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EFFECT OF ALTERNATION METHODS OF DRINKING SALINE WELL WATER WITH TAP WATER ON LIVER ENZYMES, BLOOD MINERALS AND TRIIODOTHYRONINE HORMONE IN BROILER CHICKENS

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ABSTRACT: This study aimed to determine the effects of alternation methods (AM) of drinking saline well water (SWW) with tap water (TW) on liver enzymes, blood minerals, and tri-iodothyronine in broiler chickens.

A total number 180 unsexed commercial broiler chicks Ross Alananny one day old were randomly divided into six equal groups, the first group, chicks drank TW containing 450 ppm total dissolved solids (TDS) from one day old to 36 day of age (the end of the experiment) and considered as control group (C). Treatment 1 (Tr1), chicks drank SWW containing 3100 ppm TDS from one day old to the end of the experiment, Tr2, chicks drank SWW every other day alternatively with TW from one day old to the end of the experiment, Tr3, chicks drank SWW every other week alternatively with TW from one day old to the end of the experiment, Tr4, chicks drank TW during the first 18 days of age and drank SWW from 19-36 days of age, Tr5, chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age, Tr2 to Tr5 considered as alternation methods.

Total protein significantly decreased in all treatments when compared with C. Globulin was significantly decreased in Tr2, Tr4 and Tr5 when compared with C.

There were insignificant differences between AM treatments and C in alanine aminotransferase, Aspartate aminotransferase and triiodothyronine hormone. There were insignificant differences among Tr2, Tr3 and Tr5 when compared with C in sodium level.

In conclusion, drinking SWW containing 3100 ppm TDS for broiler chickens caused some negative effects on liver enzymes, blood minerals and tri-iodothyronine, Therefore, when it is necessary to use saline water, it is preferable to alternately drink SWW with TW every other week, or drink SWW during the first 18 days of age, followed by TW, to enhance these negative effects.

Key words: Broilers, liver enzymes, tri-iodothyronine hormone, saline water, alternation methods.

INTRODUCTION

Water is essential to many aspects of chicken metabolism, and it plays a critical role in the creation of chickens (Saleh et al., 2023). Egypt's water resource is limited, and groundwater comes as the second important source after the Nile River (Abdel-Shafy and Aziza H. Kamel 2016). According to Egyptian regulations, poultry farms must be located away from populated areas. As a result, the majority of chicken farms are established on newly developed desert area, with groundwater serving as their primary source of water. (Barton 1996).

The groundwater in some areas has low, moderate or high salinity and the desalination cost is very costly, Water salinity is stated as the total concentration of soluble inorganic ions in particular sodium and chloride. (Atekwana et al., 2004 and Rusydi, 2018).

Salinity levels, Animal species and the salt minerals types in water are the main factors that effect of the ability of Animal to tolerate water salinity (Kellems and Church 1998). Several studies showed that the Animals' performance decreased as the water salinity increased (Maheri Sis et al., 2019 and Abdelsattar et al., 2020). The National Research Council (1974) stated that, for any class of poultry, water with a TDS of less than 1000 ppm should not pose a danger. However, drinking water that has a lot of dissolved salts in it might affect how well intracellular macromolecules are regulated and alter osmotic control, which can lead to declining performance (Kettunen et al. 2001). According to research, osmotic stress results in a temporary polyuria when TDS in drinking water for birds exceeds 1500 ppm, and osmoregulatory homeostasis is jeopardized when TDS exceeds 3000 ppm. (Bagley et al. 1997 and Goldstein & Skadhauge 2000). Drinking water that contains more than 3000 ppm (TDS) causes watery excrement to be removed from poultry, which can lead to moist litter issues, a decline in growth rate, and ultimately higher flock mortality (Sumano et al., 2004). Similarly, it has been shown that broiler chickens' blood pressure, water intake, and litter wetness are all increased when they consume water with high salt chloride content (Alahgholi et al., 2014). El-Bassiony et al. (2020) demonstrated that high salinity can exacerbate oxidative stress in the liver, leading to potential damage and altered enzyme activity.

So, several studies look for an effective way to reduce the harmful effects of drinking saline water (SW). From these ways, using the alternation of SW with tap water (TW), Emam et al. (2022) and Morsy et al. (2016) found that drank SW every other week alternatively with TW reduced the negative effect of drinking SW on blood parameters of laying hens and rabbits, respectively.

This study aimed to determine the effects of alternation methods of drinking saline well water with tap water on liver enzymes, blood minerals, and tri-iodothyronine hormone in broiler chickens.

MATERIALS AND METHODS

Study area

This investigation was implemented at private chicken's farm (Latitude 28° 50′36.6 N; Longitude 30° 59′58.5 E), Beni-Suef Government, Egypt, The experiment started on September up to October 2023.

Experimental design

A total number of unsexed 180 Commercial chicks Ross Alananny (one day old and body weight of 36.16±0.30) were randomly divided into six equal treatments (30 chicks of each) which in turn divided into three equal replicates (10 chicks), the first group, chicks drank tap water (TW) containing 450 ppm total dissolved solids (TDS) from one day old to 36 day of age (the end of the experiment) and considered as control group (C). Treatment 1 (Tr1), chicks drank saline well water (SWW) containing 3100 ppm TDS from one day old to the end of the experiment, Tr2, chicks drank SWW every other day alternatively with TW from one day old to the end of the experiment, Tr3, chicks drank SWW every other week alternatively with TW from one day old to the end of the experiment, Tr4, chicks drank TW during the first 18 days of age and drank SWW from 19-

36 days of age, Tr5, chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age.

Saline well water were obtained from well near the farm, desalinate water were obtained from desalination station and considered as TW because the Nile river water don't reach the area of poultry farm, SWW and TW chemically analyzed in Animal Health Research Institute - Ministry of Agriculture and Land Reclamation – Doki- Egypt, according to Muller (1995) and showed in Table 1.

Management and feeding

Chickens reared on the floor in separate pens in a closed farm and kept under the same conditions during the experimental period, Chicks fed *ad-libitum* on recommended standard rations according to the Ross broiler management guide (2002). Chicks was fed on pelleted starter diet (23 % crude protein, and metabolizable energy 3010 Kcal/Kg diet) from one day to 10 days of age, chicks were fed on a pelleted grower diet (21 % crude protein, and metabolizable energy 3150 Kcal/Kg diet) from 11-28 days of age, chicks were fed on a pelleted finisher diet (19 % crude protein, and metabolizable energy 3200 Kcal/Kg diet) from 29-36 days of age (end of the experiment). The lighting program consisted of a period of 23 h light and 1 h of darkness during the experiment. The temperature was controlled and gradually reduced from 33° C to 23° C in day 36 of age. Chicks were kept under the same managerial and hygienic conditions. Chicks were healthy and treated with vaccines.

Ethical Approval

This study was conducted according to the guidelines for care and use of laboratory Animal by Beni-suef University (BSU-IACUC). Approval number (022-471).

Measurements

At the end of experiment, blood samples (4 ml) were collected from wing vein from 15 birds which selected randomly of each treatment, samples collected into anticoagulant EDTA, samples centrifuged for 20 minutes at 3000 rpm to get plasma and it stored at –20 ^{0}C until determination of free Tri-iodothyronin*e* (T3) hormones which determined in private lab (Family lab for medical tests) by The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas immunoassay analyzers. Blood metabolites, total Protein and albumin were determined by using profitable kits (Spectrum company), creatinine was determined in chemistry lab of faculty of agriculture –Beni-Suef university by using profitable kits (Diamond diagnostics company), alanine transaminase and aspartic transaminase were determined by using profitable kits (BioMED diagnostics company), globulin was calculated by the difference between total protein and albumin. Albumin/ globulin ratio was calculated

Blood minerals as sodium, Phosphorus and Potassium were calorimetrically by using commercial kits in chemistry lab of faculty of agriculture –Beni-Suef University.

Statistical analysis

Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004) according to following model:

$Y_{ij}=\mu + Tri + e_{ij}$

Where, Yij = observations, μ = overall mean, Tri = effect of i^{th} treatment (i: 1-6),

e_{ij} = experimental error.

Duncan's New Multiple Range Test (Duncan, 1955) separated differences among treatment means.

RESULTS AND DISCUSSIONS

Blood metabolites

Table (2) showed that effect of alternation methods (AM) of drinking saline well water (SWW) with tap water (TW) on blood metabolites of broiler chickens. Drinking SWW (3100 ppm TDS) during experimental period (Tr1) or drinking SWW with TW in AM treatments (Tr2 – Tr5) led to significantly decreased ($p \leq 0.05$) of total protein (TP) when compared with C (control group).

While, there were insignificant effects of drinking SWW with TW in AM treatments (Tr2-Tr5) or drinking SWW (Tr1) on albumin concentration (Alb) compared with C, additionally, Albumin was significantly ($p \leq$

0.05) increased in Tr4 when compared with Tr2.

Globulin concentration (Glb) was significantly ($p \leq 0.05$) decreased in Tr2, Tr4 and Tr5 when compared with C, while, there were insignificant differences between treatments from Tr1 to Tr5 in Glb concentration

There were insignificant differences among treatments on albumin/globulin ratio (A/G ratio) and Creatinine concentration.

All values of TP with in normal range according to Harr et al. (2002) who reported that TP values of broiler chickens ranging from 2.5 to 4.5 g/dL, The decrease in TP in alternation treatment (Tr2 to Tr5) additionally to Tr1 might be linked to the birds' exposure to salt stress due to stress raises the blood level of the corticosterone hormone in birds. (Gharib et al.2005), which works to partially break down protein in order to convert protein sources into sugar (Malheiros et al. 2003), Also, these results might suggest that drinking SW might reduce hepatic synthesis of RNA, which in turn depresses the incorporation of amino acids for protein synthesis (Melillo, 2013, Tata and Widnell, 1966; Suckow et al. 1997), the reduction in TP may be interpreted as a physiological adaptation to limit the excessive entry of fluids into the interstitial tissue because of the increasing salinity (Abdel-Samee and El-Masry, 1992).

The results of the current study are in agreement with what was confirmed by Morsy et al., (2012) who showed a significant decrease in the concentration of TP and Glb in the serum of laying hens that were given SW containing 4000 and 6000 ppm TDS compared to the birds that were given TW.

Liver enzymes

Effect of AM of drinking SWW with TW on liver enzymes of broilers chickens showed in table (3), drinking SWW (3100 ppm TDS) in Tr1 led to significantly increased of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) when compared with C (control group), This increase in AST and ALT in Tr1 could be an indication of a liver activity reduction caused by the rising salinity of drinking water (Marai et al. 2010; Attia et al. 2015; Morsy et al. 2016). The results of the current study are in agreement with what was confirmed by Emam et al., (2019) indicated that broilers drinking SW at a level of 3398 ppm of TDS led to a significant increase in the activity of ALT and AST enzymes.

While, there were insignificant differences between AM treatments and C in ALT, while, AST was significantly increased in Tr4 when compared with C and there were insignificant differences between other AM treatments and C (Table 3). These results may be due to alternation methods led to enhance of liver activities by cleaning salinity from blood and these results refer to using SW during final period (19-36 days of age) led to harmful significant effect on AST enzyme Level. These results agreement with Emam et al., (2019) and Morsy et al., (2016) who found that alternation methods (drinking SWW alternation weekly by TW) enhance liver enzymes in broilers and rabbits, respectively.

Blood minerals

Table (4) showed that effect of AM of drinking SWW with TW on blood minerals of broilers chickens. Blood Sodium level (Na) was significantly increased in Tr1 and Tr4 when compared with Tr1. While, there were insignificant differences between Tr2, Tr3 and Tr5 when compared with C in Na level. These results may be due to chickens in Tr1 consumed more water which have more Na than other treatments (Saleh et al. 2023).

There were insignificant effects of alternation methods (Tr3, Tr4 and Tr6) and drinking saline well water (Tr2) on phosphorus (P) level when compared with Tr1, while, P level was significantly increased in Tr5 when compared with Tr3. On the other hand, blood Potassium level don't affect by drinking saline well water or by alternation methods.

These results agree with Alahgholi et al., (2014) who found that consuming SW negatively impacted blood Na in broiler chickens. This suggests that SW disrupts electrolyte balance, potentially leading to deficiencies in crucial minerals. These findings suggest that alternating water sources may help broilers regulate mineral intake and potentially

maintain better blood mineral balance when faced with SW.

Hormonal responses (Tri-iodothyronine)

Figure (1) showed effect of alternation methods of drinking SWW with TW on tri-iodothyronine (T3) of broiler chickens. T3 was significantly increased in Tr3 and Tr5 when compared with Tr1. On the other hand, there were insignificant differences among alternation methods (Tr2, Tr3, Tr4 and Tr5) and C, additionally; there were insignificant differences among Tr1, Tr2 and Tr4.

These results may be due to drinking saline well water in Tr1 might down regulate receptors for thyroid-stimulating hormone, ultimately leading to decreased T3 production in the thyroid gland (Amal, M. Hassan., 2013).

These results were agreement with Emam et al, (2019) who found that decreased T3 levels in laying hens given SW compared to TW and found that alternating SW with TW weekly improved T3 level

In conclusion, drinking SWW containing 3100 ppm TDS for broiler chickens caused some negative effects on liver enzymes, blood minerals and tri-iodothyronine, Therefore, when it is necessary to use saline water, it is preferable to alternately drink SWW with TW every other week, or drink SWW during the first 18 days of age, followed by TW to enhance these negative effects.

Table (1): Chemical analysis of tap water and saline well water.

Parameters	Tap water	Saline well water
TDS (mg/l)	450	3100
EC (µS/m)	0.236	8.42
PH	6.52	8.17
Sodium chloride (mg/l)	66	4950
Calcium (mg/l)	40	225
Bicarbonate (mg/l)	20	160
Sulphate (mg/l)	20	10
Ammonia(mg/l)	Not detected	Not detected
Nitrate(mg/l)	Not detected	0.05
Total alkalinity (mg/l)	20	160
Carbonate alkalinity(mg/l)	0	0
Hydroxide alkalinity(mg/l)	0	0
Copper (ppm)	Less than LOQ (0.008)	Less than LOQ (0.008)

TDS=Total dissolved solids; EC=Electric conductivity

Table (2): Effect of alternation methods on the blood metabolites of broiler chickens.

Traits	Treatments						±SE
	C	Tr1	Tr2	Tr3	Tr4	Tr5	
			Alternation methods				
TP (g/dl)	5.44 [a]	4.04 [b]	3.02 [b]	3.69 [b]	3.68 [b]	3.62 [b]	0.37
Alb (g/dl)	1.62 [ab]	1.50 [ab]	1.10 [b]	1.50 [ab]	2.00 [a]	1.51 [ab]	0.26
Glb (g/dl)	3.81 [a]	2.53 [ab]	1.92 [b]	2.19 [ab]	1.68 [b]	2.11 [b]	0.52
A/G Ratio	0.52	0.97	0.64	0.83	1.32	0.83	0.28
Crea (Mg/dl)	0.39	0.60	1.20	0.55	1.18	1.03	0.31

TP=total protein; Alb=albumin; Glb=globulin; A/G ratio= albumin/ globulin ratio; Crea= Creatinine; C= chicks drank tap water (TW) from one day old to the end of experiment (36 days of age). Tr1 = chicks drank saline well water (SWW) containing 3100 ppm TDS from one day old to the end of experiment, Tr2= chicks drank SWW every other day alternatively with TW from one day old to the end of experiment, Tr3= chicks drank SWW every other week alternatively with TW from one day old to the end of experiment, Tr4= chicks drank TW during the first 18 days of age and drank SWW from 19-36 days of age (end of experiment) and Tr5= chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age (end of experiment).

a, b, Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table (3): Effect of alternation methods on the liver enzymes of broiler chickens.

Traits	Treatments						±SE
	C	Tr1	Tr2	Tr3	Tr4	Tr5	
			Alternation methods				
ALT (U/L)	11.79 [b]	21.83 [a]	15.71 [ab]	10.48 [b]	17.02 [ab]	13.09 [b]	2.18
AST (U/L)	190.00 [b]	284.22 [a]	191.06 [b]	200.02 [b]	277.66 [a]	203.11 [b]	10.02

AST= Aspartate aminotransferase; ALT= alanine aminotransferase; C= chicks drank tap water (TW) from one day old to the end of experiment (36 days of age). Tr1 = chicks drank saline well water (SWW) containing 3100 ppm TDS from one day old to the end of experiment, Tr2= chicks drank SWW every other day alternatively with TW from one day old to the end of experiment, Tr3= chicks drank SWW every other week alternatively with TW from one day old to the end of experiment, Tr4= chicks drank TW during the first 18 days of age and drank SWW from 19-36 days of age (end of experiment) and Tr5= chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age (end of experiment).

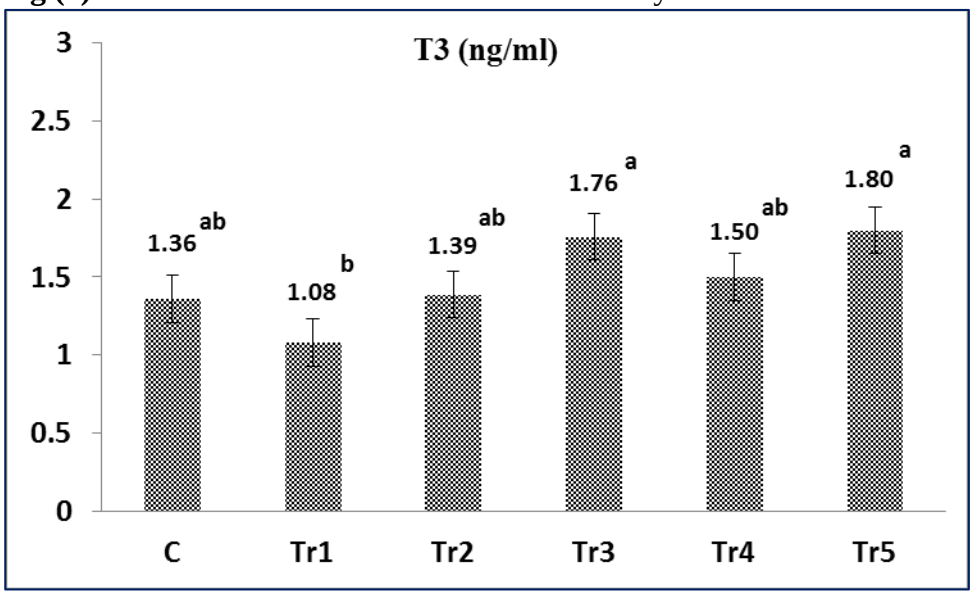
a, b, Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table (4): Effect of alternation methods on the blood minerals of broiler chickens.

Traits	Treatments						±SE
	C	Tr1	Tr2	Tr3	Tr4	Tr5	
			Alternation methods				
Na (mEq/l)	64.08 ^{c}	93.33 ^{a}	65.56 ^{c}	72.92 ^{bc}	88.99 ^{ab}	73.25 ^{bc}	6.23
P (ppm)	168.00 ^{ab}	187.25 ^{ab}	152.00 ^{b}	178.50 ^{ab}	196.00 ^{a}	173.50 ^{ab}	11.00
K (ppm)	436.50	453.00	364.75	397.75	450.50	474.75	39.86

Na= Sodium; P=phosphorus; K= Potassium; C= chicks drank tap water (TW) from one day old to the end of experiment (36 days of age). Tr1 = chicks drank saline well water (SWW) containing 3100 ppm TDS from one day old to the end of experiment, Tr2= chicks drank SWW every other day alternatively with TW from one day old to the end of experiment, Tr3= chicks drank SWW every other week alternatively with TW from one day old to the end of experiment, Tr4= chicks drank TW during the first 18 days of age and drank SWW from 19-36 days of age (end of experiment) and Tr5= chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age (end of experiment).
a, b,c, Means bearing different superscripts within the same row are significantly different (P<0.05).

Fig (1): Effect of alternation methods on tri-iodothyronine hormone.



T3= tri-iodothyronine; C= chicks drank tap water (TW) from one day old to the end of experiment (36 days of age). Tr1 = chicks drank saline well water (SWW) containing 3100 ppm TDS from one day old to the end of experiment, Tr2= chicks drank SWW every other day alternatively with TW from one day old to the end of experiment, Tr3= chicks drank SWW every other week alternatively with TW from one day old to the end of experiment, Tr4= chicks drank TW during the first 18 days of age and drank SWW from 19-36 days of age (end of experiment) and Tr5= chicks drank SWW during the first 18 days of age and drank TW from 19-36 days of age (end of experiment).
a, b, Means bearing different superscripts within the same bar are significantly different (P<0.05).

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الملخص العربى

تاثير طرق التناوب بين شرب مياة الابارالمالحة ومياة الصنبور على انزيمات الكبد والعناصر المعدنية للدم وهرمون الثيرونين ثلاثى اليود للدجاج اللاحم

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تهدف هذة الدراسة الى تحديد تاثير طرق التناوب بين شرب مياه الابار المالحة مع مياه الصنبور على انزيمات الكبد والعناصر المعدنية للدم وهرمون الثيرونين ثلاثى اليود للدجاج اللاحم.
تم تقسيم 180 كتكوت تسمين عمر يوم غير مجنس بصورة عشوائية من سلالة الروص التجارية الى 6 مجاميع .
المجموعة الاولى شربت الكتاكيت مياه الصنبور (450 جزء فى المليون أملاح كلية ذائبة) من عمر يوم وحتى ٣٦ يوم من العمر (نهاية التجربة) واعتبرت مجموعة التحكم ، المعاملة الاولى شربت الكتاكيت مياه الابار المالحة المحتوى على 3100 جزء فى المليون املاح كلية ذائبة من عمر يوم وحتى نهاية التجربة ، المعاملة الثانية شربت الكتاكيت مياه الابار المالحة تبادليا مع مياه الصنبور بصورة يومية من عمر يوم وحتى نهاية التجربة ، المعاملة الثالثة شربت الكتاكيت مياه الابار المالحة تبادليا مع مياه الصنبور بصورة اسبوعية من عمر يوم وحتى نهاية التجربة ، المعاملة الرابعة شربت الكتاكيت مياه الصنبور خلال اول 18 يوم من العمر ثم مياه الابار المالحة خلال ١٩-٣٦ يوم من العمر، المعاملة الخامسة شربت الكتاكيت مياه الابار المالحة خلال اول 18 يوم من العمر ثم مياه الصنبور خلال ١٩-٣٦ يوم من العمر.
انخفض البروتين الكلى معنويا فى كل المعاملات مقارنة بمجموعة التحكم ، انخفض الجلوبيولين معنويا فى المعاملة الثانية والرابعة والخامسة مقارنة بمجموعة التحكم، لم يكن هناك اى اختلافات معنوية بين معاملات التناوب ومجموعة التحكم فى كلا من ناقل الامين الانانين و ناقل الامين اسبرتات و هرمون الثيرونين ثلاثى اليود .
لم يكن هناك اختلافات معنوية بين المعاملة الثانية والثالثة والخامسة مقارنة مع مجموعة التحكم فى مستوى الصوديوم.
نستخلص من هذة الدراسة ان شرب مياه الابار المالحة للدجاج اللاحم المحتوى على 3100 جزء فى المليون املاح كلية ذائبة تسبب بعض التاثيرات السلبية على انزيمات الكبد والعناصر المعدنية بالدم وهرمون الثيرونين ثلاثى اليود، لذلك عند الضرورة لاستخدام المياه المالحة من المفضل استخدام طريقة التناوب اسبوعيا بين مياه الابار المالحة ومياه الصنبور او شرب مياه الابار المالحة خلال اول 18 يوم من العمر متبوعا بشرب مياه الصنبور لتحسين هذة الاثار السلبية .

الكلمات المفتاحية : الدجاج اللاحم ، انزيمات الكبد ،هرمون الثيرونين ثلاثى اليود، المياه المالحة ، طرق التناوب.