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RESEARCH ARTICLE

Evaluating the Effect of Drinking Saline Water on Fermentation Kinetics, Methane Production and Nutritional Value of Alfalfa Hay and Barley Grain Using *In Vitro* Gas Production Technique in Sheep

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Abstract: The aim of this study was to determine the effect of drinking saline water on fermentation kinetics, methane emission and nutritional value of alfalfa hay (AH) and barley grain (BG) using *in vitro* gas production technique in sheep**.** Rumen liquor collected from eight rumen cannulated Shal rams, which had received different levels of saline water as four treatment containing 480, 4000, 8000 and 12000 ppm total dissolved solids (TDS). The results showed that there were significant differences between the experimental treatments in terms of the amount of methane produced as well as total gas production and relevant parameters ($P<0.05$). The lowest amount of methane production in AH and BG was observed at the treatment containing 4000 ppm TDS. The treatment containing 12000 ppm TDS, had the highest amount of gas production in AH at the most of incubation times. Short chain fatty acids (SCFA), digestible organic matter (DOM), metabolisable energy (ME), net energy for lactation (NE_L) of AH and BG significantly differ between treatments ($P<0.05$), with the highest amount at the highest salinity level. In a general conclusion, drinking water salinity seems to affect fermentation kinetics and nutritive value of AH and BG depending on the level of salinity and the type of feedstuffs.

Keywords: *Fermentation, Gas production, Methane emission, Sheep, Water salinity*

Koyunlarda *In Vitro* Gaz Üretim Tekniği Kullanılarak Tuzlu Su İçiminin Yonca Kuru Otunun ve Arpa Tanesinin Fermantasyon Kinetiği, Metan Üretimi ve Besin Değeri Üzerine Etkisinin Değerlendirilmesi

Öz: Bu çalışmanın amacı, koyunlarda *in vitro* gaz üretim tekniğini kullanarak tuzlu su içmenin, yonca kuru otu (AH) ve arpa tanesinin (BG) fermantasyon kinetiği, metan emisyonu ve besin değeri üzerine etkisini belirlemektir. 480, 4000, 8000 ve 12000 ppm toplam çözünmüş katı madde (TDS) konsantreli tuzlu su verilerek oluşturulan 4 sağaltım grubuna ait 8 adet rumen kanüllü Shal koçundan rumen sıvı örnekleri toplandı. Bulgular, üretilen metan miktarının yanı sıra toplam gaz üretimi ve ilgili parametreler açısından çalışma grupları arasında önemli farklılıklar olduğunu gösterdi ($P<0.05$). AH ve BG'de en düşük metan üretim miktarı 4000 ppm TDS içeren grupta gözlendi. 12000 ppm TDS içeren grupta, inkübasyon sürelerinin çoğunda AH'de en yüksek gaz üretimi gerçekleşti. AH ve BG'nin kısa zincirli yağ asitleri (SCFA), sindirilebilir organik madde (DOM), metabolize edilebilir enerji (ME), laktasyon için net enerji (NEL) değerleri, en yüksek tuz seviyesine sahip grupta en yüksek olmak üzere, gruplar arasında önemli ölçüde farklılık gösterdi ($P<0.05$). Sonuç olarak, içme suyunun tuzluluğu, tuzluluk seviyesine ve yem maddelerinin türüne bağlı olarak AH ve BG'nin fermantasyon kinetiğini ve besleyici değerini etkiliyor görünmektedir.

Anahtar Sözcükler: *Fermantasyon, Gaz üretimi, Metan emisyonu, Koyun, Su tuzluluğu*

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Introduction

Due to climate change worldwide, the incidence of water scarcity and drought will increase in many regions, especially in arid and semi-arid regions [1,2]. Climate changes are reflected in heating and rainfall reduction, which successively may increase the salinity of both soil and water [3]. Saline water available in these areas may contain high concentrations of total dissolved solid (TDS), sometimes reaching levels above 30000 ppm TDS. High water salinity can have certain consequences on the animals. Sheep were reported to tolerate saline drinking water containing up to 1.3% sodium chloride without ill effects [4]. Tolerance of animals to salinity varies based on their water requirements, species, age, physiological condition, besides of time of the year, and salt content in the total diet [5,6]. Excessive level of salts may counteract one another at higher concentrations lead to limiting their availability for rumen microorganisms. As a result, the microbial activities as well as nutrients utilization may shift [4]. McGregor [7] reported that the period needed for animals to adapt to high salinity is still ambiguous. The rumen consists of complex anaerobic microbial populations such as methanogens which constitute 10^8–10^9/mL. Ruminants lose about 2-15% of their ingested energy solely as methane. Methane comprises between 20 and 30% of total gases produced within the rumen [8]. The production of methane gas in the rumen depends on factors such as pH, SCFA, diet, animal species and environmental conditions. The increased rumen passage rate due to increase osmotic pressure may reduce methane emission [8].

The *in vitro gas* production method is a useful technique for feed evaluation, which is cost effective, fast and easy to determine and suitable for use in developing countries. This method also can predict fermentation kinetics, microbial nitrogen supply, and amount of short chain fatty acids, carbon dioxide, methane production and metabolisable energy as well as organic matter digestibility of feeds for ruminants [9].

In general, water quantity and quality have a significant effect on rumen performance, and research on the effects of saline water on rumen fermentation has been neglected. Thus, the aim of this study was to determine the effect of drinking saline water on fermentation kinetics, methane emission and nutritional value of alfalfa hay (AH) and barley grain (BG) using *in vitro* gas production technique in sheep.

Material and Methods

Animals and Management

This experiment was carried out at the Animal Science Research Institute, Agricultural Education, and Extension Research Organization, Karaj, Iran; according to the "*Guide to the Care and Use of Experimental Animals" prepared by Iranian Council of Animal Care, Isfahan University of Technology, Isfahan.* Eight adult fistulated Shal rams with an initial body weight (BW) of 76±2.5 kg were used in this study. Ten days preliminary period was allowed for adaptation, feeds were offered twice daily at 8:00 and 16:00 h at a rate of 10% higher than the maintenance limit according to the standard tables of the NRC [10], and the salt-free diet was included 70% forage (alfalfa hay and wheat straw), and 30% concentrate (barley grain, soybean meal and cottonseed, mineral and vitamin supplements).

Treatments

Control group was consumed fresh water and other treatment groups contain, 3.5, 7.5, 11.5 g of salt per liter which was equal to 480 ppm total dissolved solids (TDS) for control treatment and 4000, 8000, 12000 ppm TDS for 2nd, 3rd, and 4th treatments, respectively. All rams had free access to drinking water according to their treatment. The electrical conductivity (EC) of these treatments was measured by the EC meter in the Chemical Laboratory, Institute of Animal Sciences. The value of TDS using EC data was calculated by the equation TDS = 640 * EC where TDS with ppm unit and EC with ds/m unit [5]. Chemical contents of the water showed in *Table 1*.

Table 1. *Chemical components of the fresh water (control)*

Component	Value
Na (mg/L)	119
Ca (mg/L)	39
Mg (mg/L)	7.9
Cl (mg/L)	35
SO_4 (mg/L)	116
HCO_3 (mg/L)	241
TDS (ppm)	480

TDS: total dissolved solids

Chemical Analysis

Chemical composition including dry matter (DM), ether extract (EE), crude protein (CP) and crude ash (CA) content of AH and BG were determined according to AOAC [11]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by procedures proposed by Van Soest et al.[12]. The non-fibrous carbohydrates (%NFC = 100 – (%NDF + %CP + %EE + %CA) were calculated as proposed by NRC [13].

In vitro Gas Production

Rumen fluid obtained from fistulated Shal rams before morning feeding when animals well adapted to drinking saline water (after 10 days adaptation period). Approximately 200 mg samples of dry feedstuff were weighed in triplicate and placed in a 100 mLcalibrated glass syringe. Feeds samples were incubated *in vitro* with rumen fluid-buffer mixture (30 mL) was transferred into the glass syringes of 100 mL according to the method of Menke and Steingass [14]. The samples were incubated in 100 mL syringe in a shacking incubator at 39°C. Volume of gas production was recorded at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation times and corrected for blank. In order to measuring methane (CH_4) production, after reading the syringes at the time of 24 h incubation, 4 ml of NaOH (10 M) was added to syringes and after 10 minutes, the said syringes were read again and removed. The NaOH (10 M) was introduced from the latter into incubated contents, thereby avoiding gas escape. Mixing of content with NaOH solution allowed for the absorption of CO_2, with the gas volume remaining within the syringe considered to be CH_4 [15].

Equations, Calculations and Statistical Analyses

Net gas production data were fitted to the model outlined by Ørskov and McDonald [16] and gas production parameters were estimated by the Fitcurve software version 6:

$P = A\ (1\text{-}e^{-ct})$; Where, A = potential gas production, c = the gas production rate constant for the insoluble fraction (b), t = the incubation time (h), P = the gas production at the time t

The digestible organic matter (DOM), net energy for lactation (NE_L) and metabolisable energy (ME) for tested feedstuffs were estimated using equations of Menke and Steingass [14], and short chain fatty acids (SCFA) was estimated using equation of Makkar [17].

Equations Used for Alfalfa Hay

DOM (%) = 0.889 GP + 0.45 CP + 0.651 CA + 14.88

ME (MJ/kg DM) = 0.136 GP + 0.057 CP + 0.00286 EE^2 + 2.2

NEL (MJ/kg DM) = 0.096 GP + 0.038 CP + 0.00173 EE^2 + 0.54

SCFA (mmol) = 0.0222 GP – 0.00425.

Equations Used for Barley Grain

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

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

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

SCFA (mmol) = 0.0222 GP –0.00425

Where, GP was gas production volume at 24 h of incubation time (mL/200 mg DM). General linear model (GLM) procedure of SAS [18] software was used in order to statistical analysis of data from gas production. The experiment and statistical analysis designed and performed based on complete randomized design (CRD) with four treatment and three replicates for each treatment. Treatment means was compared by Duncan multiple range tests.

Results

The chemical composition of AH and BG is given in *Table 2*. The gas production in experimental treatments at the different times of incubation of AH and BG showed in *Table 3*. There are significant differences between salinity levels and control treatment on AH and BG regarding gas production ($P<0.05$). Also, according to the results, the highest amount of AH gas production in most of the incubation times was observed in the 12000 ppm TDS treatment and the lowest in the treatment containing 4000 ppm TDS compared to the control treatment. But also, BG gas production was decreased at incubation times of 2, 4, 6, 8 and 96 in the treatments containing different levels of salinity water compared to the control treatment. The estimated kinetic parameters by exponential model are presented in *Table 4*. The significant difference was observed in A of AH ($P<0.05$). So that the highest amount of A was observe in the treatment containing 12000 ppm TDS, and the lowest amount was, in 4000 ppm TDS, but BG does not have significant differences between treatments. Significant difference was observed in c parameter of AH and BG ($P<0.05$). So that in AH, treatment containing 4000 ppm TDS has more than other groups in this fraction, and c of BG was significantly highest in the treatment containing 12000 ppm TDS and lowest in 8000 ppm salinity levels compared to the control treatment. The amounts of methane production from AH and BG under salinity levels and fresh water showed in *Table 4*. Different levels of salinity significantly affected methane emission ($P<0.05$). So that lowest methane emission of AH and BG was observed in the treatment containing

Table 2. *Chemical composition of alfalfa hay and barley grain (%)*

Constituents	Alfalfa Hay (AH)	Barley Grain (BG)
DM	93.1	92.5
CP	16.7	13.3
EE	1.1	1.8
Ash	10.1	2.7
NDF	37.0	16.5
ADF	26.5	6.7
NFC	35.1	65.5

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: non-fibrous carbohydrates

Table 3. *Effects of drinking water salinity (TDS as ppm) on gas production volume (mL/200 mg DM) at different incubation times*

Feedstuffs	Salinity Level	Incubation Time (h)								
		2	4	6	8	12	24	48	72	96
Alfalfa hay (AH)	Con. (480)	11.2±0.01	19.4±0.28^{a}	26.8±0.58^{a}	32.9±0.76^{a}	38.5±1.00^{a}	45.5±1.15^{b}	57.0±1.15^{b}	61.3±1.15^{b}	62.3±1.15^{b}
	4000	8.5±0.50^{c}	15.5±0.75^{b}	22.7±1.00^{c}	28.5±0.75^{c}	35.5±0.75^{b}	43.7±1.25^{b}	52.2±1.75^{d}	52.7±2.25^{d}	53.5±2.50^{d}
	8000	9.3±1.00^{c}	16.4±0.50^{b}	22.0±1.00^{c}	27.5±1.00^{c}	34.4±1.00^{b}	44.8±1.00^{b}	53.5±0.75^{c}	56.2±1.25^{c}	57.2±1.25^{c}
	12000	13.0±0.22^{a}	18.7±0.25^{a}	24.6±0.37^{b}	30.0±0.23^{b}	37.90±0.35^{a}	50.6±0.60^{a}	61.10±1.10^{a}	64.9^{a}±1.35	65.9^{a}±1.35
	SEM	0.032	0.282	0.454	0.428	0.473	0.596	0.717	0.903	0.957
	P- value	<0.0001	<0.0001	0.0003	<0.0001	0.0008	0.0002	<0.0001	<0.0001	<0.0001
Barley grain (BG)	Con. (480)	9.7±0.50^{a}	21.2±0.01^{a}	35.5±0.01^{a}	45.2±0.01^{a}	54.5±0.01^{a}	68.7±0.50^{ab}	85.2±1.5	92.0±2.00	95.0±1.00^{a}
	4000	6.7±0.2^{b}	15.2±0.03^{b}	27.2±0.50^{bc}	39.2±0.50^{b}	53.2±0.50^{a}	70.5±1.00^{a}	85.5±1.00	91.2±0.75	94.2±0.75^{a}
	8000	6.8±0.50^{b}	15.1±0.25^{b}	26.0±0.0^{c}	37.0±1.00^{c}	48.9±0.50^{b}	67.6±0.63^{b}	83.1±0.63	89.7±0.75	92.0±0.50^{b}
	12000	8.0±1.75^{ab}	15.5±1.5^{b}	28.5±1.50^{b}	41.0±1.75^{b}	53.5±1.75^{a}	70.5±1.25^{a}	84.2±0.5	89.2±0.50	90.3±0.45^{c}
	SEM	0.550	0.439	0.457	0.600	0.545	0.517	0.570	0.670	0.410
	P- value	0.01	<0.0001	<0.0001	<0.0001	0.0004	0.01	0.07	0.06	0.0002

a-d: *Means within a column with different subscripts differ (P<0.05),* ***Con:*** *control group, 4000: 4000 ppm salinity water, 8000:8000 ppm salinity water, 12000: 12000 ppm salinity water,* ***SEM:*** *Standard error mean*

Table 4. *Effects of different levels of drinking water salinity (TDS as ppm) on gas production parameters and methane production*

Feedstuffs	Salinity Level	A	c	CH_4 (%)	CH_4 (mL/200 mg)	CH_4 (mL/g DM)	CH_4 (mL/g OM)
Alfalfa hay (AH)	Con. (480)	60.6±0.49^{b}	0.0682±0.003^{b}	16.3±1.27^{a}	9.0±0.50^{a}	45.0±2.5^{a}	50.0±2.78^{a}
	4000	52.0±1.05^{d}	0.0912±0.005^{a}	9.8±1.65^{b}	4.3±1.04^{b}	21.6±5.20^{b}	24.0±5.78^{b}
	8000	56.2±0.90^{c}	0.0709±0.001^{b}	15.8±2.23^{a}	8.6±1.52^{a}	43.3±7.63^{a}	48.1±8.48^{a}
	12000	65.3±0.35^{a}	0.0604±0.002^{c}	14.7±2.91^{a}	7.1±1.75^{a}	35.8±8.78^{a}	39.8±9.75^{a}
	SEM	0.891	0.0017	1.219	0.750	3.754	4.171
	P- value	<0.0001	<0.0001	0.019	0.008	0.008	0.008
Barley grain (BG)	Con. (480)	91.3±0.88	0.0714±0.002^{b}	14.7±1.36^{a}	9.5±1.32^{b}	47.5±6.61^{b}	51.0±7.11^{b}
	4000	91.3±0.25	0.0721±0.003^{b}	9.6±2.90^{b}	5.3±1.53^{c}	26.6±7.64^{c}	28.6±8.21^{c}
	8000	89.9±0.85	0.0652±0.001^{c}	16.3±0.85^{a}	14.3±1.04^{a}	71.6±5.20^{a}	77.0±5.60^{a}
	12000	90.7±1.35	0.0781±0.001^{a}	15.6±0.66^{a}	11.6±0.58^{b}	58.3±2.89^{b}	62.7±3.10^{b}
	SEM	1.13	0.0013	0.975	0.677	3.38	3.64
	P -value	0.81	0.0009	0.005	<0.0001	<0.0001	<0.0001

a,b,c: *Means within a column with different subscripts differ (P<0.05);* ***Con:*** *control group (480 ppm), 4000: 4000 ppm salinity water, 8000:8000 ppm salinity water, 12000: 12000 ppm salinity water;* ***A:*** *potential of gas production,* ***c:*** *the gas production rate constant for the insoluble fraction,* ***CH_4:*** *Methane emission;* ***SEM:*** *Standard error mean*

4000 ppm TDS. The predicted SCFA, DOM, ME and NE_L are presented in *Table 5*. Significant differences were observed in AH between salinity levels and the control treatment (P<0.05). So that the highest amount of these factors in AH observed in 12000 ppm TDS and also in BG do not have significantly difference between salinity levels and control treatment, but there was significantly difference among of salinity levels.

Discussion

The chemical composition of the tested AH and BG in most cases are within the range of several studies [19-22]. Our results demonstrate that the gas production of AH and BG at different incubation times in a control treatment were in the range of previous reports [19,23-25]. The gas production parameters (*A* and *c*) for AH and BG in the control treatment was in range of the previous researches [19,20,23,26,27]. The SCFA in the control treatment of AH were higher than that of findings of Safaei et al.[20] and in agreement with Aghajanzadeh-Golshani et al.[19]. Values of SCFA production for BG (control treatment) in the present study were in the range of previous finding [25,27]. The amount of ME, NE_L and DOM of AH and BG in the control treatment were in the range of some other reports [20,24,26,27]. Regarding experimental treatment with different salinity levels, total gas production, SCFA,

Table 5. *Effects of different levels of drinking water salinity (TDS as ppm) on the amount of digestible organic matter (DOM), net energy for lactation (NEL) and metabolisable energy (ME) and short chain fatty acids (SCFA)*

Feedstuffs	Salinity Level	SCFA	DOM	ME	NE_L
Alfalfa hay (AH)	Con. (480)	1.00±0.03^{b}	69.5±1.03^{b}	9.3±0.16^{b}	5.5±0.11^{b}
	4000	0.96±0.03^{b}	67.8±1.11^{b}	9.1±0.17^{b}	5.3±0.12^{b}
	8000	0.99±0.02^{b}	68.8±0.89^{b}	9.2±0.14^{b}	5.4±0.10^{b}
	12000	1.12±0.0 1^{a}	74.0±0.53^{a}	10.0±0.08^{a}	6.0±0.06^{a}
	SEM	0.013	0.530	0.081	0.057
	P-value	0.0002	0.0002	0.0002	0.0002
Barley grain (BG)	Con. (480)	1.52±0.01^{ab}	86.1±0.50^{ab}	13.0±0.08^{ab}	8.3±0.06^{ab}
	4000	1.57±0.02^{a}	87.9±1.00^{a}	13.4±0.16^{a}	8.6±0.12^{a}
	8000	1.49±0.01^{b}	85.0±0.62^{b}	12.8±0.10^{b}	8.1±0.07^{b}
	12000	1.56±0.03^{a}	87.8±1.25^{a}	13.3±0.20^{a}	8.5±0.14^{a}
	SEM	0.011	0.516	0.081	0.059
	P-value	0.01	0.01	0.01	0.01

a,b: Means within a column with different subscripts differ (P<0.05); ***Con:*** *control group, 4000: 4000 ppm salinity water, 8000:8000 ppm salinity water, 12000: 12000 ppm salinity water;* ***SCFA:*** *short chain fatty acid (mmol);* ***DOM:*** *organic matter digestibility (%);* ***ME;*** *metabolisable energy (MJ/Kg DM);* ***NE_L;*** *net energy for lactation (MJ/Kg DM);* ***SEM:*** *Standard error mean*

DOM, ME and NE_L of the present study for AH and BG were similar, *A* parameter higher and *c* fraction lower than that of Gozali [28]. Methane emissions of AH at control treatment of the current study was in line with Safaei et al.[20] and Bhatta et al.[29]. Methane production of BG in the control treatment were in the range of Halimi-Shabestari et al.[30] and Fant et al.[31]. The difference in the results obtained in the control treatment of the present study with other studies may be due to the different chemical composition of feedstuffs, inter-laboratory variations, microbial origin and donor animals. Since the values of DOM, ME and NEL are calculated from the GP as well as CP, EE and CA content, varying amount in any of these factors can change estimated nutritive value of tested feedstuffs [19,32]. Based on the review of literatures, there is a limited number of studies regarding to the effect of water salinity on the rumen fermentability of feedstuffs. The experiments regarding the effect of salinity on the rumen fermentation process and consequently the fermentability and energy of the feeds showed that high consumption of salt, leads to an increase in salt concentration in the rumen and decrease in the ruminal production of SCFA [33]. High consumption of salt has led to a decrease in the number of bacteria, pH and rumen ammonia in cattle, but this condition does not exist in sheep, so that in sheep it can even increase the number of bacteria. However, the diversity of bacteria in cattle remains unchanged, but in sheep, rumen microbial diversity decreases [33,34]. It is expected that changes in rumen osmotic pressure in sheep that consume saline water are caused by changes in the concentration of electrolytes in the rumen, especially in the concentration of sodium and potassium. Also, high consumption of salt leads to an increase in rumen chloride concentration and osmotic pressure. Regarding to changes in rumen function, the observed effects appear to be related to increased ruminal fluid passage rates resulting from increased fluid intake [35]. All bacteria and some protozoa need sodium and potassium to grow and their tolerance to amounts of salt is different. Most rumen microorganisms have maximum growth and production in normal salt concentrations in the rumen and in some cases the number of bacteria is increased by adding a small amount of salt to the diet [33,34]. Various acid producing bacteria such as *Streptococcus bovis* can survive in salt-containing environments and it is likely that they are the dominant microbial population in sheep fed with high salinity [34]. With a sharp increase in salt concentration, *Selenomonas* will be the dominant bacteria in the rumen and the number of *Bacteroides* will decrease. *Bacteroides* are one of the major producers of succinate in the rumen and decrease in the number of this population results in a decrease in propionate production [33,34]. The results of Costa et al.[36] showed that cellulose and glucose fermenting bacteria are more sensitive to salinity than starch fermenting bacteria. So that starch fermenting bacteria were much more resistant than other microorganisms at high levels of salt in water (16000 mg/L). The population of cellulolytic bacteria decreased linearly with the increase of water salinity. The highest microbial protein production was obtained at the sodium chloride concentration of 8800 mg/L. Thomas et al.[37] also observed that with the increase of salt concentration in the rumen, the bacteria population decreases and as a result, the rumen performance is affected. Alves et al.[38]

also showed that with increasing salt concentration in the diet, the digestibility of NDF in cows, and therefore the acetate concentration in the rumen decreases linearly with increasing sodium chloride in water. The results of Costa et al.[39] also confirmed this and showed that when the available substrate for microorganisms is starch or glucose at different levels of salinity, the production of microbial protein will increase. Oliveira et al.[40] stated that fibrolytic microorganisms have an acetate pathway, so the decrease in the concentration of this volatile fatty acid in the environment can be justified by reducing NADH, reduction-oxidation, microbial growth and increasing the concentration of sodium chloride. Butyric acid producing bacteria are also sensitive to salt concentration. The imbalance of sodium, potassium, and chlorine in the rumen environment can disrupt the pH balance of the rumen and disrupt the supply of nutrients for microorganisms, thereby causing the death of some microbial populations [41].

Nowadays, methane production has received global attention due to its role as a greenhouse gas and global warming. Ruminants produce significant amounts of CH_4 as a byproduct of rumen fermentation under the anaerobic conditions and lose up to 12% of gross energy intake [42]. Ruminal methanogens are able to tolerate 1.5 percent of sodium chloride. These methanogens live with protozoa and the change in the population of protozoa can affect the number of methanogens [34]. Newbold and Ramos-Morales [43] reported that decreasing ruminal protozoan populations resulted in reduced methane production, which is often accompanied by a decrease in rumen pH. Alhraishawi et al.[44] showed that methanogenic bacteria are affected at a salt concentration of 6 grams per liter. The proportion of methane decreases significantly by increasing the dose of salt to between 10-15 g of salt. An increase in salt can increase SCFA and decrease the pH of the anaerobic digesters such as rumen. Usually, the chemical content in the feedstuffs affects the production of methane. High amounts of soluble carbohydrates in high-energy concentrates increase the production of propionate in the rumen, which prevents the growth of *methanogens* and thus reduces the production of methane per unit of fermented organic matter. Propionate acts as a hydrogen scavenger and reduces the supply of hydrogen for methane gas production [45]. In addition, the high content of ether extract helps to reduce methane because some fatty acids, especially medium chain fatty acids are toxic to *methanogens* [46]. The type of feed can also affect methane production. Alfalfa contains crude protein with high digestibility, which leads to primary fermentation and gas production. The observed difference in methane output between feedstuffs is attributed to their nutrient composition because the slow digestion of feed is associated with higher methane production [29]. With these interpretations, several factors such as breed, age, salt content in water and diet, type of feeds and diet, population of protozoa, rumen pH and rumen passage rate can affect enteric methane production [7,29,34,44].

In an overall conclusion, drinking water salinity at the level of 12000 ppm TDS led to increase gas production and SCFA, DOM, ME and NE_L of AH. Methane production was affected by saline water consumption, so that the lowest amount of methane emission in AH and BG was observed in the treatment containing 4000 ppm TDS. Different levels of salinity did not affect the gas production and amount of SCFA, DOM, ME and NE_L of BG compare with control treatment. It seems that, drinking water salinity affect fermentation kinetics and nutritive value of AH and BG depending on the level of salinity and the type of feedstuffs. The results of current study showed that saline water up to12000 ppm TDS may be used for adult sheep without negative effects on nutritional value of consumed feedstuffs. It should be noted that the high level of salt in the diet can affect the current results.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of Interest

There is not conflict of interest with any person or institute/ organization regarding this manuscript.

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Authors Contribution Statement

R.S.D.N. and N.M.S. were as the supervisors (designed the experiments and interpretation of the results as well as leading the manuscript writing and revising), M.A.P.M. operating the the experiments, collecting data and writing the manuscript, A.R.S. and A.A.G. were the thesis advisors and helped in all process of experiment and manuscript preparation as well as statistical analyses.

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