THE ROLE OF NASAL GLAND AND VITAMIN C IN ALLEVIATE THE ADVERSE EFFECTS OF OSMOTIC STRESS ON OSTRICH.

By

A. S. O. EI-Badry; Kh. A. A. Ali; W. A. H. Ali and M.A. Ahmed

Department of Rabbit; Turkey and Water Fowl Breeding Research Animal Production Research Institute. Ministry of Agriculture. Dokki. Giza. Egypt.

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Abstract: The features of functional salt glands in ostriches were investigated. Forty ostriches were randomly divided into eight groups, according to the different levels of sodium chloride $(0, 2.5, 5 \text{ and } 7.5 \text{g} \setminus L)$ with or without vitamin C (0 and $1 \text{g} \setminus L$) in drinking water. The aldosteron concentration, Na-K ATPase activity in some tissues, plasma osmotic pressure, hematological parameters, some plasma biochemical parameters and liver and kidney functions were measured at 12 months of age.

Results showed that T3 and plasma protein concentration decreased significantly with increasing saline water level. Vitamin C addition caused a significant increase in T3 compared with other treatments while, plasma cholesterol significantly increased by increasing saline water but, it significantly decreased with vitamin C addition. With respect to liver (as measured by AST and ALT) or kidney functions (as measured by uric acid and creatinine levels), birds drink high saline water recorded higher levels than the control group. While, addition of vitamin C to birds led to improve in liver and kidney function. Plasma osmotic pressure, aldosteron concentration and Na-K ATPase activity in various tissues were significantly increased with increasing saline water level but, it significantly decreased with vitamin C addition. Heterophels (H) and Heterophels / Lymphocytes % (H/L %) except Lymphocytes (L) were affected by saline level whereas; it increased significantly with increasing saline level. Ascorbic acid addition did not significantly effect on H but it caused a significant decrease in H/L% and it caused a significant increase in L.

Present data indicates that vitamin C supplementation had some beneficial effects on some physiological, hematological parameters and plasma osmotic pressure in ostriches drank high saline water (5 g / L) during growing period.

INTRODUCTION

Fluid and ions homeostasis in birds involves several organ systems including the kidneys, cloaca, ceca, and the nasal salt (nasal) glands (Braun, 1999). Saline tolerance in birds is determined by the efficiency of the reabsorption of water and sodium ions by the renal tubules and sodium secretion by the salt glands (Butler *et al.*, 1991). Avian species possessing functional salt glands by two routes of sodium chloride excretion of the renal and the extra renal salt excretion. Salt glands of birds have a mechanism of concentrated salt ions from the blood and work in conjunction with the kidneys.

According to Schmidt-Nielsen *et al.* (1963) ostriches have large, functional (nasal) salt glands which may be important for extra renal salt elimination during dehydrational stress, as water-deprived ostriches produced more secretion from the salt gland than hydrated birds. According to Cloudsley-Thompson and Mohamed (1967) these salt glands enable ostriches to utilize saline water. They found that when fresh water was not available, ostriches drank dilute sea water and even solutions of sodium chloride. They maintained their weight on 20% (0.2 Molar) sodium chloride solutions, but lose weight when given higher concentrations.

While, Skadhauge et al. (1995) found that exposed ostriches to a high salt level with 1.5% salt as drinking solution followed by an acute oral salt load, without observing any activation of nasal secretion. Gray and Brown (1995) however studied the salt glands activity of ten ostriches (five of that adapted to the fresh and five to the salty water) and had doubts about their meaning for the normal osmotic pressure support in the blood plasma for this species. After a hyperosmotic infusion in only two of the animals (from the adapted to the salty water group) had been observed a secretion of insignificant quantity of fluid for about 5-10 minutes. Sustained salt gland secretion is also dependent upon activation of the pituitary-adrenal axis. Thyroid hormones and prolactin may also

MATERIAL AND METHODS

The present study was carried out at a private ostrich farm (Naoomy Company) at El-shourook city - Egypt. During August and September, 2009. Forty growing ostriches, 10 months of age were used in this study. The ostriches were reared on wood shavings as a litter floor. The room temperature was initially 34°C and was decreased by 0.2°C every day until the age of forty-five days, and after that was regulated depending on the climatic conditions and the behavior of the birds. At ten months of age birds were reared under natural light. Diet contains 16% protein and 2400 Kcal/Kg and water were provided adlibitum throughout the experimental period. The ostriches were randomly divided into

be involved indirectly to support continued extra renal excretion via the salt glands (Holmes and Phillips, 1985).

Balnave (1989) and Khalafalla *et al.* (1998) have reviewed the responses of poultry (layer type, broiler chickens, turkey and ducks) to mineral supplements in drinking water. These authors noted that salt levels in drinking water ranged from 2.6 to 10.6 g/l and showed that high concentrations of salt in drinking water were associated with reduced growth and increased mortality in these poultry.

Dietary vitamin C has been reported to improve resistance to a variety of stressors, including toxic salts and hypoxia (Al-Taweil and Kassab, 1990), whereas, supplementation of vitamin C is reported to reduce plasma corticosterone, a stress hormone, and the heterophil / lymphocyte (H:L) ratio Lohakare *et al.*(2005).

Therefore, the objective of this study was to determine the impact of different saline drinking water levels with or without vitamin C on functional salt glands, osmoregulation, some physiological and hematological measurements of ostriches.

eight groups (5 ostrich / group), according to the different level of drinking saline water with or without vitamin C.

The drinking water treatments consisted of:

1) controls receiving tap water.

2) Tap water supplemented with 1g vitamin C / L

3) Tap water supplemented with 2.5g NaCl / L

4) Tap water supplemented with 2.5g NaCl and 1g vitamin C / L

5) Tap water supplemented with 5g NaCl /L

6) Tap water supplemented with 5g NaCl and 1g vitamin C / L

7) Tap water supplemented with 7.5g NaCl / L

8) Tap water supplemented with 7.5g NaCl and

lg vitamin C / L

The NaCl solution was prepared as required, and vitamin C was added to fresh supplies of drinking water daily. Plasma osmotic pressure, hematological parameters, some plasma parameters, liver and kidney functions were determined at the end of this experiment.

Physiological responses measurement:

All birds were taken for blood samples in the early morning hours. Blood samples were taken from a wing vein into heparinized tubes. Each blood samples was divided into two portions. The blood samples of first portion were immediately centrifuged at 3000 rpm for 15 minutes and blood plasma was separated and stored at -20°C for assaying of total protein (mg/100 ml) by the Biuret method according to Henry et al. (1974)and cholesterol (mg/100 ml) according to Richmond (1973). AST (Aspartate-aminotransferase) and ALT (Alanine-aminotransferase) activities were determined as described by White (1970), creatinine and uric acid were determined as described by Husdan, (1968) and Bogin and Keller (1987), respectively. Plasma sodium (Na) and potassium (K) were determined as described by Dean (1960).

Plasma osmotic pressure was measured by a vapor pressure osmometer (Wescor 5500, USA). Plasma concentrations of aldosterone and T3 hormone were measured by radioimmunoassay (RIA) as described by Ito *et al.* (1972) and Darras *et al.* (1992), respectively. The second portion blood samples were taken to determine Packed-cell volume (%) red blood cells (x $10^6/mm^3$) and white blood cells (x $10^3 mm^3$).

A percentage of packed cell volume (PCV %) value was determined according to Hunsaker (1969), Red blood cell's (RBC's) and white blood cell's (WBC's) were counted in fresh blood sample as described by Hawkey and Dennett (1989) using haemocytometer and counted at 400 X objective of a phase contrast microscope. Differential counts of 100 leucocytes were counted using slides stained with Wrights stain and Heterophils / Lymphocytes ratio (H: L ratio) was measured.

Determination of Na-K ATPase activity:

Tissue preparation:

At the end of experimental period three ostriches heads from each group slaughtering, immediately after were collected. The salt glands (up the eye) were dissected out after removal of the skin under sterile conditions. Also, samples from the kidney and lower intestine (colon) were taken from these birds and rapidly excised and freed from connective tissues. After the tissues had been weighed they placed into Petri dishes containing an icecold isolated medium consisting of 125mM Kcl and homogenate for tissue analysis.

Na-K ATPase activity:

The enzyme activity was measured at $37^{\circ}c$ and calculated as the difference between rates of inorganic phosphate liberated in the presence and absence of ouabain (Johnson *et al.*, 1977). The released inorganic phosphate was measured by the method of Fiske and Subbarow (1925). The protein concentration was determined by the method of Lowry *et al.* (1951). All enzyme activities were expressed as μ mol Pi / mg protein / hr.

Statistical analysis:

Data were analyzed by the least squares analysis of variance using the General Linear Models procedure of the statistical analysis model (SAS, 2001). The statistical model was as follows:

$Yijk = \mu + Ti + Aj + TAij + Eijk$

Where: Yijk. = Observation of the ij th ostrich: μ = Overall mean, common element to all observations: Ti = Effect of saline drinking water treatment (i = 1, 2, 3, 4); Aj = Effect of vitamin C supplementation (j = 1, 2): TAij = Interaction effect between ith saline drinking water treatment and j th vitamin C

supplementation: and Eijk= Random error component assumed to be normally distributed.

Data estimated in percentage were transformed with the arcsine square-root procedure to normalize variance before

RESULTS AND DISCUSSION

1- Some blood biochemical parametars:

a-Triiodothironin (T3):

Data in Table (1) showed that plasma T_3 concentration was significantly affected by saline drinking water level and vitamin C addition. It can be observed a significant decrease in plasma T_3 level with the highest level of saline. Also data in Table (1) showed the effect of interaction between saline levels and vitamin C supplementation on, plasma T_3 level whereas ostriches received the highest level of saline had the lowest T_3 level.

This result agrees with Dawson and Deeming (1997) who observed that saline injection in quail, starlings and ostriches resulted in a slow decline in thyroxin. Harvey *et al.* (1984) found that plasma T₃ concentrations decreased and consequently, reduced body weight and feed intake in ducks reared on salt water. These endocrine responses during salt water adaptation might be expected to minimize the loss of body reserves and to facilitate the extra renal excretion of sodium; these alterations in endocrine function may be partly due to salt water-induced changes in food intake.

b- Total Protein:

Data in Table (1) showed that plasma protein concentration was significantly affected by saline level and vitamin C addition whereas, plasma protein concentration was significantly decrease with increasing saline level and it was insignificantly increase with vitamin C supplementation. The beneficial effect of vitamin C supplementation on plasma analysis and were retransformed again to the original scale before presentation. The differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

protein could be attributed to vitamin C which works as coenzyme playing an important role in the metabolism of amino acid (Kutlu and Forbes, 1993) while, Rice, (2000) reported that vitamin C is effective as antioxidant, and it plays an important role in metabolic activity.

The chicks when exposed to environment stress they increased the protein catabolism associated with corticosteroid release. This might have an apparent elevation of protein levels in the high saline groups therefore, an elevation in protein-derived gluconeogenesis (Lohakare et al. (2005). Also data in Table (1) showed the effect of interaction between saline levels and vitamin C supplementation on plasma protein whereas birds drinking tap water plus vitamin C recorded significantly highest levels in plasma protein compared to other treatments.

C-Plasma cholesterol concentration:

It is well known that serum or plasma cholesterol of birds is strongly affected by heredity, nutrition, age, sex and environmental conditions (Sturkie, 1986). The results of plasma cholesterol concentration in (Table.1) showed that there was significant increase in plasma cholesterol levels due to saline drinking water. Vitamin C addition led to significant decrease in plasma cholesterol levels in control group only.

Data in Table (1) showed that, the interaction effect of saline levels and vitamin C supplementation on plasma concentrations of cholesterol, when birds drinking tap water plus vitamin C recorded significantly lowest levels plasma concentrations of cholesterol compared to other treatments.

2- Liver and kidney function:

a- Liver enzymes activities:

With respect to AST and ALT Table (2) showed that there was significant effect of saline drinking water on AST and ALT. The result also agrees with that of Abdel Rahman *et al.* (2000) who suggested that this increasing might be due to malfunction of the liver chickens which was supported by the plasma T3 decrease after saline drinking water. Ostriches receiving vitamin C showed a decrease in plasma AST and ALT levels compared to control supplementation.

Changes in blood transaminases level may depend on the rate of protein metabolism which may be due to bird's age rather than any other factor. It is well known that by the simple process of transamination, an amino radical is transferred to alfa- keto acid while the keto oxygen is transferred to the donor of the amino radical who is promoted by transaminases (Guyton, 1981). The enzyme activity in supplemented vitamin C treatments is exhibit healthy, non pathological or toxic effects of tested biological additives on liver function.

Data in Table (2) showed that, the interaction between saline levels and vitamin C supplementation on liver function, when birds drink tap water plus vitamin C recorded significantly lowest levels of AST and ALT compared to other treatments.

b- Kidney function:

Uric acid is the major nitrogenous end product of nitrogen metabolism in birds. Since, it is the excretory vehicle for four fifths of the metabolized nitrogen. Both uric acid and creatinine levels in plasma as protein metabolites followed the same manner as total plasma protein. These levels increased or decreased in response to protein metabolism. Data in Table (2) showed that there were significant differences in plasma creatinine and uric acid levels among the different saline treatments; whereas, there was significant increase in plasma creatinine and uric acid levels due to saline drinking water.

Vitamin C addition caused a decrease in plasma creatinine and uric acid levels. Suggesting that kidney performance of birds improved by vitamin were С supplementations with increasing saline level. Present results indicate that protein metabolism had significant effect on both parameters. These results agree with the results obtained by Geraert et al. (1996) in broilers. In the present study, the changes in uric acid and creatinine levels in plasma may indicate a reduction in the glomerular filration rate (GFR) as a result of high level of saline since the serum concentration of these two biological parameters depends largely on the glomerular filtration(Gavin, 1995).

Also data in Table (2) shows effect of interaction between saline levels and vitamin C supplementation on plasma creatinine and uric acid, when birds drinking tap water plus vitamin C recorded significant low level of plasma creatinine and uric acid compared to other treatments.

3. a-Sodium and potassium concentrations:

Osmoregulation is the turnover and homeostasis of water and the major electrolytes of plasma and extracellular fluids (Skadhauge, 1981). Sodium and potassium concentrations in plasma were significantly increased with increasing NaCl levels as compared with control group (Table, 3). Increasing concentration of sodium and potassium in blood may be due to the increase of water consumption containing the high level of NaCl which caused apparent increase in the blood sodium and potassium concentrations and consequently, due to stress on kidney functions. These results are in agreement with those obtained by Egwutu *et al.* (1983) and Rashwan *et al.* (1997) who found a highly significant increase of the blood sodium concentrate accomplished with increasing water salinity due to increasing retention of salt and water in both intracellular and extracellular fluids compartments. Vitamin C addition caused slight improvement in decreasing sodium and potassium concentrations (Table, 3).

The interaction effects between saline level and vitamin C supplementation were significant on plasma Na and K (Table, 3). Addition of vitamin C to the birds drinking salinity water at 5g/l showed significant decreased in plasma Na and K compared to other treatments (Table. 3).

3. b- Plasma osmotic pressure:

Plasma osmotic pressure showed different responses to saline level as indicated by its significant interaction (Table. 3), where increasing saline level significantly increased plasma osmotic pressure compared to low saline levels. These results are in agreement with those obtained by Ash (1969) who studied comparable increases in total osmolality of the plasma which produced by oral or intravenous administration of NaCl. sucrose, mannitol, KCI, urea and dextrose; but the first three only of these compounds evoked secretion of NaCl and high osmolality of the plasma. Therefore, Schmidt-Nielsen et al. (1958) suggested that the secretory mechanism responds to an osmotic load rather than to specific changes in plasma (Na+) or (Cl-).

Vitamin C supplementation also insignificantly reduced plasma osmotic pressure (Table. 3). Therefore; these results are very close to those of Yahav *et al.* (1997) who reported that the increase in concentration of plasma protein could be important in this regulatory response, which also resulted in a decrease in osmolality.

The interaction effects between saline level and vitamin C supplementation

were significant on plasma osmotic pressure (Table, 3). Birds drink high saline level with vitamin C supplementation showed significantly higher plasma osmotic pressure compared to other treatments (Table. 3). It could be concluded that, vitamin C supplementation showed no beneficial effects on plasma osmotic pressure under high saline level.

4.a- Aldosteron concentration:

Steroid aldosterone produce from the adrenal cortex which is important in osmoregulation (Sturkie, 1986). Also, he suggested that drinking salt water in birds stimulate hypothalamo-hypophyseal adrenal system, where adrenocortictrophic hormone (ACTH) is released from anterior pituitary and triggers the secretion of both aldosteron and corticosterone from adrenal cortex. This explains the increase of aldosterone level in bird plasma which in turn influences electrolyte absorption from small intestine and kidney. Increased tubular re-absorption of sodium with increase retention of sodium and water leads to expansion of the extracellular fluid compartement. Also, Arnason (1997) reported that aldosterone exerts major control of the amiloride-inhibitable Na (+)transport system in both colon and coprodeum. It can also modulate the Cl (-)secretory capacity of colon and coprodeum, but only temporarily. Also, Elbrønd et al. (1998) reported that Aldosterone stimulation increased sodium transport, the height and number of microvilli, and the brush-cell number under a high -NaCl diet.

Results showed that, aldosteron concentration was affected by saline drinking water level and vitamin C addition (Table, 4) whereas; it significantly increased with increasing saline drinking water level and decreased significantly with ascorbic acid addition. This result is in agreement with Aupetit *et al.* (1986) how show that oxygen is required in the transformation of 18hydroxycorticosterone into aldosterone, at the

cytochrome P-450 level.

Vitamin C was used to increase oxygen consumption at the cytochrome a level, thereby decreasing its availability to cytochrome P-450. Vitamin C system acts as an 'oxygen trap' and consequently vitamin C works as strong inhibitors for aldosterone biosynthesis from 18hydroxycorticosterone.

The interaction effects between saline level and vitamin C supplementation on aldosteron concentration showed that birds drink tap water plus vitamin C recorded significantly lowest levels of aldosteron concentration in blood compared to other treatments Table (4).

4.b- Na-K ATPase activity:

The hypertrophy of the gland and hyperplasia of the plasma membrane have been correlated with the level of (Na+-K+)dependent ATPase, an enzyme which is involved in the concentrating function of the salt gland to produce a hypertonic solution for secretion (Karlsson *et al.*, 1971). Also, sensitivity this Na,K_ATPase enzyme in various tissues to level of Na+ in the body was reported by Yakushev *et al.* (2008). Therefore, there was a significant increase in quail nasal gland Na-K ATPase when only saline solutions were offered to drink (200 mM NaCl) Dunson *et al.* (1976).

Results showed that, Na-K ATPase activity in various tissues (kidney and lower intestine) was affected by saline drinking water level and vitamin C addition (Table, 4) whereas; it significantly increased with increasing saline drinking water level and decreased significantly by vitamin C addition. This result is agreed with William *et al.* (1982) observed that the salt water treatment in ducks stimulated the salt gland to increased Na, K-ATPase activity.

On the other hand, Petty (1981) suggests that the activation of Na-K ATPase membrane is depend on an aldosterone that increase in the passive

entry of Na across the luminal membrane. With respect to the salt gland Na+/K+-ATPase activity with or without vitamin C addition recorded the lowest Na-K ATPase activity compared to other tissues. This result may suggest that hyperosmotic NaCl loading was unable to activate the nasal glands for secretion or it due to low relative mass nasal gland in the ostrich. This suggestion is in agreement with Skadhauge et al. (1984) who reported that ducks weighted 3 kg (salt-adapted) have nasal gland weighed approximately 0.5 g, with relative mass 20-fold higher than in the ostrich thus, were unable to stimulate the nasal glands for secretion. While, Schmidt-Nielson et al. (1963) reported that desert birds such as the Ostrich have functional salt glands, which are stimulated in response to high temperature.

The interaction effects between saline level and vitamin C supplementation on Na-K ATPase activity in various tissues showed that birds drinking tap water plus vitamin C showed an improvement in these parameters Table (4).

5- Hematological Parameters:

Data of hematological parameters are presented in (Table, 5) which indicated that saline levels did not effect significantly on RBC whereas; it decreased slightly with increasing saline level but, vitamin C addition caused a significant increase in this parameter. While, PCV % was affected by saline level whereas; it decreased significantly with increasing saline level. Vitamin C addition caused a significant increase in PCV % compared to other treatment. Also, data in Table (5) showed that, WBC was affected by saline level whereas; it increased significantly with increasing saline level. Vitamin C addition caused a significant improve in WBC compared to other treatment.

These results agreed with Mohammed (1997) who reported that drinking salt water increased the WBC count in rabbits.

They attributed that to salt poisoning with drinking salt water and lukocytosis might occur due to gastroenteritis. They added that this might be due to injury of tissue in response to irritation resulted from water saline treatment. The observed increase in PCV in experimental groups could be attributed to the effect of vitamin C in protecting the membrane integrity of the erythrocyte as demonstrated by Candan et al. (2002) and Adenkola et al. (2010) whereas, the membrane of erythrocyte is rich in polyunsaturated fatty acids which are susceptible to lipid peroxidation which results in the loss of membrane fluidity and cellular lysis (Brzezinska-Slebodzijska, 2003). This function directly affects the total erythrocyte count in the experimental groups being higher than the groups which were not supplemented with AA.

Data in Table (5) showed that, Heterophils heterophil (H) and lymphocyte (H: L %) ratio were affected by saline level whereas; (H) increased significantly with increasing saline level. While, Lymphocytes (L) decreased significantly with increasing saline level. Vitamin C addition did not significantly effect on (H) but it caused a significant decrease in H/L% and it caused a significant increase in (L) compared to other treatments. Vitamin C is reported to reduce plasma corticosterone, a stress hormone, and the H/ L%, Sergeev et al. (1990). However the lower value of total white blood cell count recorded in the experimental groups were supplemented with vitamin C could be due to the effect of vitamin C in preventing the release of the white blood cells from their body pool due to its effect in inhibiting corticosteroids which are known to increase in animals under stress (Whitehead and Keller, 2003).

Also data in Table (5) showed the effect of interaction between saline levels and vitamin C supplementation on these parameters whereas ascorbic acid addition with the highest saline level caused an improvement in these parameters. Therefore, it is not ruled out that the nasal glands may become functional in certain ostrich populations exposed to long-term loading. and/or high salt ambient temperature but such secretion cannot play any quantitative role in osmoregulation. This study may be suggests that osmoregulation of ostriches involves levels of Na⁺ and water regulation by the intestinal tract and the kidneys than the salt glands in growing period.

In conclusion, excess of any salts in drinking tap water induce adversely affect to some hematological and physiological parameters. Moreover, vitamin C addition to saline water alleviated the toxic effects of saline drinking water up to 7.5g/l. Therefore, it can be recommend that the maximum sodium chloride level in the drinking tap water can be bear safely by ostriches neither injurious on its hematological and physiological parameters were 5 g/l with vitamin C supplementation during growing period.

Table (1): Effect of different levels of sodium chloride (NaCl,g/l) with or without vitamin C (V.C, g/l) in drinking water on some blood plasma components of Ostriches.

Treatments		Some blood plasma components				
		T3 (ng/ml)	T. protein (mg/dl)	Cholesterol (mg/di)		
(NaCl)	g/l					
0		3.74*	4.04 [*]	112.05		
2.5		3.45 ^{ab}	3.62*	124.80 ^b		
5		3.25	3.34 ^{bc}	134.23*		
7.5		2.63°	3.01 ^c	136.52ª		
5	SE	±0.10	±0.14	±1.92		
(V.C)	z/ I					
0		3.06 ^b	3.43"	129.28*		
1		3.47*	3.57*	124.51 ^b		
SE		± 0.07	± 0.10	±1.36		
			Interactions			
NaCl	V.C					
.	0	3.35 ^{bc}	3.82 ^{sb}	117.94 ^d		
)	1	4.19 ^a	4.25*	106.15°		
3 6	0	3.27 ^{bc}	3.48 ^{br}	127.75 ^{bc}		
2.5	1	3.64 ^b	3.75*b	121.85 ^{cd}		
-	0	3.03	3.33 ^{br}	134.92 ^{ab}		
5	1	3.49 ^b	3.35 ^{br}	133.53 ^{ab}		
7.6	0	2.62 ^d	3.08°	136.53*		
7.5	1	2.65 ^d	2.94 [¢]	136.51*		
SE		±0.14	±0.20	±2.72		

^{a,b,c,} means in the same column within each item bearing different superscript are significantly different (P<0.05).

Table (2): Effect of different levels of sodium chloride (NaCl,g/l) with or without vitamin C (V.C, g/l) in drinking water on liver and kidney function of Ostriches.

Treatments		liver and kidney function						
		ALT (IU/L)	AST (IU/L)	Uric acid (mg/100 ml)	Creatinine (mg/100ml)			
(NaCl)) g/l							
0		5.14 [¢]	95.95 [°]	3.14 ^e	15.94°			
2.5		5.50*	95.96	3.35 ^{bc}	£5.95°			
5		5.63*	100.75 ^b	3.45*	17.50			
7.5		5.98*	118.58*	4.18"	20.97*			
S	E	±0.06	±0.59	±0.09	±0.39			
(V.C)	g/ I				· · · · · · · · · · · · · · · · · · ·			
0		5.62*	105.25ª	3.61*	18.77*			
1		5.5	100.37*	3.45*	16.41			
SE		± 0.04	±0.42	±0.06	±0.28			
			In	teractions				
NaCl	V.C							
0	0	5.33	97.66	3.33 ^{be}	17.66 ^{be}			
v	1	4.95°	94.24°	2.95	14.24 ^d			
	0	5.36 ^{cd}	97.68	3.36 ^{bc}	17.67 ^{bc}			
2.5	1	5.64 ^{bc}	94.24*	3.34 ^{be}	14.24			
	0	5.78ªb	105.31°	3.58 ^b	18.81			
5	1	5.49 ^{ct}	96.19 ^{de}	3.33 ^{br}	1619			
	0	6.02*	120.36*	4.18 ^a	20.95*			
7.5	1	5.94*	116.79	4.19 ^s	20.99*			
SE		±0.09	±0.84	±0.12	±0.56			

^{a.b.c.} means in the same column within each item bearing different superscript are significantly different (P<0.05).

Table (3):	Effect of different levels of sodium chloride (NaCl,g/l) with or without	
Ň	vitamin C (V.C, g/l) in drinking water on Sodium, Potassium concentration	
а	and osmotic pressure of Ostriches.	

Treatments		Sodium, Potassium concentration and osmotic pressure					
		Na (mmol/l)	K (mmol/l)	Osmotic pressure/kg H2O			
(NaCl)	/L						
0		106.054	3.34 ^c	315.74 ^b			
2.5		125.80°	3.45°	316.60 ^b			
5		131.54 ^b	3.83 ^b	316.60 ^b			
7.5		137.73ª	4.36*	339.229*			
SE		±1.73	±0.11	±5.58			
(V.C) g/	L						
0		122.52*	3.86*	325.38*			
1		125.03*	3.63"	319.32*			
S	E	± 1.22	± 0.08	±3.95			
		**	Interactions	······································			
NaCl	V.C						
	0	106.15 ^d	3.33 ^b	322.36 ^{bc}			
0	1	105.94	3.35	309.13 ^r			
	0	123.85	3.56 ^b	323.64 ^{bc}			
2.5	1	127.75 ^{bc}	3.34 ^b	309.57°			
	0	134.55 ^{sb}	4.18ª	323.64 ^{bc}			
5	1	128.53 ^{bc}	3.49 ^b	309.57 ^c			
	0	137.53*	4.38"	332.05 ^{ab}			
7.5	1	137.92ª	4.34	346.39ª			
SE		±2.44	±0,16	±6.08			

*.b.c. means in the same column within each item bearing different superscript are significantly different (P<0.05).

Table (4): Effect of different levels of sodium chloride (NaCl,g/l) with or without vitamin C (V.C, g/l) in drinking water on aldosterone concentration and ATPase activity in different tissues of Ostriches.

		aldosterone concentration and ATPase activity						
Treatments		Aldosterone mg/dl	ATPase in kidney (molpi/mg protein/hr)	ATPase in Salt gland (molpi/mg protein/hr)	ATPase in Lower intestine (molpi/mg protein/hr)			
(NaCl) g/L							
0		9.14 ^c	7.14 ^d	0.53*	5.02°			
2.5		9.31°c	8.97 ^e	0.48*	5.70 ^{bc}			
5		9.82 ^b	10.01	0.49*	5.70 ^{bc}			
7.5		11.14"	10.97*	0.54*	5.70 ^{be}			
7.5 11.14" SE ±0.18		±0.18	±0.23	±0.03	±0.62			
(V.C)	g/L							
0		10.22*	9.69*	0.54*	6,82*			
1		9.48	8.85*	0.50*	5.63*			
SE		± 0.13	± 0.16	±0.02	±0.43			
			Interact	ions				
NaCl	V.C							
	0	9.55 [*]	6.33 ^e	0.57*	5.23 ^{bc}			
		8.73	7.96	0.52*	4.82			
		9.54 ^b	9.45 ^{bc}	0.51"	6.48 ^{abc}			
2.5		9.08 ^b	8.48 ^{bc}	0.49*	4.92°			
		10.55*	11.02*	0.52*	7.38 ^{#b}			
5 1 9.08 ⁶ 9.01 ⁶		0.48*	6.01 *bc					
		11.25"	11.98*	0.56*	8.22*			
7.5		11.02*	9.965	0.52*	6.76 ^{#bc}			
S	Ê	±0.26	±0.33	±0.04	±0.72			

a,b,c, means in the same column within each item bearing different superscript are significantly different (P<0.05).

Treatments		some hematological parameters							
		RBC's X 10 ⁶ / mm ³	WBC's X 10 ³ / mm ³	PCV %	Hetorphils (H) X 10 ³ / mm ³	Lemphocytes (L) X 10 ³ / mm ³	H/L %		
(Na	CI) g/l				•	•			
0		2.48 ^a	26.69 ^b	31.93*	42.09 ^d	59.08 ^b	0.71°.		
2.5		2.43*	26.62 ^b	32.29ª	43.65°	61.03*	0.71		
5		2.44 ^a	27.73 ^{ab}	29.85 ⁶	44.53 ^b	57.11°	0.77 ^b		
7.5		2.44*	28.73*	29.68 ^b	46.25*	52.68 ^d	0.88*		
SE		±0.08	±0.48	±0.54	±0.23	±0.38	±0.01		
(V.	C) g/ l	1			·				
0	- H	2.35 ^b	29.83*	30.17 ^b	44.16 [#]	56.72 ^b	0.78ª		
1		2.53*	25.55 ^b	31.70ª	44.11*	58.22*	0.75 ^b		
SE		± 0.06	± 0.34	±0.38	±0.16	±0.25	±0.01		
Intera	ctions					· · · · · · · · · · · · · · · · · · ·			
NaCl	V.C						<u>.</u>		
	0	2.48 ^{ab}	27.83 ^b	31.72 ^{abcd}	41.27 ^d	58.31 ^b	0.70 ^d		
0	1	2.46 ^{abc}	25.54 ^{ed}	32.13 ^{abc}	42.29 ^c	59.84 ^{ab}	0.72 ^{cd}		
	0	2.43 ^{abc}	28.69 ^{ab}	31.72 ^{abcd}	44.22 ^b	58.31 ^b	0.72 ^{cd}		
2.5	1	2.42 ^{abc}	24.54 ^d	32.13 ^{abc}	43.08°	59.84 ^{ab}	0.72 ^{cd}		
	0	2.38 ^{bc}	30.72ª	29.29 ^d	44.74 ^b	55.49°	0.80 ^b		
5	1	2.5 ^{*b}	24.75 ^d	30.42 ^{dc}	44.32 ^b	58.72 ^b	0.74°		
	0	2.13 ^c	30.09*	26.15 ^e	44.74 ^b	51.88 ^e	0.89*		
7.5	1	2.75ª	27.36 ^{bc}	33.22 ^{ab}	44.32 ^b	53.47 ^d	0.86*		
SE		±0.11	±0.68	±0.79	±0.32	±0.51	±0.01		

Table (5): Effect of different levels of sodium chloride (NaCl,g/l) with or without vitamin C (V.C, g/l) in drinking water on some hematological parameters of Ostriches.

a,b,c, means in the same column within each item bearing different superscript are significantly different (P<0.05).

REFRENCE

- Abdel-Rahman, G.A., A.M. Abou-Ashour, F.H. Abdou, M.M.A. El-Sherif and A.H. Hassan (2000). Effect of drinking natural saline water on some physiological aspects of California rabbits. 5th Vet. Med. Zag. Conference (12-14 Sept. 2000).
- Adenkola, A.Y., F.G. Kaankuka, T.T. Ikyume, I.F. Ichaver and I.D.I. Yaakugh (2010). Ascorbic acid effect on erythrocyte osmotic fragility, haematological parameters and performance of weaned rabbits at the end of rainy season in Makurdi, Nigeria. Journal of Arimal & Plant Sciences, Vol. 9, Issue 1 1077-1085.
- Al-Taweil, R.N. and A. Kassab (1990). Effect of dietary vitamin C on ascites

in broiler chicks. International Journal Vitamin Nutrition Research, 60, 366-371.

- Arnason S.S. (1997). Aldosterone and the control of lower intestinal Na+ absorption and Cl- secretion in chickens. Comp Biochem Physiol A Physiol. Oct; 118 (2):257-269.
- ASH, R.W. (1969). plasma osmolality and salt gland secretion in the ducks. J. exp. Physiol. 54: 68-79.
- Aupetit B., N. Emeric, R. Toury, O. Racadot, J. Racadot, V. Vonarx and J.C. Legrand (1986). Stimulation of oxygen consumption at the cytochrome A3 level inhibits aldosterone biosynthesis from 18hydro::ycorticosterone. Biochim

Biophys Acta. Nov 19: 884 (2): 270-275.

- Balnave, D. (1989). Minerals in drinking water and poultry production.Monsanto Nutrition Update: 7: 1-8.
- Bogin, E. and P. Keller (1987). Application of clinical biochemistry to medically relevant animal models and standarization and quality control in animal biochemistry. J. Clin. Chem. Clin. Biochem. 25: 873 - 878.
- Braun, J. (1999). Integration of organ systems in avian osmoregulation. J. Exp. Zool., 28: 702-707.
- Brzezinska-Slebodzinska, E. (2003). Species differences in the susceptibility of erythrocytes exposed to free radicals in vivo. Veterinary Research Communication 27: 211 – 217.
- Butler, D.G., J.H. Youson and E. amplion (1991). Configuration of the medical and lateral segments of the duck (Anas platyrhynchos) salt glands. J. Morph., 207 (2): 201-210.
- Candan F., F. Gultekin and F. Candan (2002). Effect of vitamin C and zinc on fragility and lipid peroxidation in zinc-deficient haemodialysis patient. Cell Biochemistry and Function 20: 95 - 98.
- Cloudsley-Thompson, J. L. and E.R.M. Mohamed (1967). Water Economy of the Ostrich. Nature 216, pp. 1040.
- Darras, V.M., T.J. Visser, L.R. Berghman and E.R. Kuhn (1992). Ontogeny of type I and III deiodinase activities in embryonic and posthach chick: relationship with changes in plasma triiodothyronine and growth hormone level. Comparative Biochemistry and Physiology, 103 A: 131-136.
- Dawson, A. and D.C. Deeming (1997) Thyroid function in ratites compared to that in non-ratite birds. In:

Kawashima, S. and Kikuyama, S. (eds) Advances in Comparative Endocrinology. Monduzzi Editore, Bologna, pp. 439–444.

- Dean, J.A. (1960). Flame Photometry. published by McGraw-Hill Book Company, pp. 271-283.
- **Duncan, D.B. (1955).** Multiple Range and multiple F test. Biometries, 11: 1-42.
- Dunson, W.A., M.K. Dunson and R.D. Ohmart (1976). Evidence for the presence of nasal salt glands in the roadrunner and the Coturnix quail. Journal of Experimental Zoology, 198: 209-215.
- Egwuatu, C.O., J.A. Harper, O.H. Helfer and G.H. Arscott (1983). Effect of low salt, sodium and chloride levels in poult rains on growth, bone development and related factors. Poult. Sci., 62: 353-358.
- Elbrond V.S., E. Skadhauge, L. Thomsen and V. Dantzer (1998). Morphological adaptations to induced changes in transepithelial sodium transport in chicken lower intestine (coprodeum): a study of resalination, aldosterone stimulation, and epithelial turnover. Cell Tissue Res. Jun; 292 (3): 543-552.
- Fiske, C.H. and Y. Subbarow (1925). A clorimeteric determination of phosphorus. J. Biol. Chem., 66: 375-400.
- Gavin, J.B. (1995). Assessment of renal function. The Md. GR. J., 23: 102-105.
- Geraert, P.A., J.C.F. Padilha and S. Guillaumen (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens. 2- Biological and endocrine parameters. Br.J. Nutr. 75: 205-216.
- Gray, D. and C. Brown (1995). Saline infusion induced increases in plasma

osmolality do not stimulate nasal gland secretion in the ostrich (Struthio camelus), Physiological zoology, 68 (1): 164-175

- Guyton, A.C. (1981). Text Book 1-Medical Physiology W.B. Saunders Company (sixth edition), London.
- Harvey S., H. Klandorf, W.J. Radke and J.D. Few (1984). Thyroid and adrenal responses of ducks (Anas platyrhynchos) during saline adaptation. Gen Comp Endocrinol. Jul: 55 (1): 46-53.
- Hawkey, C.M. and T.B. Dennett (1989). A Color Atlas of Comparative Veterinary matology. Wolf Publishing Limited, London, England.
- Henry, R.J.; Cannon, D.C. and Winkelman, W. (1974). Clinical Chemistry rincipels and Techniques, 11th ed. Happer and Row Publishers, p. 1629.
- Holmes, W.N. and J.G. Phillips (1985). The avian salt gland, Biological Reviews, 60 (2): 213–256.
- Hunsaker, W.G. (1969). Species and sex differences in the percentage of plasma trapped in packed cell volume determination on avian blood. Poult. Sci., 48: 907-909.
- Husdan, H. (1968). Determination of creatinine in serum. Chin. Chern. 14: 222-232.
- Ito, I., J. Woo, R. Haning and R. J. Horton (1972). A radioimmuoassa for aldosterone in human purified olasma including a comparison of alternative techniques. J. Clin. Endocrinol. Metab. 34: 106-116.
- Johnson, S.L., R.D. Ewing and J.A. Lichatowich (1977). Characterization of kidney (Na-K) activated adenosine triphosphatase from mice. J. Exp. Biol. 199: 345-354.

- Karlsson, K.A., B.E. Samuelsson and G. Sheen (1971). Lipid pattern and Na+-K+ dependent adenosine triphosphatase activity in the salt gland of duck before and after adaptation to hypertonic saline. J. mem. Biol. 5, 169-184.
- Khalafalla, M.K., W. Bessei and A. Schwarzenberg (1998). Effect of mineral salts in drinking water on the domestic chicken performance D a literature review. Archiv. Gefl.gelk., 62: 6-32.
- Kutlu H.R. and J.M. Forbes (1993). Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Livestock Prod. Sci., 36: 335 – 350.
- Lohakare, J.D., J.K. Kim, M.H. Ryu, T.W. Hahn and B.J. Chae (2005). Effects of Vitamin C and Vitamin D Interaction on the Performance, Immunity, and Bone Characteristics of Commercial Broilers. J. Appl. Poult. Res., 14: 670–678
- Lowry, O.H., R.J. Rosebrough, A.I. Farr and R.J. Randall (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Mohammed, A.H. (1997). Study of some physiological, nutritional and productive aspects of rabbits as affected by natural saline water. M.Sc, Thesis, Fac., Agric., Monufiya, Univ.
- Petty, K.J., J.P. Kokko and D. Marver (1981).Secondary Effect of Aldosterone on Na-K ATPase Activity in the Rabbit Cortical Collecting Tubule. J. Clin. Invest. Vol. 68 December 1514-1521.
- Rashwan, A.A., M.A. Abdel-Razik and A.M. Baelish (1997). Performance of arbor-acres broiler chicks as affected by salinity of drinking water.

International Conference on Animal Production and Health. 491-505.

- Rice M.E. (2000). Ascorbate regulation and its neuroprotective role in the brain. Trends in Neuroscience 23: 209 - 216.
- Richmond, W. (1973). Colorimetric method for the determination of plasma cholesterol Clin. Chem. 19: 1350-1356.
- SAS (2001). Statistical Analysis System, User's Guide Version 8.2. Cary NC. USA. Lindquist. S.: and Craig, E. A., 1988.
- Schmidt-Nielson, K., A. Borut, P. Lee and E.J. Crawford (1963). Nasal salt Excretion and the possible function of the cloaca in water conservation. Science 142: pp. 1300 – 1301.
- Schmidt-Nielsen, K., B. Jorgensen and H. Osaki (1958). 'Extra-renal salt excretion in birds'. Amer. J. Physiol. 193, 101-107.
- Sergeev, I.N., Y.P. Arkhapchev and V.D. Spirichev (1990). Ascorbic acid effects on vitamin D hormone metabolism and binding in guinea pigs. J. Nutr. 120: 1185–1190.
- Skadhauge, E. (1981) Osmoregulation in Birds. Springer-Verlag, Berlin.
- Skadhauge, E., B.G. Munck and G.E. Rice (1984). Regulation of NaCl and water absorption in duck intestine. In: Pequeux, A., Gilles, R. and Bolis, L. (eds.) Lecture Notes on 80 E. Skadhauge and A. Dawson Coastal and Estuarine Studies, Vol. 9, Osmoregulation in Estuarine and Marine Animals. Springer-Verlag, Berlin, pp. 132–142.

- Skadhauge, E., R.P. Prys-Jones, D. Swart and C.N. Warüi (1995). Colonic SCFA and electrolyte absorption and nasal gland development, in relation to NaCl intake in the ostrich (Struthio camelus). Pflügers Archives European Journal of Physiology 430, R73.
- Sturkie, P.d. (1986). "Avian Physiology". 4th ed. Springer - Verlag New York, Inc. U.S.A.
- White, B.A. (1970). Determination of serum (SGOT) glutamic oxaloacetic transaminas and glutamic pyruvic transaminas (SGPT) activity. Chemistry for Medical Technologist, 3th Ed., 610-660.
- Whitehead, C.C. and T. Keller (2003). An update on ascorbic acid in poultry (Reviews). World's Poultry Science Association 59: 161 – 184.
- William, C., J.R. Eastin, W.J Fleming and C.M. Helen (1982). Organophosphate inhibition of avian salt gland Na, K-ATPase activity.Comparative Biochemistry and Physiology Part C: Comparative Pharmacology Volume 73, Issue 1, Pages 101-107
- Yahav, S., A. Straschnow, I. Plavnik, and S. Hurwitz (1997). Blood system response of chickens to changes in environmental temperature. Poult. Sci., 76: 627-633.
- Yakushev S.S., E.M. Kumskova, A.M. Rubtsov and O.D. Lopina (2008). Effect of Colchicine on Sensitivity of Duck Salt Gland Na,K_ATPase to Na+. Proceedings of the International Union of Physiological Sci., 14, 707.

الملخص العريى

دور الغدة الملحية و فيتامين ج في تخفيف التأثيرات السلبية للاجهاد الاسموزي على طيور النعام

عبد العزيز سيد عثمان - خالد عبد المعبود احمد . وانل على حسن على . محمود عاطف محمود احمد

قسم بحوث تربية الارانب والرومي والطيور المانية – معهد يحوث الانتاج الحبواني – مركز البحوث الزراعية – وزارة الزراعة – الدقي – ج.م.ع.

اجريت هذة الدراسة في مزرعة خاصة للنعام (شركة نعومي) - مدينة الشروق - القاهرة - خلال شهرى اغسطس و سبتمبر لسنة ٢٠٠٩ ، استخدم في هذة الدراسة ٤٠ طائر من النعام عمر عشرة شهور وقسمت الى ثمانية مجاميع متساوية لدراسة تأثير أربعة مستويات من كلوريد الصوديوم (صفر - ٢٠ - ٥- ٧ جرام / لتر ماء) مع اضافة فيتامين ج (صفر و ١ جرام / لتر ماء) حتى عمر اثنى عشر شهرا على بعض القياسات الفسيولوجية والمناعية والضغط السموري لبلازما الدم وايضا على وظائف الكبد والكلية وكات النتائج كالاتي:

انخفض هرمون T3 ومستوى بروتينات البلازما معنويا مع زيادة مستوى كلوريد الصوديوم فى ماء الشرب بينما اضافة الفينامين ادى الى ارتفاع معنوى فى هذة الصفات بينما ارتفع مستوى الكوليستيرول فى الدم معنويا مع زيادة مستوى كلوريد الصوديوم فى ماء الشرب بينما ادى اضافة الفيتامين الى انخفاض معنوى فى مستوى الكوليستيرول فى الدم.

أدت زيادة مستوى كلوريد الصوديوم فى ماء الشرب الى تلثير سلبى على وظانف الكبد والكلية بينما اضافة الفيتامين أدى الى تحسن معنوى فى وظانف الكبد والكلية و ايضا تحسن الضغط السموزى لبلازما الدم و انخفاض مستوى هرمون الالدوستيرون وكذلك مضخة ايون الصوديم / ايون البوتاسيم / انزيم الادينوزين ثلاثى الفوسفات والتى تلثرت هذة الصفات بزيادة مستوى كلوريد الصوديوم فى ماء الشرب.

اظهرت النتائج ان عدد خلايا الهتيروفيل ونسبة خلايا الهتيروفيل الى اللمغوسيت باستسناء خلايا اللمفوسيت ارتفعت معنويا مع زيادة مستوى كلوريد الصوديوم فى ماء الشرب اما اضبافة الفيتامين لم يؤثر على خلايا الهتيروفيل ولكنة سبب انخفاض فى نسبة خلايا الهتيروفيل الى اللمفوسيت وايضا زيادة فى خلايا اللمفوسيت .

نستخلص من هذة الدراسة ان اضافة فيتامين ج لة تاثيرات مفيدة على المكونات الخلوية بالدم وعلى بعض القياسات الفسيولوجية و الضغط السموزى لبلازما الدم للطيور التى تتناول مستويات من مجرام كلوريد المسوديوم فى ماء الشرب خلال فترة النمو وتوصى هذه الدراسة بتحليل مياه الشرب لتقدير نسبة الأملاح مع اضافة فيتامين ج عند ارتفاع مستوى الملوحة لتفادى التأثيرات الضارة.