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BLOOD PICTURE, METABOLITES AND BLOOD MINERALS OF RABBITS AS INFLUENCED BY DRINKING SALINE WATER

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ABSTRACT

This work aimed to study the effect of alternation of drinking saline well water with tap water on blood picture, metabolites and blood minerals of rabbits. A total number of 48 V-Line rabbits old 5 months (30 does, body weight of 2955.0 ± 32.6 g and 18 bucks, body weight of 2850.0 ± 31.2 g) were used in this study. Rabbits were randomly divided into three equal treatments (10 does and 6 bucks / treatment). Rabbits of the 1st treatment (T1) considered as control group drank tap water (301 ppm TDS), rabbits of 2nd treatment (T2) drank well water (5568 ppm TDS), while rabbits of 3rd treatment (T3) drank well water followed with drank fresh tap water every week until the end of experiment (alternated system).

The results demonstrated that values of red blood cells and hemoglobin decreased (P<0.05) in the doe rabbits of T2 by 27.6 and 21.4 %, respectively and by 19.7 and 19.6, respectively in the bucks when compared to T1 treatment. On the other hand, white blood cell significantly deceased in the doe rabbits of T2 by 26.1 % and insignificantly decreased in the buck rabbits of T2 by 22.2 % when compared to T1 treatment (control group). However, no significant differences in the white and red blood cells and hemoglobin were observed among the rabbits of T3 and two other treatments. In contrary, Hematocrit (%) increased (P<0.05) in doe and buck rabbits of T2 and T3 when compared to T1 treatment. Total protein, globulin and glucose concentrations of rabbits drank saline water (T2) decreased (P<0.05) in does and bucks as compared to T1 treatment. However, doe rabbits of T3 showed decreases (P<0.05) of total protein concentration by 6.7 % and globulin concentration by 18.5 % as compared to the rabbits of T1. On the other hand, rabbits of T3 showed insignificant increase in glucose concentration by 9.6 % comparing with the rabbits of T2. Cholesterol and total lipids concentrations were significantly increased in the does and bucks of T2 as compared to the rabbits of T1. Doe and buck rabbits of T2 showed significantly increased in alanine transaminase and aspartate transaminase concentrations as compared to T1 and T3 treatments. Drinking saline well water (T2) increased (P<0.05) concentration of calcium by 14.7 and 10.2 % in doe rabbits compared to T1 and T3 treatments, respectively. However, phosphorus concentration was insignificantly decreased in the rabbits of T2 as compared to other two treatments. Sodium and chloride concentrations were increased (P<0.05) in the does and bucks of T2 as compared to rabbits of T1. In contrary, potassium concentration was decreased (P<0.05) in the does and bucks of T2 as compared to rabbits of T1. Aldosterone and tri-iodothyronine hormones were decreased (P<0.05) in the rabbits of T2 than that of T1 and T3 treatments.

In conclusion, V-Line rabbits drank saline well water (5568 ppm TDS) showed harmful effects on hemato-biochemical, minerals and hormonal parameters. However, rabbits alternated drank saline water with tap water every week may alleviate the drastic effect of drinking saline well water on blood parameters and thus may be positively reflected on rabbit's performance.

Key words: V-Line rabbits, blood picture, metabolites and hormonal assay, saline water.

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1. INTRODUCTION

Farms founded in desert areas in Egypt depend on well water for drinking animals reared in. This water often contains high concentrations of dissolved salts. The consumption of saline water leads to change in the retention and excretions of minerals and may change mineral balance (Marai and Habeeb, 1994). Keeping of mineral balance in animals is essential for rabbit productive and reproductive performance. Kellems and Church (2002) reported that one of the principle factors affecting water quality is salinity, i.e. the amount of total dissolved salts (TDS) in the water. Animals' drinking water of high concentrations of some inorganic ions such as Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, SO_4^{2-} and HCO_3^{-} may cause harmful effects resulting in poor performance, illness or even death. Blood parameters and minerals are changed by drinking saline water (Marai *et al*., 1994 and Amal, 2003).

Minerals in drinking water are more readily absorbed than food because water does not usually contain chelating agents which might prevent absorption of elements (Porter *et al*., 1988). Most farms in desert area depend upon wells as a source of drinking water that may be contain some elements higher than the permissible limits, which may have an adverse effect on animal health, production and reproduction (El-Sayed, 1991). Therefore, this work aimed to study the effect of alternation of drinking saline well water with tap water on blood picture, metabolites, minerals and hormones of V-Line rabbits.

2. MATERIALS AND METHODS

The present study was carried out in a private rabbits farm at Cairo - Oasis high way, about 60 km South West Cairo from September 2013 up to February 2014. The laboratory work was executed in Lab of Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt and in the Labs belonging to Egyptian-Spanish Project titled "Raise the level of low income families and activate the role of woman in community development through the use of modern technologies in the project of Rabbits in Egypt", Faculty of Agriculture, Cairo University Research Park, CURP. The aim of this work was to study the effect of alternation of drinking saline water with tap water on blood picture, metabolites, minerals and hormones of V-Line rabbits.

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

Chemical analysis	Saline well water	Tap water
Total dissolved salts (Dacisamenz / m)	6.9	0.47
Electric conductivity (ds/m)	5568.0	301.0
Hydrogen ion (pH)	7.8	7.6
Dissolved anions (Mellimka / 1L)		
Carbonates	-	-
Bicarbonate	2.0	0.5
Chloride	40.2	3.5
Sulphates	25.1	0.68
Cations dissolved (Mellimkavii / l)		
Calcium	23.0	1.5
Magnesium	16.2	0.5
Sodium	26.1	3.5
Potassium	0.13	0.13
Residual sodium carbonate	-	-
Ratio of sodium adsorbed	5.9	2.5

A total number of 48 V-Line rabbits old 5 months (30 does, body weight of 2955.0 ± 32.6 g and 18 bucks, body weight of 2850.0 ± 31.2 g) were used in this study. Rabbits were randomly divided into three equal treatments (10 does and 6 bucks / treatment). Rabbits

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of the 1st treatment (T1) considered as control group drank tap water (301 ppm TDS), rabbits of 2nd treatment (T2) drank well water (5568 ppm TDS), while rabbits of 3rd treatment (T3) drank well water followed with drank fresh tap water every week until the end of experiment (alternated system as a biostimulation method). Well and tap water were chemically analyzed (Table 1) according to Muller (1995). Rabbits were fed, *ad-libitum*, a commercial concentrate pelleted diet containing 18.0 % crude protein, 16.3 % crude fiber, 2.5 % fat, 0.6 % minerals mixture and 2730 kcal / kg digestible energy.

Blood samples were taken from marginal ear vein into ethylenediaminetetra-acetic acid (EDTA) tubes to examine immediately hematological parameters. Red and white blood cells were counted in blood under the microscope by means hemocytometer according to (Nemi, 1986). Hemoglobin concentration was determined colorimetrically in fresh blood samples using ready-made kits. Wintrobe hematocrit tubes were used for determination of the hematocrit value. Blood was centrifuged for 15 minutes of 3000 rpm to collect plasma before being stored at -20^{o}C until blood analysis. Blood metabolites (total protein, albumin, glucose, cholesterol, alanine transaminase, aspartate transaminase) and minerals (calcium, phosphorus, sodium, potassium, chloride and magnesium) were determined calorimetrically by using commercial kits. Aldosterone and tri-iodothyronine hormones were determined by radioimmunoassay (RIA) method.

Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The design was one way analysis and the model was as follows:

$Y_{ij}=\mu + Tr_i + e_{ij}$ Where,

Y_{ij}= any observation of j^{th} animal within i^{th} treatment.

μ = overall mean.

Tr_i = effect of i^{th} treatment (i: 1-3).

e_{ij}= experimental error.

Duncan Multiple Range Test (Duncan, 1995) was used to test the level of significance among means.

3. RESULTS AND DISCUSSION

A- Hematological parameters

Values of red blood cells and hemoglobin decreased (P<0.05) in the doe rabbits of T2 treatment by 27.6 and 21.4 %, respectively and by 19.7 and 19.6, respectively in the bucks when compared to T1 treatment.

Table (2). Hematological parameters of V-line rabbits as affected by drinking saline well water.

Items	Sex	Treatments		
		T1	T2	T3
White blood cells (×10^3 mm^3)	**Doe**	8.2±1.50	6.4± 0.75	7.3±0.99
	Buck	11.1^{a}±0.49	8.2^{b}±0.75	10.4^{ab}±1.0
Red blood cells (× 10^6/mm^3)	**Doe**	7.2^{a}±0.55	5.2^{b}±0.39	6.3^{ab}±0.63
	Buck	7.6^{a}±0.39	6.1^{b}±0.41	6.9^{ab}±0.24
Hemoglobin (g/dl)	**Doe**	14.5^{a}±1.1	11.4^{b}± 1.0	13.2^{ab}± 1.3
	Buck	15.3^{a}±0.79	12.3^{b}±0.83	13.9^{ab}±0.49
Hematocrit (%)	**Doe**	40.8^{b}±1.0	48.2^{a}±1.0	45.7^{a}± 1.2
	Buck	41.9^{c}±0.71	48.1^{a}±0.89	45.5^{b}±0.53

a, b, c Means bearing different superscripts within the same row are significantly different (P<0.05).T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm); T3 = rabbits alternated drank saline well water with tap water weekly.

On the other hand, white blood cell significantly deceased in the doe rabbits of T2 by 26.1 % and insignificantly decreased in the buck rabbits of T2 group by 22.2 % when compared to T1 treatment (Table 2). However, no significant differences in white and red blood cells and hemoglobin were observed among the rabbits of T3 and two other treatments.

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In contrary, hematocrit (%) increased (P<0.05) in doe and buck rabbits of T2 (by 17.8 and 14.7 %, respectively) and T3 (by 12 and 8.5 %, respectively) when compared to T1 treatment (Table 2).

This decrement might be attributed to the decrease of erythropoitine hormone by damage kidney tissue. This hormone stimulates narrow bone to produce red blood cells **(**Guyton and Hall, 2006). Also, the results of some studies reported that it is may be attributed to increased of total body water with increase in water intake (Amal, 2003). This higher water intake would lead to hemodilution, which resulted in the relative lower white blood cells, red blood cells, hemoglobin concentration and increased hematocrit % (Attia *et al.*, 2015).

B- Biochemical parameters

Total protein, globulin and glucose concentrations of rabbits drank saline water (T2) decreased (P<0.05) in the doe (by 6.7, 22.2 and 13.1%, respectively) and in the bucks (by 11.9, 21.4 and 12.1 %, respectively) as compared to T1 treatment (Table 3). However, doe rabbits of T3 showed decreases (P<0.05) of total protein concentration by 6.7 % and globulin concentration by 18.5 % as compared to the rabbits of T1 (Table 3). On the other hand, rabbits of T3 showed insignificant increase in glucose concentration by 9.6 % comparing with T2 treatment. This result may related to the fact that water is held bach to the body fluids to dilute out the retained salts, resulting in dilution of blood proteins and so, decreases its concentration (Tietz, 1982; Ayyat *et al.*, 1991; Suckow & Douglas, 1997; Huda Qar and Abdel-Monem, 2014 and Attia *et al.*, 2015). Also, these results might suggest that drinking saline water might reduce hepatic synthesis of RNA, which in turn depressed the incorporation of amino acids for protein synthesis (Tata and Widnell, 1966 and Suckow and Douglas, 1997). The depression in total protein could be considered as a physiological accommodation to prevent excessive passage of fluids into the interstitial tissue due to the high level of salinity (Abdel Samee and El-Masry, 1992). Ahmed (1996) reported that decrease in feed intake or increase in water intake may leads to dilution of the blood components.

Table (3). Biochemical parameters of V-line rabbits as affected by drinking saline well water.

Items		Treatments		
	Sex	T1	T2	T3
Total protein (g/dl)	**Doe**	5.9^{a}±0.12	5.5^{b}±0.13	5.5^{b}±0.14
	Buck	6.2^{a}±0.11	5.4^{b}±0.13	5.8^{ab}±0.17
Albumin (g/dl)	**Doe**	3.5±0.12	3.4±0.07	3.2±0.12
	Buck	3.6±0.07	3.4±0.07	3.7±0.10
Globulin (g/dl)	**Doe**	2.7^{a}±0.21	2.1^{b}±0.11	2.2^{b}±0.12
	Buck	2.5^{a}±0.21	2.0^{b}±0.18	2.1^{ab}±0.19
Glucose (mg/dl)	**Doe**	94.4^{a}±4.5	82.0^{b}±2.9	89.9^{ab}±2.3
	Buck	91.2^{a}±3.6	80.1^{b}±4.9	87.8^{ab}±2.6
Cholesterol (mg/dl)	**Doe**	67.0^{b}±4.7	86.8^{a}± 6.9	71.5^{ab}±4.9
	Buck	53.6^{b}±4.7	67.9^{a}± 6.4	53.0^{b}±6.5
Total lipids (mg/dl)	**Doe**	845.3^{b}±4.0	898.5^{a}±27.8	876.3^{ab}±3.8
	Buck	866.1^{b}±9.2	907.0^{a}±22.4	875.6^{ab}±5.3

a, b Means bearing different superscripts within the same row are significantly different (P<0.05).
T1 (control) = rabbits drank tap water (301 ppm) ;T2 = rabbits drank saline well water (5568 ppm) ; T3 = rabbits alternated drank saline well water with tap water weekly.

Regarding decreased concentration of blood glucose as a result of increased salt intake from drinking water may attribute to increase energy requirements to maintain the sodium/potassium gradient (Ahmed, 1996; Guyton and Hall, 2006 and Attia *et al.*, 2015). Karadjole *et al*. (1999) showed that this decrease could be interpreted as a consequence of the increased transport of glucose and salt

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through membranes into tissue cells. The cellular metabolism of glucose produces energy (ATP), which is then mostly used to maintain the Na^+ and K^+ gradient via Na^+/K^+ ATP-ase. Under physiological conditions, the cells must actively release sodium and take potassium. With increased salt intake from drinking water, the body is exposed to a metabolic stress, resulting in increased energy requirements to maintain the sodium/potassium gradient, which accounts for the decreased concentration of blood glucose (Ahmed, 1996; Guyton and Hall, 2006; Morsy *et al*., 2012 and Amal, 2013).

However, rabbits of the alternating water (T3) had comparably glucose levels with control group which might indicate of the stable physiological states for rabbits as a result of applying such water system.

Cholesterol and total lipids concentrations were significantly increased in the does and bucks of T2 as compared to T1 treatment (Table 3). This result may attributed to the effect of saline water on the renal system and thyroid hormones with a defect in lipoprotein metabolism resulting in increase the high density lipoproteins in the blood, which are the cholesterol-rich lipoprotein fractions. So, the increase in total lipids due to saline well water could be attributed to the elevated cholesterol, lipoprotein and phospholipid concentrations (Ahmed and Abdel-Rahman, 2004; Ellefson & Garaway 1982; Abdel-Samee & El-Masry, 1992; Pond *et al*., 1995 and Attia *et al.,* 2015). However, using alternating water system (T3) resulted in reliving the stress of drinking high saline water on cholesterol and total lipids concentrations, where rabbits of T3 had non-significant differences comparing with control and saline water treatments (Table 3).

No significant differences between bucks of T3 and other two groups in most biochemical traits indicating that rabbits alternated drank saline water with tap water may decrease the saline stress by washing the body from excessive minerals which ingestion from drinking saline well water.

C- Alanine and aspartate transaminase parameters

Doe and buck rabbits of T2 showed increase ($P<0.05$) in alanine transaminase and aspartate transaminase concentrations as compared to T1 and T3 treatments (Table 4). This increase may reflect impairment of liver activities with the increase in level of salinity in drinking water (Abd El-Razik *et al.,* 1999; Ayyat *et al.,* 1991*;* Abdel Samee and El Masry, 1992 and Abdel Rahman *et al.,* 2000*;* Marai *et al*., 2010 and Attia *et al.,* 2015). Decreased alanine and aspartate transaminase concentrations of T3 treatments might indicate that alternating water system alleviating the saline stress on liver function.

Table (4). Alanine and aspartate transaminase parameters of V-line rabbits as affected by drinking saline well water.

Enzyme (I. U./L)		Treatments		
	Sex	T1	T2	T3
Alanine transaminase	**Doe**	$8.2^{b}\pm0.54$	$19.5^{a}\pm1.3$	$12.2^{b}\pm2.0$
	Buck	$9.2^{b}\pm1.4$	$15.6^{a}\pm0.64$	$11.5^{b}\pm1.2$
Aspartate transaminase	**Doe**	$112.1^{b}\pm2.9$	$130.8^{a}\pm0.29$	$116.1^{b}\pm1.7$
	Buck	$88.9^{b}\pm8.1$	$118.5^{a}\pm3.5$	$97.1^{b}\pm9.4$

a, b Means bearing different superscripts within the same row are significantly different ($P<0.05$).
T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm) ; T3 = rabbits alternated drank saline well water with tap water weekly.

D- Mineral parameters

Drinking well water increased ($P<0.05$) concentration of calcium by 14.7 and 10.2 % in T2 treatment as compared to T1 and T3 treatments, respectively. Meanwhile, calcium concentration was insignificantly increased in the bucks of T2 group (Figure 1 A). However, phosphorus concentration was insignificantly decreased in the rabbits of T2 as compared to other two groups (Figure 1 B).

These results agree with the results of (Tyler, 1979; Balnave *et al.,* 1989; Pourreza *et al*., 1994 and Amal, 2003) they reported that increased calcium level in natural saline well water resulted in increasing the rate of its reabsorption and so, its blood level with decreasing phosphorus level due to their reciprocal reverse relationship as the increased blood calcium level resulted in increased parathyroid hormone secretion which inhibits the renal tubules reabsorption of phosphorus.

Sodium concentration increased ($P<0.05$) in the does and bucks of T2 by 26.1 and 18 %, respectively as compared to the rabbits of T1 (Figure 1 C). In contrary, potassium concentration decreased ($P<0.05$) in the does and bucks of T2 by 25.3 and 28.7 %,

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respectively as compared to rabbits of T1(Figure 1 D). This result agree with the results of Abdel-Samee& El-Masry (1992) and Ahmed and Abdel-Rahman (2004) they attributed the contrary trend between blood sodium and potassium concentrations to protect the body against hyperkalemia and so protect the body against muscle irritability. So, increased level of sodium ions in natural saline well water resulted in increased the rate of its reabsorption and in a decrease reabsorption of potassium and/or increasing intake of saline water and consequently the increase in plasma electrolytes might result in stress on kidney function to excrete the excess of salts through urine (Crane, 1965; Grodsky, 1979; Tietz, 1982; Gary & Narang, 1985; Abdel-Samee & El-Masry, 1992; and Habeeb *et al.*, 1998). On the other hand, does and bucks drinking saline water (T2) showed increased (P<0.05) sodium/potassium ratio, sodium/ (sodium+ potassium) index and chloride concentrations as compared to T1 tratment (Figure 2 A, B and C).

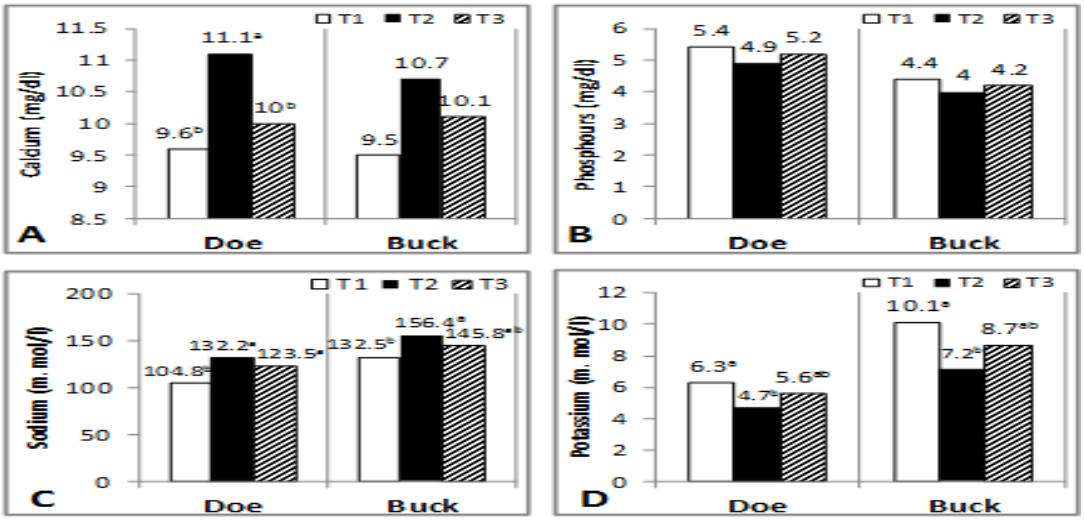

Figure 1 (A, B, C and D). Calcium, phosphorus, sodium and potassium levels of V-line rabbits as affected by drinking saline well water treatment

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

T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm); T3 = rabbits alternated drank saline well water with tap water weekly.

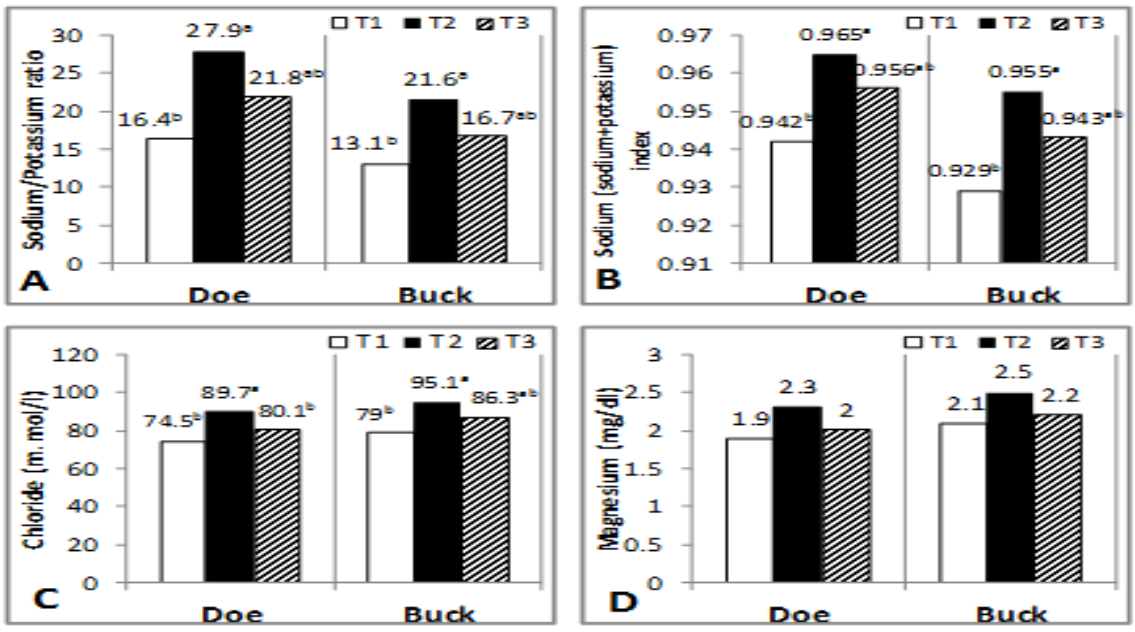

Figure 2 (A, B, C and D). Chloride, magnesium, sodium/potassium ratio and sodium/(sodium+potassium) index of V-line doe rabbits as affected by drinking saline well water.

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a, b Means bearing different superscripts within the same sex are significantly different (P<0.05).
T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm); T3 = rabbits alternated drank saline well water with tap water weekly.

The ratio sodium/(sodium+potassium) in the blood could be used as an indicator of aldosterone level, so the increase in the ratio of Na^+/K^+ and $Na^+/(Na^+ + K^+)$ index may due to increase the rate of glomerular filtration in the kidney for such electrolytes and consequently a direct increase in their concentration in the blood (Hamdi *et al*., 1982 and Amal, 2003). However, magnesium concentration was insignificantly influenced by drinking saline water (Figure 2 D). So, rabbits alternated drank saline water with tap water (T3) may capable of maintaining normal homeostasis and maintenance on acid – base balance and electrolytes system.

E- ALDOSTERONE AND TRIIODOTHYRONINE HORMONES

Aldosterone hormone decreased (P<0.05) in the doe rabbits of T2 by 27.3 % than that of T1 treatment. However, it insignificantly decreased in the doe rabbits of T3 by 18.6 % as compared to control one. Also, aldosterone hormone was decreased (P<0.05) in the bucks of T2 and T3 by 33.2 and 21.9 %, respectively than that of T1 treatment (Figure 3).

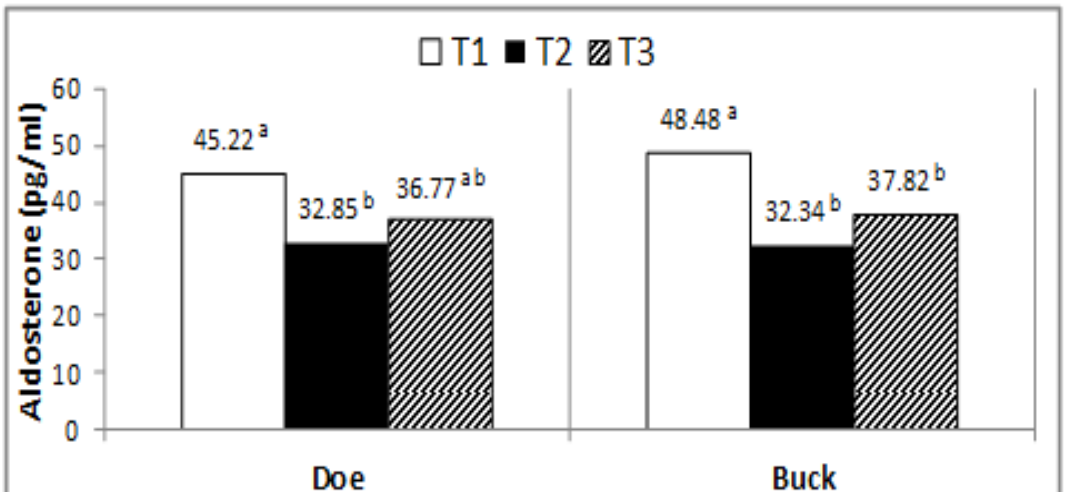

Figure 3. Aldosterone hormone of V-Line rabbits as affected by drinking saline well water.

a, b Means bearing different superscripts within the same sex are significantly different (P<0.05). T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm); T3 = rabbits alternated drank saline well water with tap water weekly.

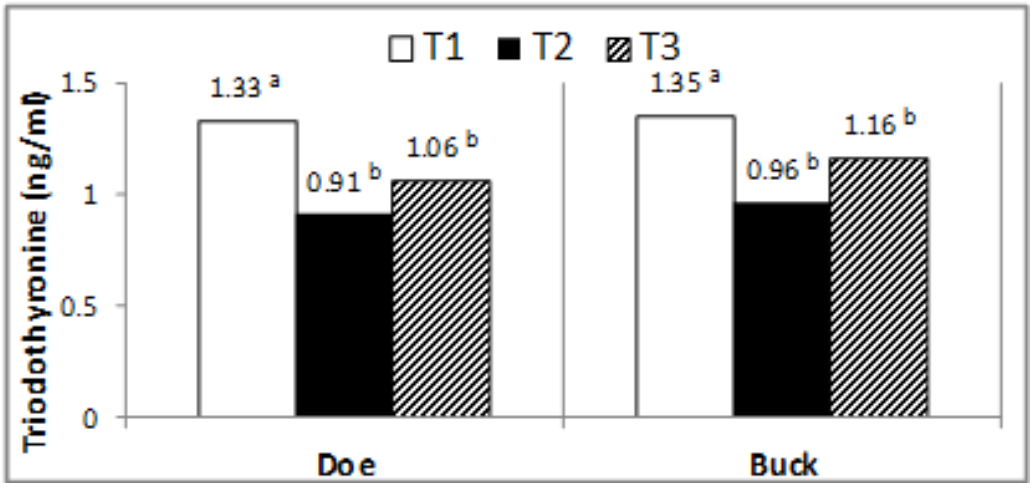

Figure 4. Tri-iodothyronine hormone of V-line rabbits as affected by drinking saline well water.

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a, b Means bearing different superscripts within the same sex are significantly different (P<0.05). T1 (control) = rabbits drank tap water (301 ppm); T2 = rabbits drank saline well water (5568 ppm); T3 = rabbits alternated drank saline well water with tap water weekly.

Rabbits drank saline water may managed the physiological of salt retention and salt excretion for an overload of salt by reducing their plasma aldosterone concentration by approximately 50% of control values.

Aldosterone is responsible for 50-70 % of total minerals corticoids activity as well as regulation and adjustment of water and electrolytes balance among the body compartments (Conner, 1962; Amal, 2003; El-Hawy, 2013 and Abd El-Galil *et al*., 2014). Rabbits drank saline water may managed the physiological of salt retention and salt excretion for an overload of salt by reducing their plasma aldosterone concentration by approximately 50% of control values. Also, Hamdi *et al*. (1982) clarified the association of aldosterone and Na+/ Na+ + K+ index in the blood suggesting that it might be attributed to an increase in the aldosterone release in response to drinking the diluted sea water.

Tri-iodothyronine (T_3) hormone recorded significantly decrease in the doe and buck rabbits of T2 when compared with T1 and T3 treatments (Figure 4). These results agreed with (Ahmed, 1996). He attributed this decrease in T_3 hormone to the increase water intake and the decrease feed intake which lead to hemodilution or increase in the osmotic pressure of body fluids which resulted in decreasing thyroid hormones (Ayyat *et al.*, 1991; Abdel-Samee and El-Masry, 1992; Marai *et al*., 2005; Amal, 2003 and 2013).

4. CONCLUSION

V-Line rabbits drank saline well water (5568 ppm TDS) showed harmful effects on hemato-biochemical, minerals and hormonal parameters. However, rabbits alternated drank saline water with tap water every week may alleviate the drastic effect of drinking saline well water on blood parameters and thus may be positively reflected on rabbit's performance.

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