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Effect of various salt concentrations on the ruminal parameters of goats

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Abstract

The objective of this study was to evaluate the effects of various concentrations of sodium chloride (NaCl), magnesium chloride ($MgCl_2$), and calcium chloride ($CaCl_2$) on the growth and in vitro fermentation of cellulolytic, glycolytic, and amylolytic microorganisms from the rumen of a goat. Six concentrations of each salt were tested separately, namely 0 mg/dL, 100 mg/dL, 200 mg/dL, 400 mg/dL, 800 mg/dL, and 1600 mg/dL in the culture medium. The experiments were conducted in a completely randomized design, in a 6 x 3 factorial arrangement of salt concentration and substrate (starch, cellulose, and glucose) with three replications of each treatment combination. Concentrations of microbial protein, ammonia (NH_3-N), and volatile fatty acids (acetate, propionate, and butyrate) were measured. A quadratic effect of $CaCl_2$ concentration on the production of microbial protein was observed in the cellulose medium. The effect of $MgCl_2$ on NH_3-N production in the cellulose medium decreased linearly. Propionate concentration decreased linearly with increasing levels of NaCl and $MgCl_2$ in the media containing starch. There was a decreasing linear effect of $MgCl_2$ on the concentration of butyrate in the media containing glucose. In conclusion, concentrations of NaCl and $CaCl_2$ up to 1,600 mg/dL did not affect the microbial activity of starch, cellulose, and glucose-fermenting organisms. However, the microbial activity of starch-fermenting microbes was inhibited at salt concentrations above 800 mg/dL. Thus, brackish water could be used by goats in semiarid regions, but its use should be managed carefully so that it does not have a negative impact on rumen microbial populations.

Keywords: cellulose, glucose, saline water, starch, ruminants
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Introduction

The supply of water has continually become increasingly scarce, especially in arid and semi-arid regions (Araújo *et al*., 2010). Brackish water represents a risk to health for animals and people. However, it is necessary to identify the tolerance of ruminants to minerals in water in some animal production systems in regions with seasonal rainfall and no access to safe drinking water (El-Keblawy & Al-Shamsi, 2008).

Ruminants can survive for up to three consecutive days without consuming water, but with some decrease in their productive performance (Aganga *et al*., 1990). This is possible only if the ruminant and the rumen microorganisms are adapted to these changes in osmolarity of the body's fluids. As a result, the microorganisms in the rumen tend to be tolerant to high concentrations of chloride (Cl^-), magnesium (Mg^{2+}), calcium (Ca^{2+}), and sodium (Na^+) ions in the rumen fluid.

Animal tolerance to salinity varies according to species, age, water requirements, and physiological conditions (NRC, 2007). Among ruminant animals, goats and sheep are more tolerant of saline water than cattle, in part because of their size (Albuquerque, 2012). High levels of salinity may inhibit water consumption and consequently food intake (Balsalobre *et al*., 2007). The consumption of brackish water can reduce animal productivity as a consequence of the effects on rumen microorganisms and result in economic losses for the producer.

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Brackish waters are characterized by high levels of Cl^-, Mg^{2+}, Ca^{2+} and Na^+ (Manera *et al*., 2016), and may be important sources of minerals for ruminants. Nevertheless, high levels of these minerals may affect the activity of rumen microorganisms negatively. Studies by Yousfi *et al.* (2017) observed that incremental additions of salt to the water did not alter the number of protozoa in sheep rumen. However, little is known about this effect in goats. Thus, information from the literature is incomplete, but is especially important in regions where people and animals compete for scarce drinking water. Therefore, the present study was designed to evaluate the effects of differing concentrations of NaCl, $MgCl_2$ and $CaCl_2$ on the growth and fermentation of cellulolytic, glycolytic, and amylolytic microorganisms in rumen fluid collected from goats.

Material and Methods

The study was undertaken in accordance with the technical norms of biosafety and ethics, and on approval by the Ethics Committee in the Use of Animals of the Centre for Biotechnology (CBiotec) of the Federal University of Paraíba (UFPB) (document 022/2012). In vitro trials were carried out at the Laboratory of Forage Crops at the Department of Animal Science of UFPB, Areia, Paraíba, Brazil, between October 2013 and August 2014. The experiment involved three rumen-cannulated goats that were housed at the Unit for Research with Large Ruminants, which is maintained by the Department of Animal Science of UFPB.

The individual effects of three salts (NaCl, $MgCl_2$, and $CaCl_2$) were evaluated at differing concentrations in the culture medium. These six levels of the salts were evaluated, namely 0, 100, 200, 400, 800, and 1600 mg/dL. The experiments were conducted as a completely randomized design in a 6 × 3 factorial arrangement consisting of six levels of salt and three substrates (starch, cellulose, and glucose), with three replicates.

Rumen fluid was collected from the goats four hours after feeding to obtain inoculant that contained an active microbial population. At the laboratory, the fluid was saturated with carbon dioxide, and left to stand at 39 °C. After the formation of liquid interfaces, the intermediate fluid was collected and centrifuged at 500 × g for 10 minutes (Oliveira *et al.*, 2012). The supernatant was discarded. The residue (pellet) from centrifugation containing predominant bacteria in the rumen fluid was re-suspended in McDougall's buffer (9.80 g $NaHCO_3$; 4.65 g $Na_2HPO_4*2H_2O$; 0.57 g KCl; 0.12 g $MgSO_4*7H_2O$; and 0.04 g $CaCl_2$ dissolved in 1000 mL of distilled water).

Incubation bottles were filled with 16 mL McDougall's buffer and 4 mL inoculant, 150 mg substrate, and the requisite amount of a salt. Starch, cellulose, and glucose were used as substrates for the rumen microorganisms. Each bottle was saturated with carbon dioxide to maintain a pH between 6.00 and 7.00, closed with a special lid, and sealed with tape to prevent contact with the incubation medium. They were then incubated at 39 °C in an agitated (35 rpm) water bath for 48 hours. A sample was obtained from each bottle at 0, 6, 12, 24 and 48 hours of incubation with a syringe that was inserted through the lid of the bottle to prevent oxygen from entering. The pH was measured with a digital potentiometer at incubation times of 0 and 48 hours. At each sampling time, a 2.0 mL sample was collected from the culture medium of each experimental unit and placed in an Eppendorf® tube, which was then centrifuged in a microcentrifuge at 1,200 × g for 10 min. The supernatant was frozen for further analyses of NH_3-N and volatile fatty acids (VFA). The pellet, which contained the predominant microorganisms from the rumen fluid, was re-suspended in 0.9% saline solution, homogenized, and re-centrifuged at 1200 × g for 10 min and the supernatant was discarded. The pellet formed during the last centrifugation was re-suspended in 0.9% saline solution and frozen at -10 °C for later analysis of microbial protein.

For the analysis of VFA, 500 μL supernatant was collected, 500 μL 25% metaphosphoric acid was added, and the mixture was poured into Eppendorf tubes. The material was analysed in a high-performance liquid chromatograph (SPD-10A VP, Shimadzu Corp., Kyoto, Japan), which was coupled to an ultraviolet detector, at a wavelength of 210 nm. Ammonia nitrogen concentration was determined by a colorimetric method, and protein concentration was quantified by Bradford's (1976) method. Electric conductivity (dS/m) was measured at zero time and at 48 hours of incubation, using a conductivity meter.

The experiment was conducted as a completely randomized 6 x 3 factorial arrangement of treatments with three replications. The results from each substrate were analysed separately. Thus, the linear model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where: Yij = dependent variable;
μ = overall mean;
αi = fixed effect of ith levels of a salt; and
εij = error associated with observation Yij was used to analyse the data.

A similar regression model was used in which the levels of salt were regarded as a continuous variate with the linear and quadratic effects being tested. All statistical analyses were performed using the GLM

procedure of the SAS® System for Windows 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Effects were declared significant at P =0.05.

Results and Discussion

The linear effects (P <0.05) of NaCl, $MgCl_2$ and $CaCl_2$ showed that conductivity of the medium increased with the level of each salt (Table 1). Thus, media with a higher concentration of salt invariably had greater conductivity. This was expected (Santos, 2012). Cellulose-based media had higher electrical conductivity with 1600 mg/dL of each salt compared with other substrates. Media that contained starch had the lowest values of electrical conductivity with 1600 mg/dL of $MgCl_2$ and $CaCl_2$ in the media.

Table 1 Values of electrical conductivity (dS/m) of the in vitro culture media with different concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)							Significance	
Salt	Substrate	0	100	200	400	800	1600	SE	Linear	Quadratic
NaCl	Starch	11.06	11.80	13.14	14.71	22.47	34.48	0.29	<.0001	<.0001
	Cellulose	10.42	11.34	12.78	14.39	22.85	35.68	0.29	<.0001	<.0001
	Glucose	11.28	11.87	13.19	14.68	22.75	34.26	0.29	<.0001	<.0001
$MgCl_2$	Starch	15.68	15.33	15.56	16.04	17.22	20.52	0.56	<.0001	0.0191
	Cellulose	19.58	18.85	19.02	19.12	21.55	25.55	0.56	<.0001	0.0006
	Glucose	15.80	16.11	16.07	16.54	17.18	22.67	0.56	<.0001	<.0001
$CaCl_2$	Starch	16.08	15.71	16.20	16.62	17.56	23.72	0.47	<.0001	<.0001
	Cellulose	19.56	18.76	19.48	21.41	23.18	28.03	0.47	<.0001	0.3159
	Glucose	16.38	16.50	16.96	17.00	18.06	25.35	0.47	<.0001	<.0001

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

Sodium chloride did not affect the growth of amylolytic microorganisms (Table 2). The $MgCl_2$ and $CaCl_2$ concentrations had an increasing linear effect (P <0.05) on the microbial protein concentration when the substrate was starch. There was no effect of NaCl or $MgCl_2$ concentration on microbial protein in the medium containing cellulose. The quadratic effect of $CaCl_2$ on the growth of cellulolytic microorganisms was significant ,with an estimated peak at a concentration of 751 mg/dL of this salt. There were no effects of NaCl and $MgCl_2$ on the concentration of microbial protein in the medium with glucose. The quadratic effect of $CaCl_2$ concentration on microbial protein production was significant in the glucose-based medium with an estimated peak at a $CaCl_2$ concentration of 550 mg/dL. However, this result might be entirely because of the markedly depressed quantity of microbial protein in the medium that contained 800 mg/dL of the salt.

Ingestion of water with up to 100 mg/dl of NaCl and $MgCl_2$ probably did not inhibit the growth of rumen microbes, whether the diet was rich in fibre or in non-fibre carbohydrates. However, high concentrations of $CaCl_2$ did inhibit microbial growth. Costa *et al.* (2019) observed that the growth of microorganisms in the starch-based medium was not affected by salt concentrations up to 1600 mg/dL. This finding was interpreted to show that amylolytic microorganisms are more tolerant to high levels of salinity.

The growth rate of ruminal microorganisms may be limited by several factors, including salt concentrations in the media. Protein synthesis appears to be the most relevant factor. Different substrates may require different metabolic pathways (enzyme, transport protein, etc.) and a substantial quantity of amino acids can be directed to this metabolic activity (Argolô, 2007).

NaCl and $MgCl_2$ levels did not affect NH_3-N concentrations in the medium containing starch (Table 3). This demonstrates that in an environment that is favourable to the growth of amylolytic bacteria, concentrations of NaCl up to 1600 mg/dL do not impede nitrogen metabolism. However, in a starch-based medium, escalating levels of $CaCl_2$ (P <0.05) increased the production of NH_3-N linearly over 48 hours of incubation. This increasing accumulation of NH_3-N in the medium containing starch was a consequence of the observed lower microbial protein synthesis, because of the inhibition of the microorganisms. The concentration of NH_3-N is thus a consequence of the balance between the production and use of microbial protein by microorganisms, according to the available energy. Likewise, the lower concentration of NH_3-N in

the medium with glucose was because of its use for growth by various bacterial species. An increasing linear effect of NaCl and a decreasing linear effect of $MgCl_2$ concentration on NH_3-N were also observed. In the cellulose-based media, in which 1600 mg/dL of $MgCl_2$ produced the lowest level of NH3-N. The levels of $CaCl_2$ did not affect the concentration of NH_3-N that was produced in this medium. Likewise, there were no effects of NaCl and $MgCl_2$ salts on NH_3-N concentration in the medium containing glucose. However, there was a quadratic effect of $CaCl_2$ on NH_3-N concentration in the presence of glucose.

Table 2 Microbial protein produced (mg/dL) during 48 hours of in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	1423.2	1372.1	1440.4	1423.2	1705.4	1493.8	228.8	0.339	0.212
	Cellulose	1346.0	1804.3	1708.2	1549.3	1447.1	1310.4	228.8	0.064	0.553
	Glucose	1383.8	1717.1	1687.1	1911.0	1666.6	1478.8	228.8	0.527	0.035
$MgCl_2$	Starch	933.2	1042.0	1093.6	1075.4	1096.3	1130.5	122.3	0.037	0.379
	Cellulose	1031.4	1048.8	1030.9	1008.8	1122.5	1144.8	122.3	0.124	0.382
	Glucose	1267.4	1162.5	1133.6	1190.9	1203.9	1214.5	122.3	0.930	0.946
$CaCl_2$	Starch	1416.2	1418.4	2098.4	1756.2	1825.1	951.8	263.3	0.008	0.004
	Cellulose	1789.6	1658.4	1876.2	1327.3	1916.2	1169.6	263.3	0.175	0.039
	Glucose	2020.7	1802.9	1896.2	1854.0	1111.8	2118.4	263.3	0.005	0.009

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

Table 3 Ammonia nitrogen produced (mM) during 48 hours in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	10.87	11.84	10.68	11.65	11.73	12.42	3.45	0.5797	0.9906
	Cellulose	9.54	10.85	9.05	8.97	11.12	15.71	3.45	0.0155	0.3206
	Glucose	13.22	10.00	9.40	6.97	10.02	12.33	3.45	0.6092	0.0792
$MgCl_2$	Starch	3.14	2.80	1.48	2.88	1.61	0.78	2.94	0.3212	0.9495
	Cellulose	15.70	19.80	10.13	5.82	2.73	4.35	2.94	<.0001	<.0001
	Glucose	6.17	1.98	1.88	1.96	1.34	1.16	2.94	0.1696	0.2449
$CaCl_2$	Starch	2.61	4.63	4.04	4.06	10.80	7.29	2.16	0.0089	0.0277
	Cellulose	1.97	4.15	29.57	7.81	6.51	10.74	2.16	0.8321	0.2326
	Glucose	19.09	14.26	12.08	6.24	12.64	9.86	2.16	0.0073	0.0073

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

The ingestion of saline water that contains high levels of $CaCl_2$, together with diets composed of soluble carbohydrates, could affect the production of NH_3-N. On the other hand, diets containing fibrous carbohydrates may interfere with NH_3-N production when animals consume water with high levels of $MgCl_2$. Thus, for fattening sheep that received high-concentrate diets, Mehrez *et al.* (1977) suggested 23 mg/100 mL ruminal fluid as the minimum concentration of NH_3-N to obtain a maximum microbial growth rate. Valtorta *et al.* (2008) found no effect of salinity on ruminal bacteria, protozoa, pH, NH_3-N, and VFA in dairy cows, illustrating the rumen buffering capacity, probably because of effects of the substrate.

The effect of NaCl concentration on acetate production by rumen microbes in the medium containing starch was curvilinear (Table 4) with a maximum at 210 mg/dL of NaCl. Similarly, Alves *et al.* (2017) observed a linear reduction in digestibility of neutral detergent fibre when cattle consumed water with a high content of NaCl. The levels of $MgCl_2$ and $CaCl_2$ in the media also produced linear decreases in the concentration of acetate. There was no observed effect of NaCl on acetate production when the substrate was cellulose. However, there was a quadratic effect of $MgCl_2$ concentration on acetate production with the cellulose-based medium. Estimated microbial production of acetate was minimized with 645 mg/dL of $MgCl_2$ in the cellulose-based medium. The effect of $CaCl_2$ levels in the medium containing cellulose on the acetate production was linear and decreasing with increasing levels of $CaCl_2$. In the media containing NaCl and $MgCl_2$ and the starch substrate the microbes produced more acetate than in media containing cellulose and glucose.

Table 4 Acetate produced (mM) during 48 hours in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	33.43	41.88	41.27	40.84	41.96	25.76	4.98	0.0022	0.0011
	Cellulose	16.21	12.84	10.95	10.81	10.78	14.38	4.98	0.9635	0.1386
	Glucose	28.89	20.73	19.79	30.75	28.62	26.59	4.98	0.3723	0.3341
$MgCl_2$	Starch	33.41	33.58	31.20	34.34	34.04	23.60	3.54	0.0009	0.0260
	Cellulose	21.94	20.37	17.13	17.30	15.49	20.33	3.54	0.7567	0.0159
	Glucose	24.75	26.34	27.91	26.68	23.96	23.25	3.54	0.1832	0.7671
$CaCl_2$	Starch	25.35	24.37	24.80	26.43	23.56	17.97	4.04	0.0148	0.2977
	Cellulose	17.39	18.27	16.90	16.39	18.10	26.45	4.04	0.0032	0.1061
	Glucose	33.61	36.55	32.96	27.72	23.41	25.77	4.04	0.0007	0.0107

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

In the medium containing starch, increased NaCl, $MgCl_2$, and $CaCl_2$ concentrations decreased microbial production of propionate linearly (Table 5). Thus, these salts inhibit the use of propionate pathways by ruminal starch microorganisms. There was a quadratic effect of $CaCl_2$ levels in the medium containing starch, which affected the concentration of propionate. It was estimated that the highest propionate level was produced with 720 mg/dL of $CaCl_2$ (23.01mM) in the media. Further, there were no effects of NaCl concentration on propionate production in the media containing cellulose and glucose as substrate. Similarly, the level of $CaCl_2$ did not affect propionate production when the medium contained glucose. However, there was an increasing linear behaviour of this salt on the propionate production in the cellulose-based medium, which invariably yielded less propionate. Microorganisms that use beta-glucose as an energy source generally use the acetate pathway instead of propionate acetate for nicotinamide adenine dinucleotide (NAD) re-oxidation, under suitable pH conditions (Moss *et al*., 2000). Thus, a lower propionate concentration was expected when the medium contained cellulose compared with media that contained starch and glucose.

Butyrate production decreased linearly as a function of the concentration of NaCl, $MgCl_2$, and $CaCl_2$ in the medium containing starch (Table 6). However, there was no effect of the level of NaCl, $MgCl_2$, and $CaCl_2$ on microbial production of butyrate in the cellulose-based medium. This demonstrates that cellulose fermenting microorganisms and butyrate producers are not sensitive to salt concentrations up to 1600 mg/dL. Rumen microorganisms that use the butyrate pathway to re-oxidize nicotinamide-adenine dinucleotide (reduced) generally do not have enzymes capable of re-oxidizing the reduced form of NAD by other pathways (Moss *et al*., 2000). Thus, the decreasing linear effects (P <0.05) of NaCl, $MgCl_2$, and $CaCl_2$ concentrations in the starch and the effect of $MgCl_2$ in the medium containing glucose are because of the inhibition of amylolytic and glycolytic microorganisms that produce butyrate.

Table 5 Propionate produced (mM) during 48 hours in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	26.43	26.80	26.03	26.23	27.99	16.94	3.61	0.0012	0.0267
	Cellulose	9.52	7.99	6.51	6.32	7.45	8.86	3.61	0.8160	0.3230
	Glucose	19.98	13.25	13.33	24.33	18.06	16.64	3.61	0.9317	0.1756
$MgCl_2$	Starch	22.94	21.94	17.51	23.54	20.90	16.10	2.35	0.0025	0.2028
	Cellulose	12.61	10.60	10.34	11.42	11.66	12.69	2.35	0.4126	0.5695
	Glucose	18.54	17.76	17.13	16.25	14.72	14.59	2.35	0.0236	0.2356
$CaCl_2$	Starch	20.80	17.90	18.30	21.39	23.01	11.79	3.23	0.0067	0.0031
	Cellulose	12.72	13.00	11.95	12.49	12.50	17.60	3.23	0.0394	0.2191
	Glucose	23.14	23.85	19.36	22.13	17.87	20.51	3.23	0.1944	0.1150

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

Table 6 Butyrate produced (mM) during 48 hours in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	14.09	13.91	14.08	13.35	14.64	8.80	2.04	0.0016	0.0573
	Cellulose	4.81	4.66	6.55	5.15	4.27	5.01	2.04	0.7699	0.8478
	Glucose	10.61	7.37	7.06	12.87	9.90	9.76	2.04	0.5007	0.2912
$MgCl_2$	Starch	12.90	13.91	10.08	11.92	10.87	8.76	1.38	0.0002	0.7392
	Cellulose	6.31	5.95	6.28	5.96	6.48	7.13	1.38	0.2978	0.7131
	Glucose	10.23	10.07	8.30	8.00	8.31	7.12	1.38	0.0089	0.2978
$CaCl_2$	Starch	11.60	10.23	8.72	11.36	11.42	6.29	1.67	0.0022	0.0292
	Cellulose	6.41	7.75	7.05	7.40	6.71	9.40	1.67	0.0645	0.3438
	Glucose	11.51	12.79	10.09	11.6	9.81	11.53	1.67	0.6693	0.1697

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

There was a quadratic effect of NaCl levels on pH in the medium containing starch (Table 7). The estimated maximum pH was predicted to be 7.03 at a NaCl concentration of 120 mg/dL. In the medium containing cellulose, pH increased linearly with the concentration of NaCl. The microorganisms that digest cellulose prefer a pH value of 6.7. Deviations above or below this pH might be inhibitory. Thus, increasing levels of NaCl can result in low cellulolytic bacteria activity (Church, 1974). There was no effect of $MgCl_2$ levels on pH in any of the three substrates. However, there was a decreasing linear effect of $CaCl_2$ on the pH in the media containing starch, cellulose, and glucose, with the lowest pH values being 6.40, 6.97, and 6.00, respectively.

Ruminal microorganisms that grew in the media containing glucose and often starch were sensitive to increasing levels of NaCl, $MgCl_2$, and $CaCl_2$. However, microorganisms in the medium obtaining cellulose were less sensitive to increasing concentrations of these salts, even though the pH increased with the concentrations of the salts. Thus, under the conditions imposed in this study, there was little variation in the microbial protein production and in the fermentation products when the microorganisms were provided with cellulose as a substrate. In general, the effect of NaCl concentration in the media was smaller than those of $MgCl_2$ and $CaCl_2$, demonstrating that rumen microorganisms are less sensitive to NaCl. In the medium containing starch as substrate, high levels of $MgCl_2$ inhibited the growth of the rumen microorganisms. In

contrast, when cellulose was used as a substrate, the effect of $MgCl_2$ on the rumen microorganisms was smaller, demonstrating that cellulolytic and glycolytic microorganisms were less sensitive to this salt. With all three substrates, the effects of $CaCl_2$ on the rumen microorganisms were comparatively small, demonstrating that the amylolytic, cellulolytic, and glycolytic microorganisms were less sensitive to $CaCl_2$ than to other salts. In the review by Umar *et al.* (2014), the quality and consumption of the drinking water was observed to decrease as excess solids were added, which resulted in reduced performance.

Table 7 Mean pH values after 48 hours of in vitro incubation in media with various concentrations of sodium chloride, magnesium chloride and calcium chloride

Media		Levels of salt (mg/dL)						SE	Significance	
Salt	Substrate	0	100	200	400	800	1600		Linear	Quadratic
NaCl	Starch	6.83	7.03	6.56	6.50	6.56	6.70	0.17	0.2022	0.0072
	Cellulose	6.90	7.00	6.83	6.83	7.30	7.46	0.17	<.0001	0.8613
	Glucose	6.53	6.73	6.56	6.56	6.63	6.56	0.17	0.8113	0.7976
$MgCl_2$	Starch	6.66	7.46	6.93	6.63	6.83	6.86	0.34	0.5804	0.5052
	Cellulose	7.15	7.27	7.10	7.00	6.99	6.91	0.34	0.2454	0.6746
	Glucose	7.14	6.94	6.78	6.56	6.96	6.66	0.34	0.2695	0.5978
$CaCl_2$	Starch	6.98	7.00	6.97	6.91	6.72	6.40	0.063	<.0001	0.3174
	Cellulose	7.15	7.14	7.02	7.11	7.14	6.97	0.063	0.0043	0.1564
	Glucose	6.98	6.95	6.87	6.74	6.55	6.00	0.063	<.0001	0.5793

NaCl: sodium chloride, $MgCl_2$: magnesium chloride, $CaCl_2$: calcium chloride

Saline water can affect microbial growth in the rumen negatively. The presence of solutes reduces the ability to dissolve additional solutes, which can influence plasma osmolarity. The solutes inhibit the movement of water through cells, causing reduced microbial activity (Al-Khalasi *et al*., 2010).

Conclusions

Rumen microbes from goats, which fermented in starch, cellulose, and glucose, tolerated NaCl and $CaCl_2$ concentrations up to 1600 mg /dL in in vitro media. Neither cellulose or glucose were sensitive to $MgCl_2$, tolerating concentrations of up to 1600 mg/dL. However, bacteria that fermented in starch were less tolerant of $MgCl_2$ concentrations above 800 mg/dL. Thus, brackish water could be used by goats in semi-arid regions, but its use should be managed carefully so that it does not have a negative impact on rumen microbial populations.

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Authors' Contributions

The idea for the study was conceived by EMS, who secured the funding and supervised GGLA, JSO, EMS and AMZ. ECBC was recruited as the student to work on the project and received her MSc cum laude. ECBC and GAP performed the experiment. AFP and FNSS were responsible for statistical analysis and structuring the manuscript formatting. EMS was the supervisor at the university and gave guidance on the study. All co-authors participated in management and discussion of the results, statistical analysis and writing, and corrected the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors declare there are no conflicts of interest

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