from both fresh and saline water) were higher (*P<0.001*) in old goats compared to young animals (Table 4.1). All goats showed a significant decrease (*P<0.001*) in respiration rates during phase 3 and 4, when exposed to lower ambient temperature.

Sodium intake and rumen temperature

Total sodium intake (g/kg body weight$^{0.75}$/day) was significantly higher during phases 2 (sensitivity test; *P* < *0.05*) and 3 (adaptation; *P* < *0.001*), while it approached control phase values in the sensitivity re-test (phase 4). Adult goats showed higher sodium intake in phase 2 and 3 compared to young goats but did not differ from the latter in control phase and sensitivity re-test (Figure 4.2A). Rumen temperature (T_r) was significantly (*P < 0.01*) decreased during the treatment phases (39.13 ± 0.15°C) compared to the control phase (39.18 ± 0.19°C) and lowest T_r was recorded in phase3 (adaptation) when goats consumed only saline water. T_r in young goats was significantly higher (*P < 0.001*) during phases 3 (adaptation) and 4 (sensitivity re-test) than in older goats (Figure 4.2B). Figure 4.3 illustrates the relationship between daily total sodium intake (TNaI, g/kg body weight$^{0.75}$/day) and rumen temperature (°C). The best fit was achieved by quadratic regressions for both old ($T_{r,,}$°C = 0.155 TNaI^2 - 0.285 TNaI + 39.17, R^2 = 0.27, *P < 0.001*; Figure 4.3A) and young goats ($T_{r,,}$°C = 0.409 TNaI^2 - 0.354 TNaI + 39.21, R^2 = 0.17, *P < 0.01*; Figure 4.3B).

Blood metabolites

The data of all measured blood metabolites are presented in the Table 4.2. The serum concentration of creatinine was significantly (*P = 0.021*) higher in phase 4 (sensitivity re-test). Potassium concentrations in serum increased from phase 2 and remained elevated until the end of the experiment (*P < 0.001*). The magnesium level decreased from phase 2 to control phase values in phase 4 (*P < 0.001*). However, no significant treatment effects were found for ALT, AST, glucose, urea, calcium, sodium and osmolality.

Table 4.1 Average body weight (BW), metabolic body weight (MBW), body condition score (BCS), dry matter intake (DMI), total water intake (TWI) and respiration rate (RR) for young (N=4) and old (N=8) Boer goats during the control (phase 1) and treatment phases (phase 2 3 and 4) of the experiment (see text for details). Values are LS-means ± SE.

Item	Phase 1 (C)[1]		Phase 2 (ST)[1]		Phase 3 (A)[1]		Phase 4 (SRT)[1]		*P-values*	
	Young goats	Old goats	Young goats	Old goats	Young goats	Old goats	Young goats	Old goats	Phase	Age
BW (kg)	43.60 ± 2.79^{a}	55.37 ± 1.90^{b}	41.50 ± 3.94^{a}	54.14 ± 2.69^{b}	40.53 ± 2.79^{a}	54.47 ± 1.90^{b}	40.77 ± 3.94^{a}	54.24 ± 2.69^{b}	0.835	<0.001
MBW ($BW^{0.75}$)	16.96 ± 0.77^{a}	20.26 ± 0.53^{b}	16.35 ± 1.10^{a}	19.93 ± 0.75^{b}	16.06 ± 0.77^{a}	20.01 ± 0.53^{b}	16.13 ± 1.10^{a}	19.96 ± 0.75^{b}	0.818	<0.001
BCS (points)	3.58 ± 0.13^{aA}	3.08 ± 0.09^{bA}	3.58 ± 0.18^{aA}	3.08 ± 0.12^{bA}	3.29 ± 0.13^{aB}	2.86 ±0.09^{bB}	3.17 ±0.18^{aB}	2.53 ± 0.12^{bB}	0.002	<0.001
DMI (kg/day)	0.94 ± 0.05^{aAB}	1.34 ± 0.04^{bAB}	0.99 ± 0.03^{aA}	1.36 ± 0.02^{bA}	0.92 ± 0.03^{aBC}	1.27 ±0.02^{bBC}	0.90 ± 0.03^{aC}	1.19 ± 0.02^{bC}	<0.001	<0.001
DMI (g/kg $BW^{0.75}$/day)	54.37 ± 2.27^{aA}	65.34 ± 1.55^{bA}	60.03 ± 1.46^{aB}	67.67 ± 1.00^{bB}	56.09 ± 1.17^{aA}	63.24 ± 0.71^{bA}	56.09 ± 1.46^{aA}	59.73 ± 1.00^{bA}	<0.001	<0.001
TWI (kg/day)	2.07 ± 0.23^{aA}	2.86 ± 0.16^{bA}	2.57 ± 0.15^{aB}	3.81 ± 0.10^{bB}	2.50 ± 0.12^{aB}	3.80 ± 0.07^{bB}	2.08 ± 0.15^{aA}	2.79 ± 0.10^{bA}	<0.001	<0.001
TWI (g/kg $BW^{0.82}$/day)	91.65 ± 8.27^{A}	105.46 ± 5.65^{A}	120.16 ± 5.34^{aB}	143.45 ± 3.65^{bB}	118.03 ± 4.28^{aB}	142.95 ± 2.58^{bB}	100.26 ± 5.34^{A}	104.82 ± 3.65^{A}	<0.001	<0.001
RR (breath/min)	31.90 ± 1.08^{A}	33.49 ± 0.74^{A}	33.94 ± 0.70^{A}	33.28 ± 0.47^{A}	28.28 ± 0.56^{B}	27.48 ± 0.34^{B}	27.63 ± 0.70^{B}	27.37 ± 0.47^{B}	<0.001	0.946

^{ab} significant differences between ages P < 0.05; ^{ABC} significant differences between phases P < 0.05

[1]C = Control; ST = Sensitivity test; A = Adaptation to saline water; SRT = Sensitivity re-test

Table 4.2 LS-means for blood serum concentrations of liver enzymes (ALT, AST), urea, creatinine, glucose, electrolytes (calcium, potassium, magnesium, sodium) and osmolality of Boer goats (N = 12) during the control (phase 1) and experimental phases (phase 2,3 and 4) of the experiment. See text for details.

Item[1]	Phase 1 (C)[2]	Phase 2 (ST)[2]	Phase 3 (A)[2]				Phase 4 (SRT)[2]	Effects		Reference values
			Week 1	Week 2	Week 3	Week 4		SEM	*P*-value	
ALT (U/l)	13.94	13.92	13.34	14.24	13.02	13.68	13.97	1.02	0.983	<14[3]
AST (U/l)	30.88	28.46	28.19	27.98	27.42	29.00	29.46	1.37	0.654	10 – 50[3]
Glucose (mmol/l)	2.72	2.70	2.68	2.76	2.65	2.75	2.73	0.06	0.901	2.4 – 4.0[4]
Urea (mmol/l)	3.64	3.29	2.75	3.42	3.09	2.89	3.59	0.28	0.194	4.5 – 8.6[4]
Creatinine (µmol/l)	82.92^{a}	78.94^{a}	79.36^{a}	83.56^{a}	84.58^{a}	84.97^{a}	93.39^{b}	2.85	0.021	54 – 123[4]
Calcium (mmol/l)	2.60	2.60	2.53	2.55	2.53	2.53	2.53	0.04	0.523	2.3 – 2.9[4]
Potassium (mmol/l)	5.14^{a}	5.83^{b}	5.18^{a}	5.62^{bc}	5.51^{c}	5.60^{bc}	5.45^{c}	0.10	<0.001	3.4 – 6.1[4]
Magnesium (mmol/l)	0.95^{a}	0.89^{be}	0.86^{bd}	0.83^{cd}	0.80^{c}	0.83^{cd}	0.92^{ae}	0.02	<0.001	0.8 – 1.3[4]
Sodium (mmol/l)	144.36	145.14	144.83	145.39	144.67	143.94	143.53	0.73	0.561	135 – 156[4]
Osmolality (mosmol/kg)	295.08	296.27	295.09	297.96	295.08	293.52	293.37	1.51	0.611	270 – 305[3]

[abcde]Values with different superscripts within the same row differ ($P < 0.05$).

[1]ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase.

[2]C = Control; ST = Sensitivity test; A = Adaptation to saline water; SRT = Sensitivity re-test

[3,4]Reference values according to: [3]Laboklin (2017); [4]Jackson and Cockcroft (2002)

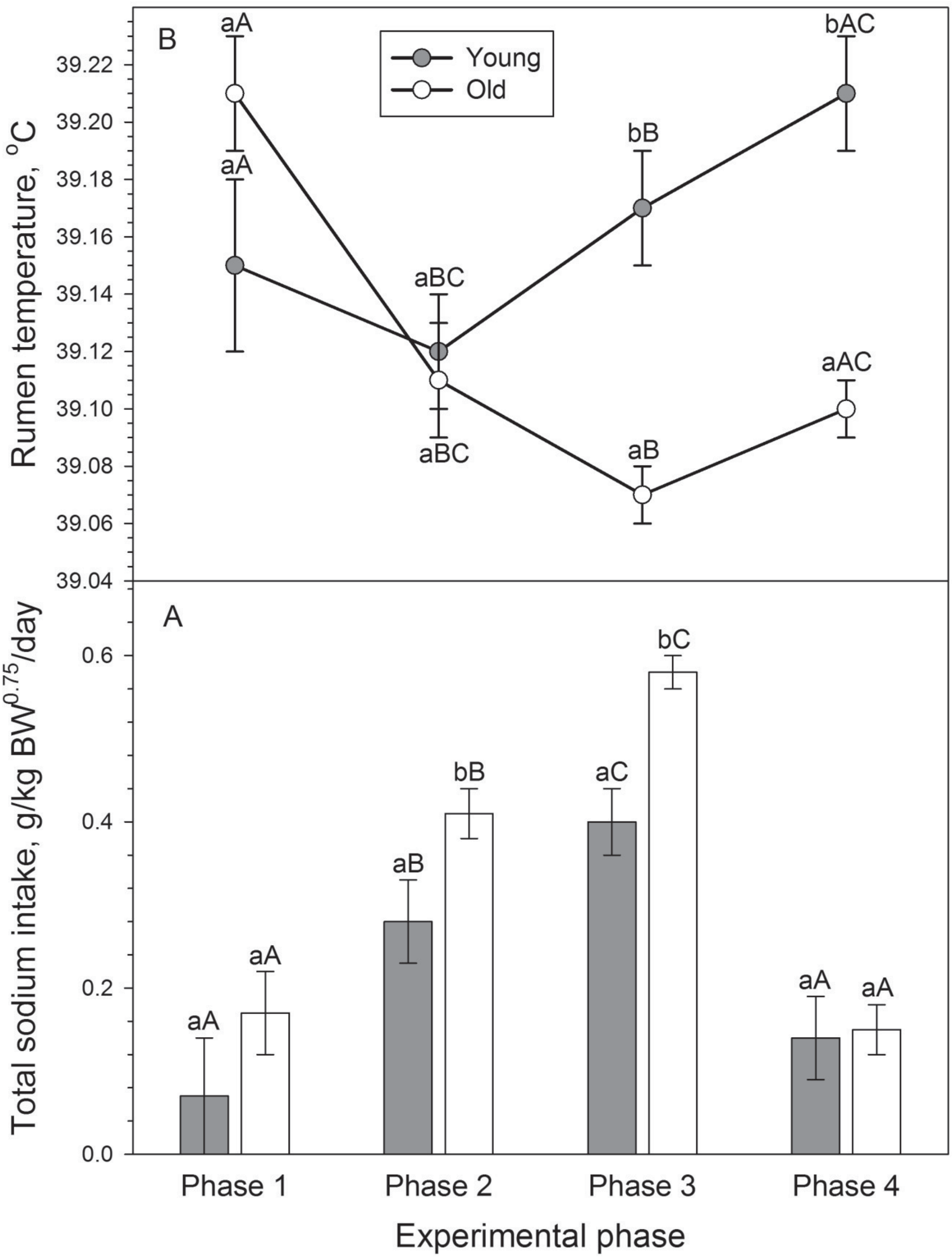

Figure 4.2 Total sodium intake (A) and daily mean rumen temperature (B) in Boer goats during four experimental phases (phase 1=control; phase 2=sensitivity test; phase 3=adaptation and phase 4=sensitivity re-test, see text for details), by age. Grey and white column indicateyoung (N=4) and old (N=8) goats, respectively. Values are presented as means ± SE. [ab] significant differences between ages P < 0.05; [ABC] significant differences between phases P < 0.05.

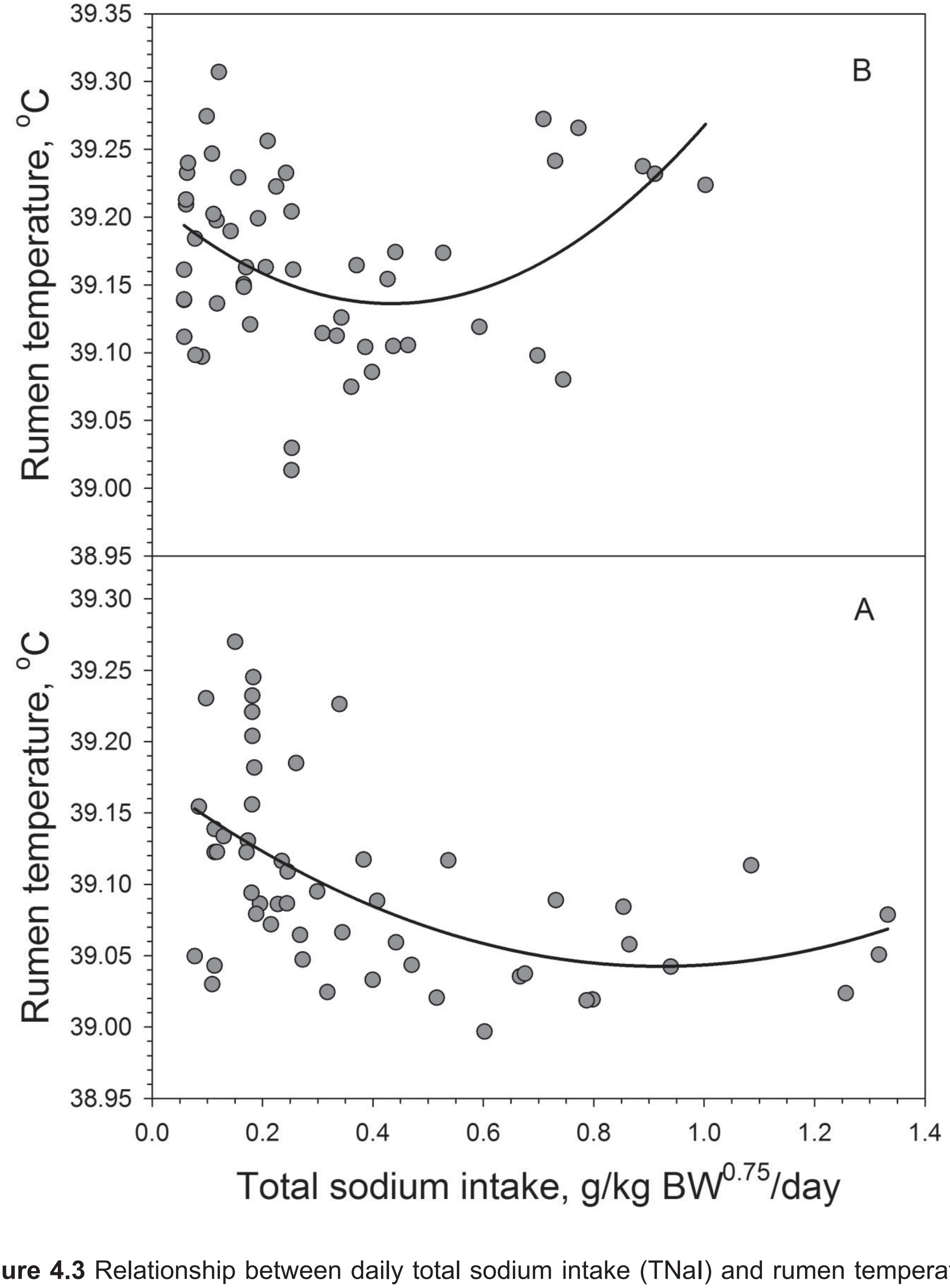

Figure 4.3 Relationship between daily total sodium intake (TNaI) and rumen temperature (T_r) for both old (A: $T_{r,}$ °C = 39.17 - 0.29TNaI + 0.16TNaI^2, $P < 0.001$, $R^2 = 0.27$) and young goats (B: $T_{r,}$ °C = 39.21 - 0.35TNaI + 0.41TNaI^2, $P < 0.01$, $R^2 = 0.17$) during the entire experiment.

Discussion

To our knowledge our study is the first analyzing the effects of a stepwise adaptation to saline water ingestion on blood parameters and rumen temperature in Boer goats. The measured blood metabolites were within the reference range indicating that animals were able to regulate sodium balance without health impairment. Studies in Nguni goats (Mdletshe *et al*., 2017) also support our results.

Body weight and body condition score

Continuous intake of high saline water with concentrations of 2% or more depressed appetite and reduced feed intake and digestibility, therefore resulting in body weight loss in sheep (Peirce, 1957; Masters *et al*., 2005). In our study, the stepwise adaptation to saline drinking water had no effect on body weight. Similar results were found in sheep receiving 1% salt in drinking water (Peirce, 1957). However, the lower body condition score found in our treatment phases could indicate that water retention may have contributed to constant body weight (Masters *et al*., 2005). Similarly, sheep increased their body water content in the intracellular space to excrete excess sodium from the body fluids (El-Sherif and El-Hassanein, 1996; Assad and El-Sherif, 2002).

Blood metabolites

With the exception of urea, all measured blood parameters were within the reference ranges for healthy goats (compiled by Laboklin, 2017; Table 4.2). In all phases, serum urea concentrations were below the reference values of goats given by Jackson and Cockcroft (2002) and Omidi *et al*. (2018), even in the control phase. When based on the lower reference values of 3.6–7.1mmol/l given by Fielder (2018) for goats, urea values in the control phase and phase 4 were in the lower normal range. Urea concentration in serum reflects the balance between urea production and urea elimination via urine. Lower serum urea concentration may indicate insufficient protein intake by feed which is associated with reduced urea production and consequently reduced serum urea concentrations (Higgins, 2016). However, this explanation is not plausible for our study as all goats were fed a sufficient protein rich diet throughout the experiment. On the other hand, total water intake was significantly increased during the treatment phases 2 and 3. Overhydration induces increased glomerular filtration rate, leading to increases in renal urea excretion and decreases in blood urea concentrations (Meintjes and Engelbrecht, 2004). The reduced serum urea concentration during the adaptation phase of our study might be partly explained by this mechanism. Similarly, Eltayeb (2006) found decreasing serum urea concentration in goats when exposed to increasing saline concentrations in drinking water.

No significant changes in serum sodium concentrations were found in this study after prolonged exposure to saline water which is consistent with other studies in deer (Ru *et al.,* 2005), sheep (Peirce, 1959; Potter, 1963) and heifers (Weeth *et al*., 1960) exposed to sodium concentrations in drinking water of 1 to 1.3%. However, sodium concentration in the blood increased in goats (Eltayeb, 2006), sheep (Tomas *et al*.,1973) and heifers (Weeth *et al*., 1960) receiving saline water with more than 1.3% salt. It is noteworthy, that even after 4 weeks of continuous ingestion of saline water, sodium concentrations in the serum of our goats were still in the reference range of goats reported by Jackson and Cockcroft (2002). Our goats might have kept their serum sodium concentration constant by increasing sodium output via urine and faeces as reported in goats (Eltayeb, 2006) and sheep (Tomas *et al*., 1973). In response to consumption of too much salt the body tries to dilute excess salt by increasing water reabsorption in the kidney tubules (Sherwood *et al*., 2005). This mechanism could also explain the constant body weight of our goats despite of their reduced BCS.

In the adaptation phase, when only saline water was offered, serum creatinine levels were within the reference range (Jackson and Cockcroft, 2002), indicating no adverse effects on kidney function due to continuous ingestion of saline water. Interestingly, the highest concentration of serum creatinine was observed in phase 4 (sensitivity re-test). Zoidis and Hodjigeorgiou (2017) likewise found maximum plasma creatinine concentrations in the freshwater period following a salt water ingestion phase (up to 20‰ salt). This observation might be explained by changes in glomerular filtration rate (GFR). Significantly higher GFR and effective renal plasma flow were found in goats receiving higher concentrations of salt which leads to increased filtration load of the waste products such as urea, uric acid and creatinine in the kidneys (Godwin and Williams, 1986; Meintjes and Engelbrecht, 2004). As our animals quickly reduced their water intake when they had access to the choice between fresh and saline water again (phase 4), the GFR was probably decreased and less creatinine was released by the kidneys via urine (Richard *et al*., 2015) as indicated by findings of Zoidis and Hadjigeorgiou (2017). The authors found lower urinary creatinine concentrations in goats consuming fresh water after a four-week period of saline water intake. It is open to question, whether only GFR was associated with the changes of creatinine levels or muscle degeneration related to continuous ingestion of saline water was involved.

In sheep and camels, ALT and AST were significantly affected by salinity (1.35 to 1.45%) of drinking water (Metwally 2001; Assad and El-Sherif, 2002). However, in our study, both enzymes remained constant during the entire experiment and did not exceed the reference range (Khan *et al*., 2016; Omidi *et al*., 2018) suggesting that liver functions were not impaired during the experiment. Similarly, Kattnig *et al*. (1992) found no significant changes of liver enzymes (ALT and AST) in steers after ingestion of water containing 0.23% total dissolved salt.

It is known that potassium and sodium function as paired nutrients that maintain osmotic balance and cell signaling of the body through the Na^+-K^+ pump (Sherwood *et al*., 2005). Magnesium is required to absorb potassium into the cell membrane (Haas, 2013) and the magnesium and potassium concentrations are positively correlated in all the tissues (Richard *et al.,* 1992). This mechanism explains the significant changes of serum potassium and magnesium concentrations in different phases of our study. However, we found a negative relation between serum potassium and magnesium concentrations which was in accordance with a previous study in sheep (Tomas *et al.*, 1973). Higher salt water intake increases urinary volume and an increase in the filtered load can lead to an increase in excretion of electrolytes in sheep (Potter, 1963; Tomas *et al*., 1973). The altered filtration rate may partly explain the reduced level of magnesium concentrations in our goats, but does not explain the slightly increasing levels of potassium. An increase in the concentration of potassium was also evident in sheep (Potter, 1963) and cattle (Weeth *et al*., 1960; Weeth and Haverland, 1961) while Zoidis and Hadjigeorgiou (2017) did not found changes in plasma potassium concentration with increasing salt water intake presumable enabled by the increased urinary potassium output.

Salt and water balance is maintained in the body through the renin-angiotensin-aldosterone system. Renin activates angiotensinogen into angiotensin I which is converted into angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II triggers the secretion of aldosterone which stimulates Na+ reabsorption by the kidneys, and controls arginine vasopressin (AVP) secretion that promotes the water reabsorption in the kidneys (Sherwood *et al*., 2005). High salt intake either by feed or water leads to an increase in both plasma volume and osmolality (Sherwood *et al*, 2005) which exert a negative feedback on the secretion of renin. When drinking water with higher osmolality than the maximum urine osmolality (for example sea water) is ingested salt will remain in the body independent of the kidneys capacity and the body reacts via osmoreceptors that in turns leads to thirst. If only saline water is available salt concentration in the body will increase further and cause antidiuresis that leads to reduction of the intracellular space and an increase of the extracellular and plasma volume. Ultimately, high salt consumption causes a decrease in the aldosterone concentration and the body regulates the excess salt ingestion by reducing Na+ reabsorption and increasing Na+ excretion via urine (Digby *et al*., 2011). Cowley *et al*. (1986) stated that high salt intake by both feed and water does not change arginine vasopressin (AVP) concentration if fresh drinking water is available and osmolality is controlled by increased drinking and renal sodium excretion. In our study, osmolality was even within the reference range when only saline water was available. Moreover, the stable serum glucose concentration during the entire experiment is in agreement with previous studies in sheep (Goodwin and Williams, 1986; Assad and El-Sherif, 2002) and goats (Eltayeb, 2006). The stability of serum glucose level could be related to a decreased insulin level induced by reduced feed intake associated with increased saline water intake (Brinkmann *et al*., 2017; Yousfi and Ben Salem, 2017). Despite the observed lower feed intake in the present study, plasma glucose concentration was not affected, presumably because of higher feed digestibility achieved, or expanded mobilization of body reserves (Silanikove, 1992).

Effect of age

During the adaptation phase of our experiment, when salinity concentration exceeded 0.5%, younger animals exhibited mild signs of physiological disorders such as soft feaces and lower appetite. One young goat aged 1 year and 9 months showed most adverse signs and was excluded from the experiment. The animal was supplied with hay and fresh drinking water only, and completely recovered within 3–4 days without any medication. Similar disorders were observed in adult Merino sheep (aged 4 years; Pierce, 1957) and in rusa deer stags (aged 4 to 4.5 years; Kii and Dryden, 2005) when receiving 1.5% and 0.85% saline water, respectively. Compared to the younger ones, the adult goats used in our study were less sensitive and more resistant to increased salinity concentration which is in agreement with previous studies in goats and sheep (Pierce, 1968; Wilson and

Dudzinski, 1973; Runa *et al*., 2018). Thus, the adaptation of younger animals to saline water needs to be carried out with increased caution in order to avoid health problems. The higher susceptibility to sodium excess can be explained by the fact that growing animals contain a higher percentage of body water (Riek and Gerken, 2010), which may enhance the transport of Na^+ to cell tissues with possible adverse effects. In addition, the fractional water turnover is higher in younger that in older animals (Riek *et al*., 2007).

Rumen temperature

It is known that rumen temperature arises from the balance between heat production and heat loss in the rumen and creates a heat load that is dissipated from the rumen to the body surface by the blood (Beatty *et al.,* 2008a). Across our experiment, the rumen temperature fluctuated within the range of 37.56°C to 39.84°C. Similar values have been reported for goats (Castro-Costa *et al*., 2015), sheep (Beatty *et al*., 2008a) and cattle (Beatty *et al*., 2008b; AlZahal *et al.,* 2011). Earlier results indicate that salt intake is associated with an increased heat production in the rumen and also increases the energy requirement to excrete excess salt (Rumpler and Johnson, 1987; Arieli *et al*., 1989). In particular, the energy consumption by the kidney is commonly associated with the sodium pump (Summers *et al*. 1988).

Our study showed divergent results. While in young goats, rumen temperature significantly increased with prolonged saline water intake as expected, it decreased in adult goats. Increased water intake along with increasing salinity concentration is a physiological response of goats to dilute excess salt and reduce possible metabolic burden of salt loading. Older goats had a higher body mass corrected water intake when only saline water was offered which may partly explain their lower rumen temperature. Apparently, other factors such as feed intake, water intake and environmental conditions (Brod *et al*., 1982; Conrad, 1985; Beatty *et al*., 2006) influence rumen temperature and may mask possible rumen temperature increases due to increased energy metabolism to excrete excess salt. Thus, the higher temperature found in our younger goats might indicate a higher metabolic impact due to salt excretion. However, this remains to be confirmed in further studies based on both, rumen and rectal temperature.

Conclusions

Our study reveals that the stepwise adaptation to saline drinking water applying maximum concentrations between 1.25 and 1.5% was a suitable method. Body physiology was not affected by the high saline water intake and goats were able to regulate salt intake without health impairment. The measured blood parameters remained within reference ranges and indicate remarkable adaptation capacities of Boer goats to saline water without damage to liver and kidney functions. The differences between young and old animals among all phases of the study underline that young animals are less resistant to salt toxicity compared to older ones. However, impacts of continuous long term ingestion of saline water on health status of ruminants cannot be ruled out and need further investigation.

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CHAPTER 5

GENERAL DISCUSSION

GENERAL DISCUSSION

The present studies were conducted to investigate individual preferences and adaptation capacities of goats towards saline drinking water. In our experiments, goats were exposed to different concentrations of salted water. Different choice tests were used to explore the goats' capacities to adjust their choices in regulating excess salt intake (Chapters 2 and 3). Additionally, we evaluated the influence of high saline water intake on body physiology and health (Chapter 4). In the following chapter, the suitability of different choice methods to evaluate sensitivity threshold to salt will be discussed. Further, the responses and adaptation strategies to saline water in different livestock species will be assessed.

Choice Tests

Choice tests are used as an important tool in the study of animal health, behavior and welfare (Fraser and Matthews, 1997; Keskin *et al*., 2004; Boga *et al*., 2009). In general, a choice or preference test is an experimental procedure in which animals get free access to multiple situations which differ in one or more ways. The principle approach is to provide a simultaneous choice of selections and then measure the animals' responses to each (Raffa *et al*., 2002). This method can be used to test the preferences between two contents commonly referred as two-choice test, or multiple contents referred as free-choice test or cafeteria test. Preference tests have been commonly used to determine animals' choices for housing options such as ambient temperature (Morrison et al., 1987), preferred type of bedding (Blom *et al*., 1996), flooring (Hughes and Black, 1973) or housing systems (Dawkins, 1977). Some studies also applied preference tests in ruminants and non-ruminants to assess different preferences for diets (Mackie *et al*., 1978; Kyriazakis and Oldham, 1993) or flavors (Nolte and Provenza, 1991; Robertson *et al*., 2006).

Two choice preference tests were used to investigate the taste responses and thresholds to saline drinking water in sheep (Goatcher and Church, 1970a, b), goats (Bell, 1959), and cattle (Bell and Williams, 1959). The present experimental procedures are partly similar to these previous studies (explained in Chapter 3 and 4). However, in our study, the use of a free choice test as well as a stepwise adaptation technique is a novel approach in assessing the reactions and thresholds to saline drinking water (detailed description in chapter 2 and 3). Compared to a two choice test, the used free choice test (Chapter 2) was more suitable to determine the animals' responses to saline drinking water. This approach was closer to natural conditions as individuals had a range of options to choose, which allows animals to establish their preferences more clearly (Fraser and Matthews, 1997).

It has been proven that in a choice test, animals can self-select a balanced diet to meet their physiological and nutritional requirements (Emmans, 1977; Kyriazakis and Oldham, 1993; Fedele *et al.,* 2002; Yurtseven and Görgülü, 2004) and to avoid diets containing secondary metabolites like tannin (Provenza and Malechek, 1984). This choice may be associated with sensory components (e.g. smell, taste) present in the feed (Arnold, 1966) and the post-ingestive effects, like positive and negative gastrointestinal feedback (Kyriazakis *et al*., 1997). The food flavor also plays an important sensory role in determining the preferences of different foods in ruminants (Provenza, 1995). For example, Robertson *et al*. (2006) noted, when feed pellets treated with different food-flavouring agents representing strawberry, apple, orange, maple, caramel, truffle, garlic and onions, sheep and goats can discriminate the flavoured foods and show preferences for different flavours. Another study by Görgülü *et al*. (1996) found that Awassi lambs attained better growth performance by selecting adequate diets when allowed a free choice among an option of feeds (barley, wheat, bran, cotton seed meal and alfalfa straw). Similarly, during our present study, Boer goats showed a remarkable shift in preferences to saline water in a free choice test (Chapter 2), indicating that goats physiologically can regulate their excess sodium intake from drinking water and manage salt ingestion by timely adjustment in self-selection.

Prior to making final choices animals go through a decision making process guided by their preferences and motivations (Amdam and Hovland, 2011). This adaptive process is advantageous to the animals and supportive to their evolutionary process. The present results revealed that the choice was specific for each individual and was influenced by factors such as age, experience, and availability of contents. The previous experience of animals is important to determine their preferences. Animals may show a temporary attraction or avoidance to unfamiliar objects initially, which may change for longer duration as animals gain experience of the different options (Fraser and Matthews, 1997). For example, Phillips *et al*. (1996) housed sows for three weeks in a preference apparatus where they could choose different types of flooring. Initially the pigs strongly preferred concrete flooring followed by metal and plastic products. Studies on dwarf goats also revealed a remarkable learning capacity to solve complex learning tasks (Langbein *et al*., 2007). Variations in choice with regards to saline water among different experimental weeks of the present study can be explained by the gained experiences or the learning capacity of the animals.

Responses and adaptation strategies of different livestock species to saline water

Sensitivity thresholds to saline water

Irrespective of taste modalities, sensitivity threshold is defined as the minimum detectable concentration, and the recognition threshold is referred to the concentration where the taste can first be recognized (Goatcher and Church, 1970c). Investigation on taste responses can be performed based on electrophysiology of nerve, physiological responses and animal behavior (Goatcher and Church, 1970c). In our study, we focused on behavioural and physiological responses to determine sensitivity thresholds to saline water.

The results of this thesis that show responses and sensitivity thresholds to saline water in goats varied with the application of choice tests. The experimental findings (Chapter 2) confirmed that goats can tolerate higher concentration of saline water when they have access to fresh water *ad libitum.* Goats appeared to be able to tolerate higher salt levels in water (about 3%) when adequate green herbage and shade was provided (McGregor 2004). Some additional factors also play an important role in individual tolerance to various degrees of salt load in drinking water or in feed, e.g., salt ingestion period (Peirce 1957 and 1959), age (Wilson and Dudzinski, 1973), environmental condition (Weeth and Haverland, 1961), physiological adaptation, thermal stress, and even foetal programming (Digby *et al*., 2011).

The differences in saline water intake before and after the habituation period of the present study (Chapter 3) confirmed that there was a change in sensitivity responses of goats towards salted water after habituation. However, prolonged higher intake of sodium chloride did not lead to a higher salt acceptance as we expected from this study (Chapter 3). During the stepwise adaptation phase of the study (Chapter 3), the preference of goats to saline water was indifferent until 0.5%, followed by strong rejection for higher concentrations of saline water, which may be related to animals’ aversive behavioral responses (Lindemann, 2001; Chandrashekar *et al*., 2010). Strong aversion was also reported in gulls when they were supplied hypertonic (0.7%) salt solutions (Harriman, 1967). Goats' choices for lower concentrations during the adaptation period (Chapter 3) indicate that they are capable to regulate their sodium intake even when they had only a single option of choosing drinking water containing different salt concentrations.

Our results (Chapter 2 and 4) also confirm that sensitivity responses of goats to saline drinking water vary with their age and are in agreement with earlier reports in goats and sheep (Peirce, 1968; Wilson and Dudzinski, 1973). The lowered intake of total sodium and saline water at each offered concentration by young animals during the experimental phases of the present study indicates that young goats are more sensitive to saline water compared to older ones. In a free choice system, young goats showed a higher sensitivity to saline water with a lower rejection threshold at 0.75% salt concentration (Chapter 2). This observation may be explained by the high learning capacity of young goats to find fresh water in a choice situation and may be also associated with higher sensitivity of their salt taste receptors as reported for younger animals (Grzegorczyk *et al*., 1979) and children (Fukunaga *et al*., 2005). Compared to older ones, younger animals contain a higher percentage of body water (Riek and Gerken, 2010), which enhances intra-cellular transport of sodium, thus activating respective ionic regulation mechanisms even at lower salt ingestion.

Comparison with other livestock species

There are big differences among different species with regard to tolerance of drinking saline water. A number of studies (Abou Hussien *et al*., 1994; Marai *et al*., 1995; Assad *et al*., 1997; McGregor, 2004; Ru *et al*., 2005) indicate the tolerance order of sodium chloride in drinking water as follows: camels > goats = sheep > cattle > deer > poultry.

Our findings underline that goats differ in their salt tolerance capacity from other livestock species. Nassar and Moussa (1981) reported that the acceptance thresholds of goats for sodium chloride in drinking water were 1.5% to 3.2%, while 1.1% is considered as safe concentration (McGregor, 2004). Sheep can tolerate 1.5% (Wilson, 1978) or even 2% salt in water (Wilson, 1966), but recommended upper limit of the safe concentration is 1.3% (Peirce, 1957 and 1959). Water containing more than 2% salt was detrimental to the production and survival of sheep (Wilson, 1978). However, it is also reported that Merino sheep became partly intoxicated by water containing 1.3% salt (Peirce, 1968). Goats appear to be more adapted to higher salt loads by having better sodium pumps than sheep, for instance, Turkana goats tolerated 1.5% of salt in drinking water (Macfarlane, 1982). The tolerance levels of saline water in growing cattle lie between 1–2% (Weeth and Haverland, 1961). Studies showed that the general appearance and behavior of heifers receiving 1% salted water did not differ from those receiving tap water (Weeth *et al*., 1960). The salt tolerance in drinking water for fallow deer and red deer is lower (1.2% and 0.8%, respectively; Ru *et al*., 2005) than that of cattle, sheep and goats. However, when comparing species the different approaches used to evaluate their salt tolerances in drinking water need to be considered.

Camels demonstrated a remarkable high salt tolerance and tolerated more than 1.5% salt in water (Abou El-Nasr *et al.,* 1988), compared to sheep and goats (Abou Hussien *et al*., 1994; Assad and El-Sherif, 2002). Abou Hussien *et al*. (1994) reported that camels were less affected by increased salinity than goats and sheep as they showed low water intake and reduced urinary water loss. However, it is necessary to consider that the salt load controlling mechanisms of sheep and goats are different to that of camels (Abou Hussien *et al*., 1994). Sheep and goats reduce high salt load by increased glomerular filtration rate and renal salt excretion through urine (Potter, 1968; Dunson, 1974), whereas camels protect themselves from salt stress by reducing water consumption per unit of body size (Abou Hussien *et al*., 1994; Assad and El-Sherif, 2002).

Compared to ruminants, horses are quite variable in their responses to particular chemical tests (Randall *et al.,* 1978). The responses of horses to saline water were indifferent up to the concentration of 0.63%, afterwards they started to avoid salt solutions and clearly rejected salt concentrations of 1.25–2.5% (Randall *et al.,* 1978). Among all livestock species, pigs are most sensitive to increased water salinity (Ellis, 1942; Thompson, 2018) and 1.5% salinity concentration in drinking water was toxic for pigs (Heller, 1933).

Total water intake increases with increased water salinity and is related to the amount of salt ingested irrespective of source of ingestion (Peirce, 1957; Wilson, 1966; Wilson and Dudzinski, 1973, Ru *et al*., 2004; Kii and Dryden, 2005). Our study results confirm this adaptive strategy (Chapter 2 and 3). A comparative study on different species by Abou Hussien *et al*. (1994) shows that increasing water salinity up to 1.7% increased total water intake in camels, goats and sheep by 130 $ml/kg^{0.82}$, 376 $ml/kg^{0.82}$ and 500 $ml/kg^{0.82}$, respectively. Higher water intake is also associated with greater urinary water loss and minor changes in faecal and insensible water loss through sweating and breathing (Abou Hussien *et al*., 1994).

Health indicators

The measured respiration rate, rumen temperature, and different blood parameters (physiological health indicators) of goats in the present study (Chapter 4) did not vary largely over the course of the experiment. The changes in respiration rate were mainly attributed to changes in environmental temperature during the experiment. Our study found decreased respiration rates along with increased water salinity, indicating that saline water intake for a shorter period did not cause harm to the goats, which is also in agreement with the findings of Mdletshe *et al*. (2017) in Nguni goats.

The measurement of body temperature through the rectum (rectal body temperature) is adversive to the animals and also the vaginal introduction of temperature loggers makes animals more vulnerable to vaginal mucosal inflammation. Considering these difficulties, we used rumen boli with miniature data loggers (detailed description in Chapter 4) which is a more suitable and animal friendly method for measuring rumen temperature (T_r), as an approximation of core body temperature. In previous studies, rumen boli were successfully applied in goats (Castro-Costa *et al*., 2015), sheep (Singer *et al*., 2010), cattle (Lin *et al*., 2010; Singer *et al.*, 2010; AlZahal *et al*., 2011) and Alpine ibex (Singer *et al*., 2010) to monitor T_r and rumen pH. Continuous data recording using rumen boli allows monitoring intra-ruminal changes caused by different feeding and drinking regimes as well as different aspects of animal health.

According to Parsons (2003), core body temperature refers to the inner body temperature. The greater part of the body heat (about 60%) is generated from the main organs such as heart, brain, liver and kidneys (Nadine *et al*., 2014). In ruminants, heat is also generated in the rumen during microbial fermentation that creates heat load and increases T_r (Beatty *et al.,* 2008a). T_r exceeds rectal temperature by about 2^{o}C and could be considered as core temperature according to Dale *et al.* (1954). Beatty *et al.* (2008a) stated that T_r and core body temperature remained constant in cattle despite changes in heat load, water and feed intake. In contrast, T_r were consistently higher by 0.45 – 0.75^{o}C than core body temperature in sheep as reported by Beatty *et al*. (2008b).

Until now, only changes in rectal temperature have been used to assess physiological effects of saline drinking water in goats. In our study, for the first time, we used T_r as an indicator of physiological changes induced by salt intake through drinking water. El Gawad (1997) reported that rectal temperature of goats was slightly increased by water containing 0.8% of salt. On the contrary, Mdletshe *et al*. (2017) found that saline water consumption (0.55% and 1.1%) did not influence rectal temperature of goats, but when goats were offered saline (1.2%) water only, rectal temperature increased significantly (Eltayeb, 2006). We observed decreased T_r during the adaptation phase of our study (Chapter 4) due to increased water intake. Higher water intake along with increased water salinity allows greater water turnover. Thus, the body regulates excess salt balance through increased urinary salt excretion. This in turn reduces the possible metabolic burden of salt loading in the rumen.

In the present study (Chapter 4), it was found that there was no increase of serum sodium concentration in goats even after continuous ingestion of saline water for four weeks. Values were still within the reference range (135–156 mmol/L) for goats as reported by Jackson and Cockcroft (2002). However, this finding differs from previous reported observations in goats (Eltayeb, 2006), sheep (Tomas *et al*., 1973) and heifers (Weeth *et al*., 1960). Sodium functions with potassium as pair nutrients for maintaining osmotic balance of the body through the Na^+-K^+ pump (Sherwood *et al*., 2005). There is a correlation between magnesium and potassium concentrations as magnesium is required to absorb potassium into the cell membrane (Richard *et al.,* 1992). This mechanism explains the concomitant changes of serum potassium and magnesium concentrations in our study (Chapter 4), similar to earlier findings in sheep (Potter, 1963) and cattle (Weeth *et al.*, 1960; Weeth and Haverland, 1961).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes are commonly used markers for hepatic diseases. They are usually elevated with the inflammation or injury of hepatocytes. These enzymes may also be increased due to disorders of other organs such as cardiovascular and musculoskeletal diseases (Giannini *et al.,* 2005). As both ALT and AST concentrations remained similar across the experiment, it is suggested that liver functions were not impaired and the nutritional status of the goats was not affected by high saline water intake. This is, however, in contrast to other studies in sheep and camel receiving drinking water containing 1.35% and 1.45% salt, respectively (Metwally, 2001; Assad and El-Sherif, 2002).

During the adaptation phase, when only saline water was offered (Chapter 4), the serum creatinine and urea concentrations were also not significantly changed, indicating that the kidney function was not affected by continuous ingestion of saline water. The observed higher concentration of serum creatinine in the sensitivity re-test might be due to decreases in glomerular filtration rate (GFR) and a lower release of creatinine by the kidneys through urine (Richard *et al*., 2015).

In order to prevent health problems due to saline water intake, suitable physiological parameters should be monitored continuously to detect changes in physiological health and well-being of the animals. Body temperature (core body and surface temperature), respiration rate, heart rate, blood pressure, body electrolytes, metabolic enzymes and hormones related to salt and water balance are the most important health indicators in this context. As the gastrointestinal tract and the central nervous system (CNS) are mainly impaired by salt intoxication, the adverse effects are recognized by lower appetite, soft faeces, muscle tremor and aggressive behaviour (Ellis, 1942; Peirce, 1957; Thompson, 2018). Health impairment due to excess salt could be overcome by removing the source of salt intake (feed or water) immediately and providing fresh water at frequent intervals to minimize salt load in the body.

Conclusions

From the results of the present study, it is concluded that Boer goats are capable of distinguishing between fresh and saline water in different designs of choice tests. They adapted to varying concentrations of saline water by making quick changes in their drinking choices as well as avoiding excessive salt ingestion through timely adjustments in their selection. This might have been caused by properties of the water that stimulate the senses (e.g. smell and taste). The higher sensitivity to saline water showed by young goats suggests at least some involvement of intake regulation via taste sensitivity. Interestingly, it was observed that goats had developed a stable preference in a free choice test after only one week of exposure and their acceptance of saline water was adjusted over time. Results also suggest that goats are able to balance their sodium intake from different sources (feed, water and mineral supplement) according to their nutritional requirements. However, these observations warrant further research to answer the question whether salt intake is regulated only by innate regulation mechanisms of the animal or also through involvement of other genetic factors.

In a two choice test, when offered the choice between fresh water and different concentrations of saline water, goats tolerated 1% saline water without any health impairment, while the most preferred concentration was 0.25–0.5% when only saline water was provided. Interestingly, goats showed a lower rejection threshold towards saline water after the adaptation period as opposed to our expectation. Accordingly, choice discrimination and rejection thresholds were not constant but depend on the individual sensitivity to salt, learning capacity, total sodium balance, and age of the animal.

The stepwise adaptation to saline drinking water is an effective method to habituate the exposed animals to increasing water salinity. However, the applied stepwise habituation for a shorter period (4 weeks) did not demonstrate an overall higher tolerance of saline water. Thus, more detailed investigation is required to firstly, determine the goats ability to tolerate higher salinity for longer periods and secondly, to study the factors involving their adaptation capacities to saline water under tropical conditions (>30°C).

Our results showed that the physiological parameters (blood metabolites, body temperature and respiration rate) remained within the normal values. This suggests that goats have a good adaptation capacity towards saline water and can regulate salt intake without health impairment for short durations. It would be interesting to investigate long term impacts of water salinity on health status of small ruminants in arid and coastal areas where increased water salinity potentially threatens local livestock production. Further physiological and behavioral parameters, e.g. heart rate, blood hormones related to salt regulation or drinking behavior should be determined. Comparative studies regarding the preferences and adaptation capacities between camelids, ruminants and monogastric animals (e.g., pigs, horses) reared under free ranging housing systems would be of particular interest.

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CURRICULUM VITAE

Surname:	Runa
First name:	Rukhsana Amin
Date and place of birth:	02 April 1982, Netrakona, Bangladesh
Sex:	Female
Status:	Married and have a son (Raed Mahbeer)
Nationality:	Bangladeshi
Address:	Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh E-mail: ramin.dso@gmail.com
Education:	
2014 – 2018	Doctoral student, Department of Animal Sciences, Ecology of Livestock Production, Georg-August University of Goettingen, Germany
2006 – 2008	Master Degree, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Bangladesh
2001 – 2005	Bachelor Degree, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh
1997 – 1999	Higher Secondary, Government Girls' College, Khunla, Bangladesh
1992 – 1997	Secondary School, Rajghat Secondary Girls' School, Jessore, Bangladesh
Work Experience:	
2009 – to date	Academic staff, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Bangladesh
2010 – 2011	Veterinarian (Surgery), Veterinary Teaching Hospital, Bangladesh Agricultural University, Bangladesh
Grants and Awards:	
2014	DAAD Scholarship
2011	University Gold Medal Award and University Prize, Bangladesh Agricultural University, Bangladesh
2009	Professor Dr. Abdus Salam Mian Trust Gold Medal Award, Bangladesh Agricultural University, Bangladesh
2006	Prime Minister Gold Medal Award, University Grants Commission, Bangladesh

Göttingen, August 2018