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Effects of saline water stress on *in vitro* and *in situ* ruminal degradation kinetics of soybean meal in sheep

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Abstract The study was conducted to determine the soybean meal (SBM) fermentation and degradation kinetics using gas production (GP) and nylon bag techniques in sheep under saline water stress. Eight rumen-cannulated Iranian Shaal rams that received different levels of saline water, including the control group (480), 4000, 8000, and 12000 mg/kg total dissolved solids (TDS) were used. The results showed significant differences between the experimental treatments in terms of the amount of methane produced, total GP, dry mater (DM) and crude protein (CP) degradation and the relevant parameters (P<0.05). The treatment containing 12000 mg/kg TDS had the highest GP at 48, 72, and 96 h of incubation times. Short-chain fatty acids, digestible organic matter, metabolizable energy, and net energy for lactation of SBM significantly differ between treatments (P<0.05), with the lowest amount at the 4000 mg/kg salinity level. The lowest amount of methane emission was observed in the treatment containing 8000 and 12000 mg/kg TDS. The results demonstrated that drinking water salinity significantly influenced the DM and CP degradability in SBM. The highest effective degradability values for DM and CP were observed in the treatment containing 12000 mg/kg TDS. The highest values of *b* fraction for DM and CP were observed in the treatment containing 8000 mg/kg TDS. Also, slowly degradable protein and effective rumen degradable protein significantly (P<0.05) increased by increasing the salinity levels. In contrast, the undegradable protein, digestible undegradable protein and metabolizable protein were decreased with increasing water salinity. In conclusion, drinking water salinity affected the soybean meal fermentation and degradation kinetics and nutritional value. The treatment containing 12000 mg/kg TDS in drinking water decreased methane production and metabolizable protein in sheep.

Keywords: degradability, methane emission, nylon bag, water salinity

Introduction

In arid and semi-arid regions worldwide, access to water is a limiting factor for livestock production. This problem can be related to a decrease in rainfall in the area and the geological structure of the region, which does not allow the optimal storage of underground water. Underground water is the largest water source available in these areas (Albuquerque et al., 2020). Also, important factors that contribute to water scarcity include climate change, increased economic growth, drought, environmental degradation, and rapid human population growth (Mdletshe et al., 2017). Therefore, due to global warming, groundwater

and surface water have been exposed to increased salinity levels. The most critical challenge of livestock systems is water quantity and quality (Tulu, 2022). However, the animal's ability to tolerate and adapt to the quantity and quality of water depends on factors such as species, breed, age, gender, environmental conditions, physiological state, and production status (Kaliber et al., 2016; Zovidis and Haji Georgiou, 2017; Tulu, 2022). Ruminants are sensitive to sudden and severe changes in the composition of the diet, and habituation must be done step by step. Similarly, acclimation to saline water in ruminants should be done gradually, which helps the animal to adapt to water with higher salinity levels without harming health (Runa et al., 2019). Sheep and goats can tolerate water and feed with high salt concentrations (Leite et al., 2019; Pishdadi-Motlagh et al., 2023). Based on the findings of Attia et al. (2008), sheep can tolerate saline water containing 1.3% sodium chloride without adverse effects. In semi-arid areas, goats and sheep are among the most efficient animals in water consumption due to their smaller size and better water use. This feature of their adaptation is related to increasing the efficiency of water consumption and reabsorption in the digestive tract, which maximizes this metabolism when using saline water (Albuquerque et al., 2020). High salinity levels may alter rumen microbial activities and nutrient utilization (Attia et al. 2008). El-Shaer and Squires (2016) stated that diets and water containing high salt levels in ruminants increased the concentration of salt and decreased the pH, ammonia, and volatile fatty acids (VFA) in the rumen. In general, not only was there no change in the number of bacteria in the rumen of sheep due to water salinity, but it also caused an increase in the population of bacteria, while the diversity of bacteria was reduced. The profile of rumen fermentation changes when animals drink saline water, which affects the animal performance.

The *in vitro* GP technique is a helpful method for determining the nutritional value of feed. This method can also predict the fermentation kinetics, microbial nitrogen supply and amount of SCFA, carbon dioxide, methane production and ME, as well as the digestibility of organic matter (OM) in ruminant animals (Mirzaei-Aghsaghali et al., 2011; Ansah et al., 2016). On the other hand, the nylon bag (*in situ*) method is an important tool for feedstuffs evaluation as well as for improving our understanding of degradation kinetics within the rumen. Also, it is a more efficient method for measuring the ruminal degradation rate and extent of dry matter (DM), crude protein (CP), and OM (Ørskov and McDonald,1979; Maheri-Sis et al., 2011). The information obtained by this method has shown the potential ability to predict digestibility, feed intake, and livestock performance (Wood and Badve, 2001). Despite the importance of water in animal husbandry, the issue of water quality has not been researched sufficiently (Araujo et al., 2019). Therefore, the current research was carried out to study the impact of saline water stress on *in vitro* and *in situ* ruminal fermentation and degradation kinetics of soybean meal (SBM) in sheep.

Materials and methods

Animals and management

This experiment was carried out at the Animal Science Research Institute (ASRI), Karaj, Iran, according to the Iranian Council of Animal Care (1995). In this study, we used eight fistulated Shaal rams with an initial body weight (BW) of 76 ± 2.5 kg. The preliminary period for adaptation was allowed for about ten days. The salt-free diet which included 70% forage (alfalfa hay and wheat straw), and 30% concentrate (barley grain, SBM, cottonseed, and mineral and vitamin supplements) were offered twice per day at 8:00 and 16:00 h at a rate of 10% higher than the maintenance level according to the NRC (2007).

Treatments

Fresh water containing 480 mg/kg total dissolved solids (TDS) was considered as the control group, and the other treatments contained 4000, 8000, and 12000 mg/kg TDS. The rams had full access to drinking water according to their treatments. The electrical conductivity (EC) of treatments was measured by the EC meter in the chemical laboratory of the Animal Science Research Institute (ASRI). The value of TDS using EC data was calculated by the equation TDS = 640 * EC, where TDS with mg/kg and EC with ds/m (Rusydi, 2018; Khalilipour et al., 2019). The mineral and bicarbonate ion contents of fresh water (control: 480 mg/kg TDS) used in this study including, Na^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, $SO4^{2-}$, $HCO3^{-}$ as mg/L were 119, 39, 7.9, 35, 116 and 241, respectively.

Chemical analysis

The chemical composition, including DM, ether extract (EE), CP, and crude ash (CA) content in SBM, was determined according to AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by procedures proposed by Van Soest et al. (1991) and acid detergent insoluble nitrogen (ADIN) was estimated according to Van Soest et al. (1994). The non-fibrous carbohydrates (%NFC = 100 – (%NDF + % CP + % EE + % ash) were calculated as proposed by NRC (2001). The determined chemical composition of SBM including, DM, CP, EE, Ash, NDF, ADF, ADIN, and NFC in the current study were 93.5%, 49.9%, 0.7%, 7.6%, 18.5%, 12%, 4.2% and 23.2%, respectively.

In vitro gas production

Rumen fluid was obtained from two fistulated Shaal rams before morning feeding. Approximately 200 mg samples

of dry feedstuff were weighed in triplicate and placed in a 100 mL calibrated glass syringe. Feed samples were incubated *in vitro* with a rumen fluid buffer mixture (30 mL) and transferred into glass syringes of 100 mL capacity, according to Menke and Steingass (1988). The samples were incubated in a shaking incubator at 39°C. The GP amount was recorded at 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation and corrected for blank. To measure methane production at 24 h incubation, 4 mL of NaOH (10 M) were added to each syringe, and after 10 minutes, the syringes were read again and removed. Mixing of content of syringes with NaOH solution caused the absorption of CO_2, the remaining gas volume in the syringe was considered as methane (Anele et al., 2011). Net GP data were fitted to the model outlined by Ørskov and McDonald (1979) and GP parameters were estimated by the Fitcurve software version 6 (Chen, 1995):

$P = A\ (1\text{-}e^{-ct})$

P = the GP at the time t

A = potential GP

c = the GP rate constant for the insoluble fraction

t = the incubation time (h)

The digestible OM (DOM), net energy for lactation (NE_L), and metabolizable energy (ME) for soybean meal were estimated using equations of Menke and Steingass (1988), and short-chain fatty acids (SCFA) were calculated using the following equations (Makkar, 2005):

SCFA (mmol) = 0.0222GP–0.00425

DOM (%) = 0.9991GP+0.595CP+0.181ash+9

ME (MJ/kg DM) = 0.157GP+0.084CP+0.22EE–0.081ash+1.06

NE_L (MJ/kg DM) = 0.115GP+0.054CP+0.14EE–0.054ash–0.36

Where GP is the gas production volume at 24 h of incubation (mL/200mg DM).

In situ degradation procedures

To determine the disappearance of DM and CP, 5g dried SBM were transferred to nylon bags (14×7 cm; about 50-micron pore size), and incubated in the rumen for 0, 2, 4, 6, 8, 16, 24 and 48 hours. After incubation, the bags were washed out with cold water, dried at 55ºC in an oven for 48 hours, and weighed (Ørskov and McDonald,1979). Then, the CP content was determined. Rumen degradation kinetics of DM and CP were calculated by the equation [$P= a+b\ (1\text{-}e^{-ct})$] described by Ørskov and McDonald (1979) using Fitcurve software version 6 (Chen, 1995); where P= potential degradability for response variables at time t. a= highly soluble and readily degradable fraction (%). b= insoluble and slowly degradable fraction (%). c= rate constant for fraction b (/h) degradation. e= 2.7182 (natural logarithm base). t= time relative to incubation (h). The amounts of quickly degradable protein (QDP), slowly degradable protein (SDP), effective rumen degradable protein (ERDP), undegradable protein (UDP), digestible undegradable protein (DUP), and metabolizable protein (MP) were calculated based on AFRC (1993) equations.

QDP = a×CP

SDP = [(b×c)/(c+r)]×CP

ERDP = 0.8 (QDP)+SDP

UDP = CP [1-a-(bc/(c+r))]

DUP = 0.9 [(UDP)–(6.25×ADIN)]

MP = 0.6375 (ERDP)+DUP

a= Degradability of water-soluble fraction (DM%), b= Degradability of water-insoluble fraction (DM%), c= Degradation rate (h%), r= Passage rate, ADIN= Acid detergent insoluble nitrogen.

Statistical analyses

The PROC GLM (SAS, 2001) was used for statistical analysis of the in vitro GP and *in situ* degradability data. The experiment and statistical analysis were designed and performed based on a complete randomized design (CRD) with four treatments and three replicates for each treatment. The Duncan's multiple range test was used to compare the treatment means (P≤0.05).

Results

In vitro gas production

Significant differences existed between the salinity levels and control groups for GP in SBM (Table 1, P<0.05). The highest amount of GP at most of the incubation times was observed in the treatment containing 12000 mg/kg TDS and the lowest in the treatment containing 4000 mg/kg TDS compared to the control group. The estimated kinetic parameters, methane production, and SCFA, DOM, ME, and NE_L are presented in Tables 2 and 3, respectively. A significant difference was observed in potential GP (A), rate of GP (c), SCFA, DOM, ME, and NE_L between experimental treatments (P<0.05). The highest and lowest values of A were observed in the treatment containing 12000 and 4000 mg/kg TDS, respectively. The c fraction in the treatment containing 12000 mg/kg TDS, was lower than that of other groups and the highest amount of the c fraction was observed at 4000 mg/kg TDS. The lowest methane emission of SBM was found in the treatments with higher salinity levels (8000 and 12000 mg/kg TDS). The highest and lowest amounts of SCFA, DOM, ME, and NE_L were obtained at 12000 and 4000 mg/kg TDS, respectively.

In situ degradability

The ruminal degradability of DM and CP at different incubation times is reported in Tables 4 and 5, respectively. There was a significant difference amongst the experimental treatments (P<0.05) for DM degradability (DMD) and CP degradability (CPD) in SBM at most of the incubation times. The highest DMD and CPD was observed in the treatment containing 12000 mg/kg TDS, and the lowest values in the control group.

Significant differences were observed among the experimental treatments in parameters *a*, *b*, and *c* for DM in SBM, and in *b* and *c* for CP (Table 6, P<0.05). The lowest amount of *a* parameter of DM and the highest amount of *b* parameter for DM and CP were related to the treatment containing 8000 mg/kg TDS. The highest value of *c* fraction for DM and CP was recorded at 12000 mg/kg TDS, and the lowest one in the control treatment.

A significant difference in ED for DM and CP was noted among the experimental treatments at all passage rates, with the highest value observed at 12000 mg/kg TDS, and the lowest value for the control group (Table 7, P<0.05).

There were significant differences between treatments regarding the QDP, SDP and ERDP parameters, which increased at all passage rates with increasing salinity levels. The highest amount for SDP and ERDP was found at 12000 mg/kg TDS and the lowest amount was observed in the control treatment (Table 8, P<0.05). As the salinity level increased, the UDP, DUP, and MP values decreased at all passage rates, being lowest at 12000 mg/kg TDS and highest in the control treatment (Table 9).

Table 1. Effects of drinking water salinity on *in vitro* gas production volume (mL/200mg DM) at different incubation times of soybean meal

	Salinity levels (TDS as mg/kg)					
Incubation time (h)	Con (480)	4000	8000	12000	SEM	*p*- value
2	9.4^{a}	6.5^{b}	6.8^{b}	9.1^{a}	0.302	0.0002
4	16.5^{a}	12.2^{c}	12.1^{c}	13.7^{b}	0.317	<0.0001
6	21.3^{a}	17.0^{c}	16.7^{c}	18.5^{b}	0.431	0.0003
8	26.0^{a}	22.4^{b}	21.7^{b}	23.2^{b}	0.455	0.0007
12	35.3^{a}	31.9^{b}	30.1^{c}	32.3^{b}	0.500	0.0006
24	49.7^{ab}	46.8^{c}	49.0^{b}	50.7^{a}	0.447	0.001
48	65.5^{b}	58.5^{d}	63.0^{c}	68.6^{a}	0.513	<0.0001
72	69.8^{b}	61.1^{d}	66.2^{c}	72.2^{a}	0.455	<0.0001
96	70.8^{b}	62.6^{d}	67.2^{c}	73.7^{a}	0.594	<0.0001

a-d: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. SEM: standard error of the mean

Table 2. Effects of different levels of drinking water salinity on *in vitro* gas production parameters and methane production (CH_4) of soybean meal

	Salinity levels (TDS as mg/kg)					
Parameters	Con (480)	4000	8000	12000	SEM	*p*- value
A	71.4^{b}	62.4^{d}	68.2^{c}	75.3^{a}	0.498	<0.0001
c	0.0498^{b}	0.0584^{a}	0.0513^{b}	0.0455^{c}	0.0005	<0.0001
CH_4 (%)	20.9^{a}	18.5^{a}	8.4^{b}	5.8^{b}	0.880	<0.0001
CH_4 (ml/g DM)	55.0^{a}	52.5^{a}	20.0^{b}	12.5^{b}	2.5	<0.0001
CH_4 (ml/g OM)	59.7^{a}	57.0^{a}	21.7^{b}	13.5^{b}	2.7	<0.0001

a-d: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. *A*: potential gas production, *c*: the gas production rate constant for the insoluble fraction, SEM: standard error of the mean

Table 3. Effects of different levels of drinking water salinity on the amount of digestible organic matter (DOM), net energy for lactation (NE_L), metabolizable energy (ME) and short chain fatty acids (SCFA) of soybean meal

	Salinity levels (TDS as mg/kg)					
Items	Con (480)	4000	8000	12000	SEM	*p*- value
SCFA	1.1^{ab}	1.03^{c}	1.08^{b}	1.12^{a}	0.498	0.001
DOM	89.7^{ab}	86.8^{c}	89.0^{b}	90.7^{a}	0.446	0.001
ME	12.6^{ab}	12.1^{c}	12.4^{b}	12.7^{a}	0.070	0.001
NE_L	7.7^{ab}	7.4^{c}	7.6^{b}	7.8^{a}	0.051	0.001

a-c: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. SEM: standard error of the mean

Discussion

The chemical composition of the SBM, in most cases, was within the range of the findings of other researchers (Ibaneza et al., 2020; Yang et al., 2020). Based on the literature review, limited studies were found about the effect of saline water consumption on the rumen fermentability and degradability in SBM. Most published studies were often concerned with the impact of drinking water salinity on the performance, blood parameters, nutritional behavior, and apparent digestibility of feedstuffs in ruminant animals. In the current study, rams showed different responses to the drinking water salinity, mainly in term of *in vitro* fermentation and methane emission as well as *in situ* degradation.

In the current study, the highest amounts of GP, SCFA, DOM, ME, and NE_L were obtained in the treatment containing 12000 mg/kg TDS. In contrast with our results, Vosooghi-Postindoz et al. (2018) expressed that, the high level of salinity reduced the production of SCFA but did not affect rumen pH and ammonia nitrogen

production. An increase in TDS in water, led to reduced feed consumption and poor animal performance (Umar et al., 2014). According to Costa et al. (2019), water with a salinity level of greater than 7000 mg/kg TDS can cause problems in young, pregnant, and lactating animals. Saline water may harm rumen microbial growth; however, different strains of rumen microbes can tolerate saline water over 7000 mg/kg TDS. Hemsley et al. (1975) described that high salt intake (150 grams per day) in sheep leads to a decrease in the population of protozoa due to an increase in the osmotic pressure and reduced amount of organic matter fermented in the rumen. Most researchers reported unequivocal results concerning the impact of saline water consumption on the rumen ecosystem. Feed degradability and apparent digestibility are related to the changes in microbial population, pH, concentration of minerals, osmolarity, passage rate, microbial fermentation products and rumen movements (Attia et al., 2008; Valtorta et al., 2008; Elshaer and Squires, 2016; Yousfi et al., 2016). Valizadeh et al. (2019) said that the majority of ruminal bacteria populations and some protozoa need sodium and potassium for growth, which may explain their tolerance to different levels of salt in water and diet. Most rumen microorganisms show maximum growth and production under normal salt concentrations. Ruminal microorganisms in sheep may even tolerate 5% salt in the rumen, while the protozoa population may decrease with increasing salinity levels. Since protozoa have high proteolytic activity, reducing their population may increase the protein passing from the rumen to the intestine Also, the increase in sodium chloride increases the rumen movements by stimulation of the receptors in the rumen wall. Increasing water consumption also increases the protein passing through the rumen. El-Shaer and Squires (2016) demonstrated that when the rumen is under salt stress, *Selenomonas* and *Bacteroids* are the dominant bacteria in the rumen. The main fermentation product of *Bacteriodes* and other rumen bacteria is succinate, and the reduction of *Bacteriodes* leads to the reduction of succinate production, which can also be responsible for the reduction of propionate production. In contrast, *Streptococcus bovis, Butyrivibrio fibrisolvens, Selenomonas ruminantium,* and *Megasphaera elsdenii* survive in environments with high osmotic pressure. The habituation of rumen bacteria to consumption of diet or water containing high salinity is also of particular importance to ruminal function (Mayberry, 2003; Thomas et al., 2007; Valizadeh et al., 2019). Valtorta et al. (2008) reported that drinking water salinity did not affect ruminal parameters (pH, population of microorganisms and volatile fatty acids) in dairy cows, but growth of cellulolytic bacteria was reduced with increasing salinity levels of 1000 to 10000 mg/kg TDS. El-Shaer and Squires (2016) found that high salt stress increased the fermentation efficiency by increasing the rate of fluid dilution and microbial growth, and reducing the substrate required for microbial maintenance.

Table 4. Effects of drinking water salinity on *in situ* rumen dry matter degradability (DMD) of soybean meal at different incubation times (%)

	Salinity levels (TDS as mg/kg)					
Incubation time (h)	Con (480)	4000	8000	12000	SEM	*p*- value
0	23.3	23.3	23.4	23.2	0.078	0.55
2	26.4^{b}	29.0^{a}	25.8^{c}	29.0^{a}	0.121	<0.0001
4	33.8^{b}	38.7^{a}	32.9^{b}	40.2^{a}	0.473	<0.0001
6	38.4^{c}	46.2^{b}	38.9^{c}	48.3^{a}	0.384	<0.0001
8	43.4^{b}	52.9^{a}	44.4^{b}	54.3^{a}	0.629	<0.0001
16	61.3^{c}	69.7^{ab}	67.0^{bc}	74.8^{a}	1.794	0.004
24	74.5^{c}	81.2^{bc}	84.0^{ab}	90.6^{a}	1.149	0.005
48	86.0^{c}	96.0^{b}	97.2^{a}	98.5^{a}	0.391	<0.0001

a-c: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. SEM: standard error of the mean

Table 5. Effects of drinking water salinity on *in situ* rumen crude protein degradability (CPD) of soybean meal at different incubation times (%)

	Salinity levels (TDS as mg/kg)					
Incubation time (h)	Con (480)	4000	8000	12000	SEM	*p*- value
0	9.2	9.2	9.4	9.2	0.104	0.68
2	11.0^{d}	15.5^{b}	11.9^{c}	18.2^{a}	0.240	<0.0001
4	16.5^{d}	23.7^{b}	20.9^{c}	32.0^{a}	0.486	<0.0001
6	21.3^{c}	30.3^{b}	30.0^{b}	39.9^{a}	0.680	<0.0001
8	25.7^{c}	37.3^{b}	39.0^{b}	48.0^{a}	1.053	<0.0001
16	40.8^{c}	61.6^{b}	62.6^{b}	72.6^{a}	2.477	0.0001
24	52.2^{b}	78.0^{a}	80.0^{a}	86.5^{a}	3.132	0.0003
48	72.3^{b}	95.2^{a}	97.7^{a}	98.6^{a}	0.964	<0.0001

a-d: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. SEM: standard error of the mean

Based on the results of the present study, the lowest methane emission of SBM was found in the treatments with higher salinity levels (8000 and 12000 mg/kg TDS). Methane emission has received global attention due to its role as a greenhouse gas and global warming (Eckard et al., 2010). Ruminants lose about 2 to 15% of their feed

energy as methane, which comprises between 20 and 30% of all gases produced in the rumen (Attia, 2015; Mirzaei-Aghsaghali and Maheri-Sis, 2016). Attia (2015) stated that methane production in the rumen is influenced by factors such as pH, VFA, diet, feeding frequency, animal species, and environmental stress. Also, increasing the passage rate in the rumen due to increased osmotic pressure may reduce methane production. Methanogenic microorganisms are halophilic, thermophilic, and mesophilic, and can tolerate up to 1.5% NaCl. These microorganisms feed on protozoa, and changes in the protozoal population could impact on the number of methanogens (Sorensen et al., 2004; Valizadeh et al., 2019). Kaushik et al. (2015) reported that the type of feedstuff and amount of carbohydrates affect methane production by changing the microbial population in the rumen. High amounts of soluble carbohydrates in high-energy concentrates enhance propionate production in the rumen, which prevents the growth of methanogens and thus reduces methane production. Propionate acts as a hydrogen scavenger and reduces the hydrogen supply for methane gas production. In addition, the high levels of ether extract help decrease methane emissions because some fatty acids, especially medium-chain fatty acids, are toxic to *methanogens* (Jayanegara et al., 2017). Factors such as breed, age, water and diet salinity, type of feed and ration, protozoa population, rumen pH and rumen passage rate can affect methane gas emission (McGregor, 2004; Bhatta et al., 2006; Alhraishawi et al., 2018; Valizadeh et al., 2019; Pishdadi-Motlagh et al., 2023). Liu et al. (2016) showed that salt supplements effectively reduced enteric methane production, which is in line with our present results. Also, Lee et al. (2009) demonstrated that methane emission was reduced when water salinity levels were increased. Therefore, methane emission reduction can be attributed to the very high salt concentration gradient between the internal and external cell environments. This condition removes water and other necessary nutrients from inside the cells and ultimately decreases the growth and activity of rumen microorganisms significantly. According to Alhraishawi et al. (2018), the complete digestion of materials enhances biogas accumulation, but factors such as salt or salinity inhibit the methane emission process. Thus, methane emission was reduced by 12, 55, and 95% with enhancing salt concentrations of 6.5, 14.2, and 22 g/L, respectively. Anyway, *methanogenic* microorganisms are affected at a salt concentration of 6 g/L. The effect of salt on digestion is not limited to methane emission as it also impacts on the efficiency of the determining factors such as pH. A high concentration of salt activates carbon dioxide and inhibits methane emission.

Table 6. Effects of different levels of drinking water salinity on *in situ* ruminal degradation parameters of dry matter (DM) and crude protein (CP) of soybean meal

		Salinity levels (TDS as mg/kg)					
Parameters		Con (480)	4000	8000	12000	SEM	*p*- value
DM	*a*	20.8^{b}	22.3^{a}	19.2^{c}	20.8^{b}	0.419	0.005
	b	73.1^{d}	78.4^{c}	90.0^{a}	82.6^{b}	0.663	<0.0001
	c	0.0498^{bc}	0.0590^{ab}	0.0462^{c}	0.0676^{a}	0.0033	0.007
CP	*a*	7.3	6.8	5.1	7.7	0.602	0.06
	b	84.1^{c}	98.5^{a}	99.4^{a}	94.7^{b}	0.523	<0.0001
	c	0.0311^{c}	0.0489^{b}	0.0495^{b}	0.0703^{a}	0.0039	0.0008

a-d: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. *a*: highly soluble and readily degradable fraction (%), *b*: insoluble and slowly degradable fraction (%), *c*: degradation rate of fraction *b* (/h), SEM: standard error of the mean

Table 7. Effects of different levels of drinking water salinity on effective degradability (ED) of dry matter (DM) and crude protein (CP) of soybean meal at different rumen outflow rates

		Salinity level (TDS as mg/kg)					
	ED	Con (480)	4000	8000	12000	SEM	*p*- value
DM	2%/h	73.1^{c}	80.6^{b}	82.2^{b}	84.6^{a}	0.701	<0.0001
	5%/h	57.4^{c}	64.6^{b}	62.7^{b}	68.3^{a}	0.868	0.0001
	8%/h	49.0^{d}	55.4^{b}	52.4^{c}	58.7^{a}	0.809	0.0002
CP	2%/h	58.6^{c}	78.9^{ab}	77.3^{ab}	81.9^{a}	1.167	<0.0001
	5%/h	39.7^{c}	56.6^{b}	55.9^{b}	63.4^{a}	1.375	<0.0001
	8%/h	31.0^{c}	44.6^{b}	44.5^{b}	52.3^{a}	1.249	<0.0001

a-d: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. SEM: standard error of the mean

According to the *in situ* degradation data, and in most incubation durations, the treatment containing 12000 mg/kg TDS resulted in the highest amount of DMD and CPD, and, conversely, the lowest level of UDP, DUP, and MP. However, Katting et al. (1992) reported that water containing 350 and 2300 mg/kg TDS caused no significant differences in the DMD of mixed fodder in Holstein calves. Alves et al. (2017) did not observe a significant difference in microbial protein synthesis in heifers by adding NaCl (up to 8326 mg/kg TDS). Other researchers reported a significant increase in the apparent digestibility of protein, where the efficiency of microbial protein synthesis increased significantly with increasing salinity (Yapekii and Dryden, 2005; Yousfi et al., 2016; Alves et al., 2017; Vosooghi- Postindoz et al., 2018). According to Costa et al. (2019), up to 8800 mg/kg TDS did not interfere with the growth of cellulolytic microbes. Microorganisms grown in a medium

containing starch, were not affected by water salinity up to 16000 mg/kg TDS, indicating that amylolytic microorganisms are more tolerant to high salinity levels. Attia et al. (2008) illustrated that drinking saline water can affect the population of microorganisms and their activity through changes in rumen osmotic pressure. Hence, the protein-degrading microorganisms are more affected by the drinking water salinity, which is consistent with the results of our study. Potter et al. (1972) declared that salt stress increased the rumen osmotic pressure, which can be caused by altering the concentration of rumen electrolytes, especially sodium and potassium concentrations. El-Shaer and Squires (2016) proposed two options for lower ruminal digestibility under salt stress: (1) reducing the residence time of particles in the rumen; and (2) impaired metabolism of rumen microbes that digest fiber. They also demonstrated that the osmotic pressure of the rumen fluid in salt-stressed sheep was high (331 mOsm/kg), being higher than the normal rumen osmolality of 260-280 mOsm/kg. The optimal osmotic pressure for ruminal ciliate protozoa is 260 mOsm/kg. Therefore, higher osmotic pressure may decrease the protozoal population and impair digestion, particularly cellulose degradation. Potter et al. (1972) also stated that osmotic pressure was increased by increasing the drinking water salinity. Moreover, there seems to be a relationship between chloride and osmotic pressure in the rumen, as observed in sheep that consumed saline water and diet. Regarding changes in the ruminal function, the observed effects seem to be related to increased ruminal fluid passage rates due to increased water consumption. Also, an effect associated with increased osmolarity probably leads to an increased flow rate. In the study of Hemsley et al. (1975) with the high salt intake by sheep, ammonia production in the rumen decreased, and the percentage of organic matter and protein passage into the intestine increased the digestibility of protein. Yousfi et al. (2016) also showed that the consumption of saline water containing 7000 mg/kg TDS decreased the protozoal population and increased the efficiency of microbial nitrogen utilization. Costa et al. (2019) stated that cellulose and glucose-fermenting bacteria were more sensitive to salinity than starch-fermenting (amylolytic) bacteria; amylolytic bacteria were more resistant to higher water salinity (up to 16000 mg/kg TDS) than other microorganisms. They concluded that water salinity that can be used by ruminants, was related to the type and the amount of salt in water and diet. Notably, the population of cellulolytic microorganisms decreased linearly with the increased amount of salt in water. Based on the regression equations, the highest microbial protein production was obtained at the sodium chloride concentration of 8800 mg/kg TDS. El-Shaer and Squires (2016) stated that the lower propionate production at salt stress may have occurred because of the washout of readily fermentable materials. They claimed that the dilution rate was negatively related to the molar proportion of propionate and positively related to the molar proportion of acetate in the ruminal fluid in some sheep. On the other hand, Alves et al. (2017) described that acetate concentration in the rumen decreased linearly due to increasing water salinity. Based on the literature review of Pishdadi-Motlagh et al. (2023), in salinity conditions, the growth rate of amylolytic bacteria in the rumen is higher than that of fibrolytic bacteria. Any factor (e.g., salt) that increases the amylolytic population in the rumen will also increase microbial protein production. Furthermore, Costa et al. (2019) stated that production of microbial protein will increase when the available substrate for microorganisms is starch or glucose at different salinity levels. Contradictory, Valtorta et al. (2008) showed that a gradual increase in ruminal ammonia levels is decreased with increasing water salinity levels in dairy cows. As the water salinity increases, microbial protein production reduces linearly, and the concentration of ammonia in the rumen increases when the dominant substrate is cellulose. Ammonia is essential for the growth of fibrolytic bacteria; therefore, decreasing cellulolytic microorganisms by salinity may lead to ammonia accumulation in the rumen.

Table 8. Effects of different levels of drinking water salinity on the amount of crude protein fractions of soybean meal

	Salinity level (TDS as mg/kg)					
Crude protein fraction	Con (480)	4000	8000	12000	SEM	*p*- value
QDP	36.7^{a}	33.9^{b}	25.5^{c}	38.6^{a}	3.0007	0.006
SDP 2%	255.8^{b}	348.4^{a}	352.7^{a}	367.5^{a}	7.644	<0.0001
SDP 5%	161.2^{c}	242.6^{b}	246.3^{b}	275.6^{a}	8.956	0.0001
SDP 8%	117.7^{c}	186.2^{b}	189.3^{b}	220.6^{a}	8.523	0.0002
ERDP 2%	285.1^{c}	375.6^{b}	373.2^{b}	398.4^{a}	5.816	<0.0001
ERDP 5%	190.6^{c}	269.8^{b}	266.8^{b}	306.5^{a}	6.949	<0.0001
ERDP 8%	147.1^{c}	213.4^{b}	209.7^{b}	251.5^{a}	6.459	<0.0001

a-c: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. QDP: quickly degradable protein, SDP: slowly degradable protein, ERDP: effective rumen degradable protein in different rumen outflow rates, SEM: standard error of the mean.

Conclusions

Overall, drinking water salinity seems to affect the fermentation and degradation kinetics and nutritive value of SBM. The treatment containing 12000 mg/kg TDS might have affected the rumen ecosystem which resulted in decreasing metabolizable protein and increasing DM and CP degradability at most incubation times for SBM in sheep. Salinity positively affected methane emission, so the lowest amount of methane

was related to the treatments containing 8000 and 12000 mg/kg TDS.

Table 9. Effects of different levels of drinking water salinity on the amount of metabolizable protein (MP) parameters of soybean meal

	Salinity level (TDS as mg/kg)					
MP parameters	Con (480)	4000	8000	12000	SEM	*p*- value
UDP 2%	206.7^{a}	116.9^{b}	120.9^{b}	93.1^{c}	5.430	<0.0001
UDP 5%	301.3^{a}	222.6^{b}	227.3^{b}	184.9^{c}	6.489	<0.0001
UDP 8%	344.8^{a}	279.0^{b}	284.3^{b}	239.9^{c}	5.983	<0.0001
DUP 2%	162.4^{a}	81.5^{b}	85.2^{b}	60.2^{c}	4.887	<0.0001
DUP 5%	247.5^{a}	176.7^{b}	181.0^{b}	142.8^{c}	5.840	<0.0001
DUP 8%	286.7^{a}	227.5^{b}	232.3^{b}	192.3^{c}	5.385	<0.0001
MP 2%	344.2^{a}	321.0^{b}	323.1^{b}	314.2^{c}	1.229	<0.0001
MP 5%	369.1^{a}	348.7^{b}	351.1^{b}	338.3^{c}	1.917	<0.0001
MP 8%	380.5^{a}	363.6^{b}	366.0^{b}	352.7^{c}	1.297	<0.0001

a-c: Within rows, mean values with common letters are not different (P>0.05). Con: control group, 4000: 4000 mg/kg water salinity, 8000:8000 mg/kg water salinity, 12000: 12000 mg/kg water salinity. UDP: undegradable protein, DUP: digestible undegradable protein, MP: metabolizable protein in different rumen outflow rates, SEM: standard error of the mean

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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