

EVALUATION OF WATER POLLUTED WITH NITRITE ON EGG PRODUCTION AND SOME HEMATOBIOCHEMICAL PARAMETERS IN LAYING HENS FARMS

EMAN, I. M. ISMAIL¹; OSAMA E. RAMDAN¹; MAGDA N. ABD EL HAMID¹; EL-NILE, M.B.¹; NAHED A.KAMOURA² AND SANNA M. SALEM³

¹Department Biochemistry, Animal Health Research Institute (Zagazig Branch)

²Department Clinical Pathology, Animal Health Research Institute (Zagazig Branch)

³Department Histopathology Animal Health Research Institute (Zagazig Branch)

Received: 14 September 2020; **Accepted:** 5 October 2020

ABSTRACT

This study was designed to determine adverse effect of nitrite on some hematobiochemical parameters and egg production in laying hens. Sample from underground well water from different region in Sharkia province (Diarp Negm, Kafr Sakr, El Salhia and Zagazig) was examined for detection nitrite level. Analysis of water sample revealed presence of nitrite in level of 0.34, 0.013, 0.05 and 0.63 ppm nitrite in Zagazig, Diarp Negm, Kafr Saker and El Salhia Cities. A total of 60 laying hens (22 week age) were divided into 3 equal groups (20 of each). First group laying hens received one liter of water contains 1mg nitrite/liter for 30 day (control group), second group laying hens received one liter of water contains 4mg nitrite/lite for 30 day and third group laying hens received one liter of water contains 8mg nitrite/lite for 30 days. At 1st day post supplementation three blood samples were collected from 5 birds for hematobiochemical study. Hens received one liter of water contains 4mg sodium nitrite/liter drinking water for 30 days showed non-significant reduction in RBCs, Hb, PCV, MCHC, WBCs, lymphocyte, Phagocytosis, total protein, albumin, globulin and egg production and Calcium beside insignificant increase in MCV, MCH, Platelet count, heterophils, AST, ALT, ALP, uric acid and creatinine. Meanwhile hens received one liter of water contains 8 mg nitrite/liter show significant reduction in RBCs, Hb, PCV, MCHC, WBCs, lymphocyte, Phagocytosis, total protein, albumin, globulin, egg production%, egg weight, Shell thicknes, Egg shell weight and Calcium beside significant increase in MCV, MCH, Platelet count, heterophils, AST, ALT, ALP, uric acid and creatinine. Residues of nitrite in liver and kidney beside its dose dependent laying hens received one liter of water contains 0.1% sodium nitrite showed normal histological structures in all body organs (liver, Kidney and spleen) but laying hens received one liter of water contains 4% and one liter of water contains 8% sodium nitrite showed moderate to severe lesion in liver, Kidney and spleen. It could be concluded that high nitrite in water induce alterations in hematobiochemical parameters and egg production as well as pathological lesions. So, it is recommended not use water contains high level of nitrite in laying hens farms.

Key words: Water Pollution by Nitrite – Hematololgy- Biochemistry– Egg production-Laying hens.

INTRODUCTION

Water is vital to life of the organism that water content of the body be

maintained, an animal will die more rapidly if deprived of water than if deprived of food (Mc Donald *et al.*, 2002). Water help to maintain homeostasis and control pH, osmotic pressures and other functions necessary for life (Scott *et al.*, 1982). Environmental pollution is deleterious of biological life. Chemical Pollution

Corresponding author: EMAN, I. M. ISMAIL
E-mail address: dr.emanismail201@gmail.com
Present address: Department Biochemistry, Animal Health Research Institute (Zagazig Branch)

(pesticides, heavy metals, organic compounds, toxic gases and fumes) widely spread and threatens biological balance (Forstner and Wittman, 1983). Pure water does not exist in nature (Jennings *et al.*, 1996). Water pollution is one of world problems, which results from increased population growth, urbanization and industrialization (Ma *et al.*, 2009). Rain water collect impurities while passing through air, dangerous products from industry and agriculture reaches the rivers, lakes, and underground water (Salem *et al.*, 2000).

Sodium nitrite is important antimicrobial, flavoring, coloring and preservative agent in meat. Nitrite may cause methemoglobinemia (Gihan *et al.*, 2014). Nitrite is formed naturally by nitrogen cycle during process of nitrogen fixation and possess mutagenic, teratogenic and embryotoxic. Two main nitrite salt forms occur, sodium and potassium nitrite (Atef *et al.*, 1991). Nitrite is a natural body constituent and is important for biological functions (Jensen, 2007) which performs a major role in immunology (Lundberg *et al.*, 2008). High levels of nitrite is considered a toxic as it can bring about physiological disturbances effects upon health, including impairment of reproductive function, endocrine disturbances and hepatorenal toxicity (Abu Aita and Mohammed 2014). Nitrite toxicity may be attributed to its potency to produce oxidative stress (Ansari *et al.*, 2015). Low levels of nitrite may play a potential role as an antioxidant and has several health benefits (McNally *et al.*, 2016).

Nitrites are used as human food additives mainly for production of specific flavor and for preservation of meat products. Several organic nitrites have been used clinically but only inorganic nitrites of therapeutic are sodium nitrite (Heibashy and Abd El Moneim, 1999). Nitrites and nitrates are environmental pollutants present in food and water and it is suggested that they may contribute to the etiology of liver and

kidney diseases and problems related of immunity in domestic fowls (Ibrahim *et al.*, 1999).

The objective of this study is to determine the effect of Nitrites on egg production, blood picture and blood biochemistry in laying hens and associated with pathological change.

MATERIALS AND METHODS

Water samples and Chemical analysis

Underground well water samples (one liter of water) from Zagazig, Diarp Negm, Kafr Sakr and El Salhia Cities areas were collected for detection of Ph calculated by jenway apparatus (pH meter) model number 3510. Nitrite was determined used spectrophotometer model 690 according to Haimin *et al.* (2016). Total dissolved solid were analyzed according (APHA, 1999) and calculated according to equation

$$W_1 - W_2 \times 10$$

Where W_1 weight of empty flask

W_2 weight of flask after heating with 100ml water untill boiling then evaporatd all water and remining salt only

Birds and Experimental design

About 60 laying hens, 22 week age were divided into 3 equal groups (20 each). First group hens received one liter of water contains 1mg nitrite/liter drinking water for 30 day (control group), second group hens received one liter of water contains 4mg nitrite/liter drinking water for 30 day (Safary and Daneshyar, 2012) and third group hens received one liter of water contains 8mg nitrite/liter drinking water for 30 day with (Eman and Fahm 2006).

Blood samples

At 1st day post supplementation 5 laying hens from each group were selected and three blood samples were collected.

First sample was taken on tube contain EDTA for estimation blood picture (Jain, 1993).

Second sample was taken in tube contain heparin for estimation phagocytosis Heparinized blood samples used for phagocytosis assay:-

- a. Separation of Peripheral Blood Mononuclear Cells (PBMC) using ficollplaque density gradient was carried out (Boyum, 1986 and Godeeris *et al.*, 1986).
- b. Phagocytic assay (Wilkinson, 1976).
- c. Measurement of phagocytic activity of Peripheral Blood Monocyte (PBM) using *Candida albicans* was performed (Anthony *et al.*, 1985 and Chu and Dietert, 1989).
- d. Evaluation of phagocytic activity (Wilkinson, 1976).

Third samples was taken without anticoagulant for estimation (AST and ALT) (Ritman and Frankle 1957), ALP (John 1982), total protein (Doumas *et al.*, 1981), albumin (Bauer, 1982), globulin (Kapale, 2008), uric acid (Coalombe and Faurean 1963), creatinine (Husdan and Roporpot, 1968) and calcium (Gindler 1972)

Egg production, egg weight and Shell thickness

Egg produced by each groups were collected during and 1st month post supplementation for recorded egg production% and egg weight and Shell thickness (Summers *et al.*, 1976).

Examination

Specimens were taken from liver, kidneys, intestine, spleen and bursa of the sacrificed duckling and directly fixed in 10% neutral buffered formalin. Five-micron thick paraffin sections were prepared stained with hematoxylin and eosin and examined microscopically (Bancroft and Gamble, 2002).

Measurements of tissue nitrite in liver and kidney

Residues of nitrite in liver and kidney were determined according method described by (Carlstrom *et al.*, 2010)

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). Duncan's Multiple Range Duncan, (1955) was used to determine differences among treatments

mean at significance level of 0.05. Statistics were run using SPSS program (SPSS 2004)

RESULTS

Analysis of underground well waters from different region in Sharkia Province revealed levels of PH, total dissolved solid and nitrite in Diarp Negm were 8.28, 608 and 0.013, Kafr Sakr 8.46, 207.25, 0.05, El Salhia, 8.69, 722.5, 0.63 and Zagazig, 9.85, 1764.33, 34 ppm respectively table (1).

The obtained results for some egg parameters were summarized in table (4). The results revealed laying hens received one liter of water contains 8mg sodium nitrate/liter drinking water decreased egg production%, egg weight, shell thickness and shell weight.

Hens received one liter of water contains 4mg sodium nitrite/liter drinking water for 30 days showed non-significant reduction in RBCs, Hb, PCV, MCHC, WBCs, lymphocyte, Phagocytosis, total protein, albumin, globulin and egg production and Calcium beside insignificant increase in MCV, MCH, Platelet count, neutrophils, AST, ALT, ALP, uric acid and creatinine. Meanwhile hens received one liter of water contains 8 mg nitrite/liter show significant reduction in RBCs, Hb, PCV, MCHC, WBCs, lymphocyte, Phagocytosis, total protein, albumin, globulin, egg production%, egg weight, Shell thickness, Egg shell weight and Calcium beside significant increase in MCV, MCH, Platelet count, neutrophils, AST, ALT, ALP, uric acid and creatinine table(3).

Residues of nitrite in liver and kidney is dose dependent, Table (5) showed that the nitrite residues were detected in high levels in kidney more than liver post administration.

Gross pathological lesion appeared in laying hens received one liter of water contains 0.1% sodium nitrite showed liver, Kidney and spleen normal histological characterization of different structures but laying hens received one liter of water contains 4% and one liter of water contains

8% sodium nitrite showed sever lesion in liver, Kidney and spleen.

Residues of nitrite in liver and kidney is dose dependent, Table, (5) showed nitrite residues were detected in high levels in kidney more that liver post supplementation.

Sodium nitrite in dose 1mg/liter water showed normal histological structures of different organ. Liver, portal area, hepatocytes were seen small masses around central veins. a few round cells were seen as a natural immune response around portal area (Fig. 1 A, B) Kidney showed normal nephron, including tubular structures, Uri nephric duct and avian glomeruli. The stromal and vascular structures were apparently normal. (Fig.1C, D). Spleen showed normal white and red pulp with central arteriole, germinal centers and red pulp sinusoids with their reticulo-endothelial linings. (Fig.1 E, F)

Hens received one liter of water contains 4mg sodium nitrite /liter show: Liver dilated, congested poenrtal blood vessels, moderate biliary proliferation, portal round cells aggregations and predominance of eosinophils in some areas (Fig 2. A,B). Scale bars 50 um, 25um Kidney (Fig 2. C,

D). Renal vascular congestion, tubular degeneration, focal tubular hyperplasia and focal glomerular endothelial cellular proliferation. Scale bars 50 um, 25 um. Spleen (Fig. 2. E, F). Moderate germinal centers hyperplasia and peri-arteriolar sheath lymphoid proliferation with a normal structurally organized Red pulp. Scale bars 50 um, 25 um

Hens received one liter of water contains 8 sodium nitrite/liter Liver show hepatic portal round cells aggregations, biliary proliferative hyperplasia with focal metaplastic change together with portal Fibroplasia and infiltration of eosinophils. Interstitial round cells aggregations, eosinophils infiltration and hepatocellular degeneration and individual cellular apoptosis. (Fig. 3 A, B). Kidney. Show peri-vascular edema, intestinal hemorrhages perivascular and interstitial round cell aggregations and renal tubular degeneration with focal necrotic changes (Fig.3 C, D). Spleen showed germinal centers hyperplasia and peri-arteriolar sheath lymphoid proliferation, diffuse replacement of red pulp by mature and immature lymphocytic and eosinophils infiltration. (Fig.3 E, F).

Table (1): Analysis of underground well water in different centers in Sharkia Governorate (n=5).

Centers	Zagazig	Kafr Sakr	El Salhia	Diarp Negm
PH	9.85±0.87	8.46±0.98	8.69±0.79	8.28±0.83
Total dissolved solid	1764.33±3.18	207.25±2.37	722.5±3.94	608±2.58
Nitrite(ppm)	0.34±0.05	0.05±0.004	0.63±0.12	0.013±0.006

Table (2): Effect of sodium nitrite on blood picture and phagocytosis in laying hens (N=5)

Groups		1mg nitrite	4mg nitrite	8mg nitrite	
Erythrogram	RBCs(×10 ⁶)	4.20±0.65a	3.90±0.65a	2.52±0.65b	
	Hb(g/dl)	9.40±0.92a	8.60±0.65a	6.00±0.92b	
	PCV (%)	27.78±2.14a	26.74±0.65a	19.24±2.14b	
	Blood indices	MCHC(%)	35.94±1.21a	35.57±0.65a	32.77±1.21b
		MCH(Pg)	27.63±1.20b	28.48±0.65b	30.53±1.20a
		MCV(FL)	31.85±1.12b	33.22±0.65b	36.22±0.65
Platelet (103/cmm)		268.23±2.14b	269.45±0.65b	284.18±2.14a	
leukogram	Total WBCs count 10 ³ /cm	9.21±0.41a	8.51±0.23a	9.21±0.43a	
	Diferential count %	Heterophils	28.03±0.39b	51.03±0.26a	39.23±0.21a
		Lymphoyte	52.12±0.23a	51.03±0.26a	48.06±0.42b
		Eosinophils	3.11± 0.19a	2.15±0.21a	2.03±0.11a
		Basophils	3.20± 0.16 a	2.03±0.26a	1.07±0.27b
		Monocyte	13.41±0.21b	13.41±0.21b	8.08±0.18a
Phagocytosis (%)		22.32±0.33a	21.42±0.40a	19.61±0.34b	

Table (3): Effect of sodium nitrite on liver and kidney functions in laying hens (n=5)

Groups		1mg nitrite/liter	4mg nitrite/liter	8mg nitrite/liter	
Liver function (u/l)	Liver enzymes	ALT	18.34± 0.89b	19.18± 0.95b	25.09± 0.89a
		AST	53.18±0.78b	55.34±0.98b	59.18±0.78a
		ALP	31.21±0.88b	31.21±0.88b	37.26±0.98a
	Protein picture (gm/dl)	T. protein	5.54±0.42a	5.08±0.21a	3.69±0.52b
		Albumin	2.99±0.45a	2.78±0.33a	2.15±0.28b
		Globulin	2.55±0.32a	2.30±0.40a	1.54±0.21b
		A/G ratio	1.17±0.28b	1.21±0.38ab	1.40±0.23a
Kidney function (mg/dl)	Uric acid	7.23±0.72b	7.99±0.47b	9.41±0.55a	
	Creatinine	1.02±0.21b	1.52±0.42b	2.13±0.27a	
	Calcium	19.42 ± 0.89a	18.67 ± 0.91a	14.18 ± 0.79b	

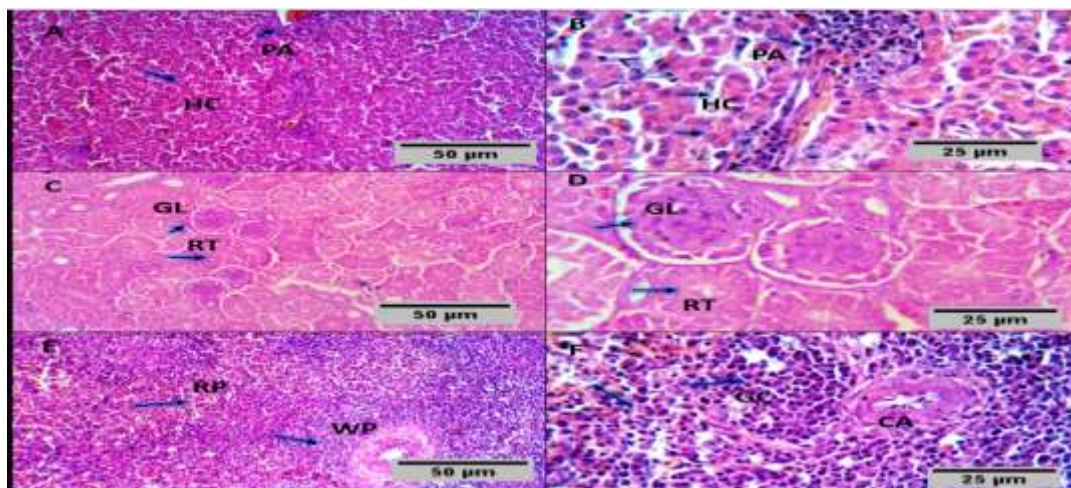
Table 4: Effect of sodium nitrite on egg production%, and egg characters in laying hens (n=5)

Groups		1mg nitrite/liter	4mg nitrite/liter	8mg nitrite/liter
egg production%	During suppl	78.21 ±1.98a	77.26±1.56a	69.31±1.87b
	1 st month post suppl	78.86 ±1.44a	77.98±1.80a	57.60±1.36b
Egg weight(gm)	During suppl	55.11±1.03a	53.42±1.31a	50.10±1.41b
	1 st month post suppl	55.08±1.12a	54.13±1.22a	52.12±1.21b
Shell thickness(gm)	During suppl	28.2±1.03a	26.13±1.13a	23.14±1.07b
	1 st month post suppl	28.14±1.12a	27.35±1.18a	26.89±1.29a
shell weight (gm)	During suppl	7.37 ± 0.34a	7.03 ± 0.28a	6.87 ± 0.52a
	1 st month post suppl	7.04 ± 0.29a	7.01 ± 0.38a	6.68 ± 0.28a

During supplementation 1st month post suppl.= 1st month post =During suppl
supplementation

Table 5: Nitrite residue in liver and kidney (mg/kgm) in laying hens (n=5)

Groups	1mg nitrite/liter	4mg nitrite/liter	8mg nitrite/liter
Liver	00	00	30.18±1.59
Kidney	00	0.59±0.17	48.43±1.40

**Fig. 1:** Hens received one liter of water contains 1mg sodium nitrite /liter drinking water show

Liver (A,B). Normal histological of different structures, including portal area (triads) (PA, arrow), hepatocytes (HC, arrow) which are seen as a small masses around the central veins. a few round cells are seen as a natural immune response around the portal area (PA, arrow). Scale bars 50 um, 25 um. **Kidney** (C,D). Normal histomorphology of the nephron unites, including the tubular structures (RT, arrow), uri-nephric duct and avian glomeruli (GL, arrow). Scale bars 50 um, 25 um. **Spleen** (E,F). Apparently normal whit (WP, arrow) and red pulp (RP, arrow) with a keeping features of the central arteriole (CA, arrow), germinal centers (GC, arrow) and red pulp sinusoids with their reticulo-endothelial linings. Scale bars 50 um, 25 um.

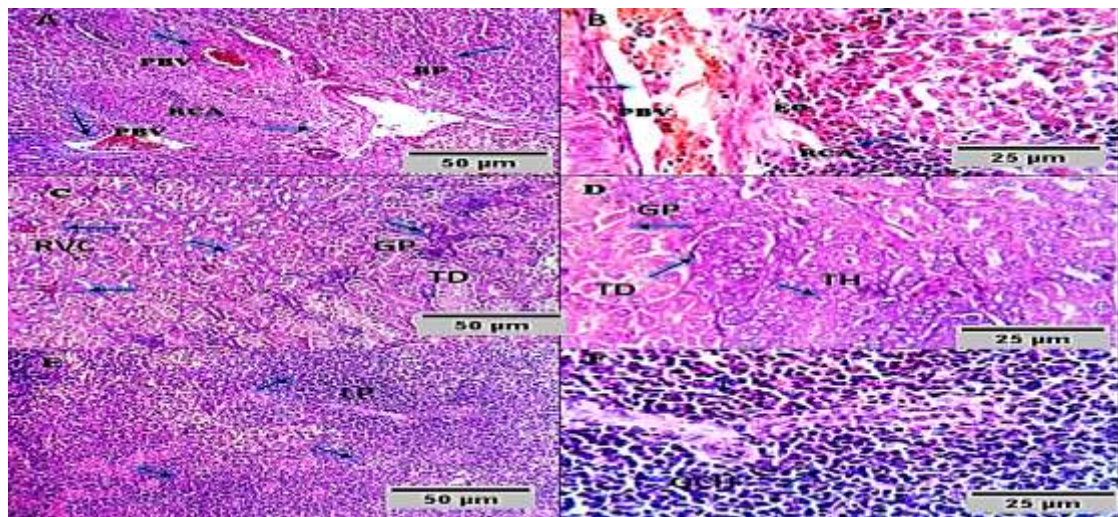


Fig. 2: Hens received one liter of water contains 4mg sodium nitrite /liter drinking water show

Liver (A, B). Dilated, congested portal blood vessels (PBV, arrow), mild to moderate biliary proliferation (BP, arrow), portal round cells aggregations (RCA, arrow) with a predominance of eosinophils (EO, arrow) in some areas are seen. Scale bars 50 µm, 25 µm.

Kidney (C, D). Renal vascular congestion (RVC, arrow), tubular degeneration (TD, arrow), focal tubular hyperplasia (TH, arrow) and focal glomerular endothelial-mesangial cellular proliferation (GP, arrow) are seen. Scale bars 50 µm, 25 µm.

Spleen (E, F). A reactive mild to moderate germinal centers hyperplasia (GCH, arrow) and peri-arteriolar sheath lymphoid proliferation (LP, arrow) are observed with a normal structurally organized Red pulp (RP, arrow). Scale bars 50 µm, 25 µm.

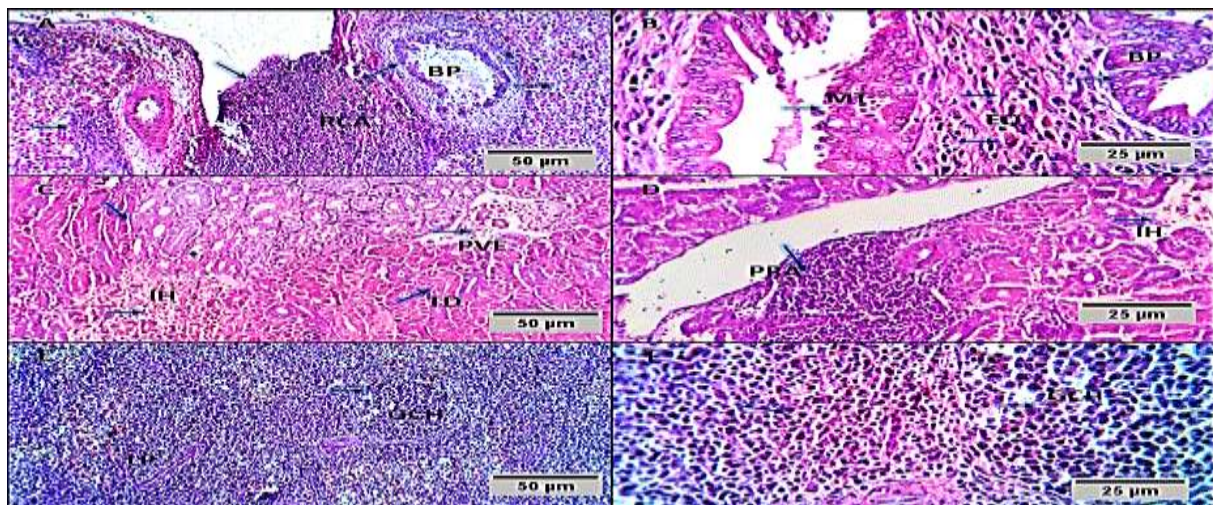


Fig. 3: Hens received one liter of water contains 8 mg sodium nitrite /liter drinking water show

Liver. Demonstrated characteristic marked hepatic histopathologic changes as portal round cells aggregations (RCA), biliary proliferative hyperplasia (BP) with focal metaplastic change (MT) together with portal Fibroplasia and infiltration of eosinophils. Marked interstitial round cells aggregations, eosinophils infiltration and hepatocellular degeneration and individual cellular apoptosis were also encountered. (Fig.3 A, B). **Kidney.** Histopathological changes were marked in this group; there were perivascular edema (PVE), intestinal hemorrhages (IH) perivascular and interstitial round cell aggregations (RCA) and renal tubular degeneration (RTD) with focal early necrotic changes. (Fig.3 C D). **Spleen** germinal centers hyperplasia (GCH) and peri-arteriolar sheath lymphoid proliferation (LP) together with focal or diffuse replacement of red pulp by mature and immature lymphocytic, eosinophils infiltration was encountered.

DISCUSSION

Analysis of underground well water from different region in Sharkia Province revealed levels of PH, total dissolved solid and nitrite in Diarp Negm were 8.28, 608 and 0.013, Kafr Sakr 8.46, 207.25, 0.05, El Salhia, 8.69, 722.5, 0.63 and Zagazig, 9.85, 1764.33, 34 ppm respectively. The lowest level of these parameters were present in underground well water in Diarp Negm followed by Kafr Sakr then El Salhia but high levels of these parameters were present in underground well water in Zagazig city (Table 1). Same result was recorded by (Talha *et al.*, 2008) stated that Physical, and chemical properties (Phvalues, total dissolved solid and nitrite) were increased in well water due to pollution. Presence and increased concentration of nitrite in underground water well is correlated to high industrial and agriculture wastes (Jennings *et al.*, 1996).

The results of this study revealed that hens received one liter of water contains 4mg sodium nitrite/liter drinking water for 30 days showed non-significant reduction in RBCs, Hb, PCV, WBCs, lymphocyte, Phagocytic activity, total protein, albumin, globulin and egg production beside insignificant increase in MCH, MCHC, platelet count, AST, ALT, ALP, uric acid and creatinine and significant increase heterophils,. Same change in egg production was observed by Atef *et al.* (1991) in laying hens. Laying received 4 mg sodium nitrate/liter water reduced in egg production and blood chemistry (Safary and Daneshyar, 2012).

Results of the present study indicate that laying hens received one liter of water contains 8mg sodium nitrite/ liter drinking water for 30 days showed insignificant reduction in WBCs beside significant reduction in RBCs, Hb, PCV, MCHC, lymphocyte, Monocyte eosinophils, basophils Monocyte and Phagocytic activity coupled with increase in Platelet count, MCV MCH, hetetrophils (table, 2 and 3). This observation was previously recorded by Kundsén and Jensen (1997) who reported that nitrite induce decrease in RBCs Hb PCV and WBCs. This reduction in erythrogram may be due to fragility of erythrocytes induced by nitrite (Atef *et al.*, 1991). The decrease in leukocytic count post using sodium nitrite may

be due to failure of hematopoietic tissues to produce new leukocyte (Tan *et al.*, 1992). Reduction in blood picture parameters may be due to Oxidative damage of RBCs and lysis or shrinkage of erythrocytes in the blood (Beaupre and Schiffman, 1994). Similar findings were reported by Diazi *et al.* (1995) in chickens exposed to nitrite. Reduction of Hb level may be due to nitrites convert the ferrous ion of hemoglobin to ferric ion (Ganong, 1997). Our data clearly reinforced by those obtained by Abuharfeil *et al.* (2001) reported that mice received sublethal doses of sodium nitrite in drinking water for 21 days revealed significant decrease in dose-dependent in WBCs, lymphocyte % and Phagocytosis coupled with increase in neutrophil count. Mice received 25 to 100 mg sodium nitrite orally for 21 day induced dose dependent decrease of lymphocyte (Chow and Hong, 2002). Our results came in agreement with (Eman and Fahmy 2006 and Eman *et al.*, 2008) stated that sodium nitrite induced significant decrease in RBCs, Hb, PCV and WBCs in rats. Our data clearly reinforced by those obtained by Gluhchevaa *et al.* (2012) who concluded that rats received sodium nitrite showed significantly decrease in RBCs, Hb, PCV coupled with significant increase in MCH and MCHC. Our results were supported by Abu Aita and Mohammed (2014) stated rats received sodium nitrite showed significant decreases in RBCs, Hb, PCV, WBCs, MCHC and increase MCV. Nitrite causes methemoglobinemia and reduction in hemoglobin (Gihan *et al.*, 2014). Our data is agreed with Azab *et al.* (2015) found that Guinea pigs received 80 mg/kg body weight sodium nitrite daily for 35 days showed significant reduction in RBCs, Hb, PCV and WBCs, MCH, MCHC beside increase in MCV. Sodium nitrate induces decrease in RBCs, Hb, PCV and WBCs, lymphocyte and increase in neutrophils (Ashmore *et al.*, 2016 and Khaled *et al.*, 2019).

In the current work, it has been found that laying hens received one liter of water contains 8 mg sodium nitrite /liter drinking water for 30 days revealed significant decrease in total protein, albumin and globulin (Table 3). Nearly similar results were recorded by Ibrahim *et al.* (1999) mentioned that nitrate toxicity in duck revealed significant decrease in total protein, albumin and globulin. Another explanation for

reduction of total protein, albumin and globulin come from Salama *et al.* (2013) who stated that Sodium nitrite-induced hepatic damage by intensification of oxidative stress and DNA damages and inhibition of mitochondrial function leading to reduction of albumin. Similar result was recorded previously by Eman and Fahmy (2006) and Eman *et al.* (2008) in rat received of sodium nitrite. The obtained results agree with those reported by Hassan *et al.* (2009) and Gihan *et al.* (2014) who stated that Guinea pigs exposed to sodium nitrite were significantly decreased serum total proteins, albumin, and globulin concentration. Reduction in total protein, albumin and globulin may be due to toxic effect of nitrite inducing impaired albumin synthesis by damaged liver cells (Abdel-Rahman *et al.* 2018). Also, Nagla *et al.* (2020) reported that adult rats received sodium nitrite induces dose dependent decrease serum total protein, albumin and globulin levels.

The obtained results for serum liver enzymes summarized in table, (3) revealed a significant increase AST, ALT, ALP, uric acid and creatinine beside reduction in calcium level in laying hens received one liter of water contains 8 mg sodium nitrite/ liter drinking water for 30 days. Our results go hand in hand with those reported by Eman and Fahmy (2006) and Eman *et al.*, (2008) stated that sodium nitrite induced increase in liver enzymes in rats. Elevation in uric acid and creatinine is due to kidney injury (El-Sheikh and Khalil, 2011). Nearly similar findings were reported by Safary and Daneshyar (2012) they found that liver enzymes were increased and reduction in calcium level in serum of laying hens received sodium nitrite. Similar result was recorded previously by Imam and Mohammed (2013) stated that sodium nitrite induced liver damage and increase AST, ALT and ALP in rats. Our results were recorded by Mohammed *et al.* (2014) and Gihan *et al.* (2014) stated that nitrite significant increase in AST, ALT and ALP in rats. Elevation in liver enzymes may be due to toxic impacts of nitro so-compounds formed in acidic environment of the stomach causing severe hepatic necrosis (Abdel-Rahman *et al.*, 2018). In the same direction, Jeppe *et al.* (2018) observed that sodium nitrite impaired renal function, and increased uric acid and creatinine. Nitrite induces significant increase AST, ALT, ALP, uric acid and creatinine (Nagla *et al.*, 2020).

The obtained results for some egg parameters were summarized in table (4). The results revealed laying hens received one liter of water contains 8mg sodium nitrate/liter drinking water decreased egg production%, egg weight, shell thickness and shell weight. Layers received water high in nitrite produced significant decreased in egg production% and egg weight and shell thickness (Talha *et al.*, 2008).

Residues of nitrite in liver and kidney beside its dose dependent, Table, (5) showed that the nitrite residues were detected in high levels in kidney more than liver post supplementation. The obtained results nearly coincide with those reported by Kohn *et al.* (2002) who mentioned nitrite excretion rapidly in urine. These results agreed with Lee *et al.* (2018) reported that nitrite were detected in high levels in kidney than liver and this may be due to nitrite excreted through urine.

The main gross pathological lesion appeared in laying hens received one liter of water contains 0.1% sodium nitrite showed liver, Kidney and spleen normal histological characterization of different structures but laying hens received one liter of water contains 4% and 8% sodium nitrite showed moderate to severe lesion in liver, Kidney and spleen. Same gross pathological lesion was observed previously in mice received sodium nitrite (Sadiq *et al.*, 2018). Goats received 4 mg potassium nitrite/kg b. wt orally for 32 days caused dilatation of central vein and sinusoids, degenerative changes in periarterial zone, beside hyperplasia, hypertrophy and detachment of epithelium lining of bile duct with mononuclear cell infiltration (Mondal *et al.*, 1999 and Kohn *et al.*, 2002). Pathological finding in our work revealed severe lesion in laying hens received one liter of water contains 8mg sodium nitrate /liter drinking water but laying hens received one liter of water contains 4mg sodium nitrate/liter drinking water lesions were mild. Same pathological changes in rat liver (Hakki *et al.*, 2009) received sodium nitrite. Sodium nitrate induced shrunken glomerular tuft, degeneration of some tubules and epithelial lining cells. Formation of focal fibrosis and infiltrated with a number of inflammatory cells. Liver showed hydropic degenerated

hepatocytes, necrotic areas infiltrated with a number of inflammatory cells (Sanaa and Mahmoud 2012). The previous observations were supported by Hasan *et al.* (2014) found that sodium nitrite induced degenerative changes in liver, kidney and spleen in mice. Same pathological lesions were reported by (Lee *et al.*, 2018) in dogs received nitrite. Pathological changes observed in liver and kidney post using sodium nitrite may be due to reactive oxygen and nitrogen species produced by nitrite (Sherif and Al-Gayyar 2015). Same pathological lesions were reported by Nagla *et al.* (2020) in rats received sodium nitrite.

From the previously mentioned results it could be concluded that, high levels of nitrite induce alterations in some hematobiochemical parameters, egg production and induce many pathological lesions. So, not use water contains high levels of nitrite in laying farms.

REFERENCES

- Abdel-Rahman, H.; Abd-El-Fattah, M.; Youssef, M.; Essawi, E. and Elsedawy, M. (2018):* the protective effect of different doses of alpha lipoic acid against hepatotoxicity of sodium nitrite in rat. *Int J Dev Res* 08(11): 24140-24145
- Abu Aita, N. and Mohammed, F. (2014):* Effect of marjoram oil on clinicpathological, and pathological alterations induced by nitrite in rats. *Glob Vet* 12 (5) 606-616.
- Abuharfeil, N.; Sarsour, E. and Hassuneh, M. (2001):* the effect of sodium nitrite on some parameters of the immune system. *Food Chem Toxicol* 2001; 39:119-124.
- American Public Health Association (APHA) (1999):* Standard method for examination of water and wastewater (20thed). Washington: AWWA/ WPCE. American Water Works Association, Water Environment Federation.
- Ansari, F.; Ali, S. and Mahmood, R. (2015):* Sodium nitrite-induced oxidative stress causes membrane damage, protein oxidation, lipid peroxidation and alters major metabolic pathways in human erythrocytes. *Toxicol Vitr* 29(7):1878-1886.
- Anthony, T.; Twin, K.; Erin, M. and Michael, E. (1985):* "Phagocytic and killing capacities of urine derived leukocytes. From mares, resistance and susceptible to chronic endometritis". *Am. J. Vet. Res.* 46 (9): 1938-1940.
- Atef, M.; Abo-Norag, M.; Hanafy, M. and Agag, A. (1991):* Pharmacological aspects of nitrate and nitrite in laying hens. *British Poultry Science*, 32, 99-104.
- Ashmore, T.; Fernandez, B.; BrancoPrice, C.; Griffin, J. and Murray, A. (2016):* Suppression of erythropoiesis by dietary nitrate. *The FASEB J.* 29(3): 1102-1112.
- Azab, E.; Nuri, M. and Mohamed, O. (2015):* Haemato-protective and hypolipidemic effects of libyan propolis against sodium nitrite induced haematotoxicity and hyperlipid-emia in Guinea Pigs. *Amer J. of Bios and Bioengineering*, 3(4) 22-32
- Bancroft, J. and Gamble, M. (2002):* Theory and practice of histological technique, 5th Ed livingston, London, Edinburgh, New york, Philadelphina, St Louis, Syndeg.
- Bauer, J. (1982):* Determination of Serum albumin. *Clinical Methods*, 4th Ed., 495-496.
- Beaupre, S. and Schiffman, F. (1994):* Rush hemolysis. A "bite-cell" hemolytic anemia associated with volatile liquid nitrite use. *Archives of Family Medicine* 3, 545-548
- Boyum, A. (1986):* "Isolation of mononuclear cells and granulocytes from human blood". *Canadian. J. Clin. Invest.* 21: 77-89.
- Chow, C. and Hong, C. (2002):* Dietary vitamin E and selenium and toxicity of nitrite and nitrate. *Toxicology* 180: 195-207.
- Carlstrom, M.; Larsen, F.; Nystrom, T.; Hezel, M.; Borniquel, S. and Weitzberg, E. (2010):* Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc Natl Acad Sci USA*, 107, 17716 -17720
- Chu, Y. and Dietert, R. (1989):* "Monocytes function in chicken with hereditary dystrophy". *Poult. Sci.* 68: 226-232
- Coalombe, J. and Faurean, I. (1963):* determination of urea. *Clin. Chem.*, 9:102-108.
- Diazi, G.; Julian, R. and Squires, E. (1995):* Effect of dietary nitrite on pulmonary hypertension in chickens and dilatory

- cardiomyopathy in turkey Avian Patho 24, 19-29
- Doumas, B.; Cartor, R.; Peers, T. and Schafer, R. (1981):* A candidate reference method for determination of total protein in serum Clin Chem, 27, 1642.
- Duncan, D. (1955):* Multiple ranges and multiple "F" test. Biometrics, 11:10.
- El-Sheikh, N. and Khalil, F. (2011):* L-Arginine and l-glutamine as immunonutrients and modulating agents for oxidative stress and toxicity induced by sodium nitrite in rats. Food Chem Toxicol 49(4): 758-762.
- Eman, H.; Zahkok, S.; Ghada, Z.; Al-Kassas, M. and Abdel, H. (2008):* Biochemical studies on the effect of sodium nitrite and/or glutathione treatment on male rats. The Egyptian J. of Hospital Med. 30 (1) 25- 38.
- Eman, G. and Fahmy, G. (2006):* management the action of sodium nitrite on albino rats by aqueous garlic extract. Res. J. of Medicine and Med. Sci, 1(3)85-89
- Forstner, N. and Wittman, G. (1983):* "Metal pollution in the aquatic environment" Springer-Verlag, Berlin.
- Ganong, W. (1997):* Review of Medical Physiology. 8th ed. Libraire du liban, Appelton of Longe, Lebanon, California: 296 - 311.
- Gihan, M.; Hammoud, S.; Nail, K.; ELSawy, S.; Abd El-Rahman, S.; Youssef, M. and Mohamed, S. (2014):* Protective effect of grape seed extract against sodium nitrite induced toxicity and oxidative stress in albino rats. Az. J. Pharm Sci. 49, (1) 1-9
- Gindler, E. (1972):* Determination serum calcium level. Am. J. Clin, Path (58) 376.
- Gluhchevaa, Y.; Ivanovb, I. and Vladova, I. (2012):* Sodium nitrite-induced hematological and hemorheological changes in rats. Series on Biomechanics, 27 (3-4) 53-58
- Godeeris, B.; Boldwin, C. and Morrison, W. (1986):* "Improved methods for purification and depletion of monocytes from bovine peripheral blood mononuclear cells. Functional evaluation of monocytes in response to Lectins". Imm. Meth. J. 89(2): 165-73.
- Haimin, Z.; Shenghong, K.; Yunxia, Z. and andHuijun, Z. (2016):* fluorescene determination of nitrite in water using prawn-shell derived- Nitrogen-Doped carbone Nondateas fluorophores ACS sensors 1(7) 875-681
- Hasan, Ö.; Ufuk, K.; Musa, K.; Süleyman, G.; Emine, A.; Kadir, Ö. and Onur, A. (2014):* Histopathologic, Biochemical and Genotoxic Investigations on Chronic Sodium Nitrite Toxicity in Mice. Exp Toxicol Path 66 (8):367-75
- Hassan, H.; El Agmy, S.; Gaur, R.; Fernando, A. and Raj, M. (2009):* In vivo evidence of the hepato-and renoprotective effect of garlic oil against sodium nitrite-induced oxidative stress. Int J Biol Sci; 5: 249-55.
- Heihashy, S. and Abd El-Moneim F. (1999):* Blood lipid profile and serum free thyroidal hormone concentrations in growing rats fed diets enriched with sodium nitrate for short and long terms. J. Egypt. Ger. Soc. Zool.; 30 (A): 93- 103.
- Hakki, C.; Fatih, F. and Hayati, Y. (2009):* protective potential of Yucca schidigera against nitrite-induced oxidative stress in rats..J Natur Med. 63(3): 311-317
- Husdan, H. and Roporpot, A. (1968):* Estimation of creatinin. Clin. Chem14, 22
- Ibrahim, A.; Zaky, Z.; Sharkawy, A. and Mubarak, M. (1999):* Effect of vit. C on nitrate toxicity in ducks.15th annual conf for Egy. Soci of Toxicol. Alex. Univ.
- Imam, O. and Mohammed, M. (2013):* Antioxidant and hepatoprotective of silymarin on hepatic dysfunction induced by sodium nitrite. Eur. Cytok Netw. 24(3)14-21
- Jain, N. (1993):* Essentials of Veterinary Hematology. Lea & Fibiger. Philadelphia. USA.
- Jennings, G.; Sneed, R. and Clair, M. (1996):* Metals in Drinking Water. Published by: North Carolina Cooperative Extension service Publication.
- Jepppe, B.; Erling, B. and Jesper, N. (2018):* the effect of sodium nitrite infusion on renal function, brachial and central blood pressure during enzyme inhibition by allopurinol, enalapril or acetazolamide in healthy subjects: a randomized, double-blinded, placebo-controlled, crossover study. BMC Nephrology. 19, 326-332
- Jensen, F. (2007):* Nitric oxide formation from nitrite in fish. J Exp Biol 210(19)387-394
- John, D. (1982):* Determination of Alkaline Phosphates. 9th Ed. 580-581.
- Khaled, A.; Mohamed, M.; Yasmine, M.; Gehan, M.; Mona, M. and Ali, Q. (2019):*

- Effect of sodium nitrite exposure on the immune responses against rift vally fever vaccine in mice. *Inter. J. of Pharm. Pharm. Sci.*, 11(7) 28-31
- Kapale, P.; Badkale, D. and Sahatpre, S. (2008):* serum total protein and serum total cholesterol levels in Gaolao veterinary world. 1 (4):15-16
- Kohn, M.; Melnick, R.; Ye, F. and Portier, C. (2002):* Pharmacokinetics of sodium nitrite-induced methemoglobinemia in the rat. *Drug Metabo and Disposiz.* 30:676-683.
- Kundsén, P. and Jensen, F. (1997):* Recovery from nitrite induces dmethaemoglobinaemia and potassium balance disturbances in carp. *Fish Physiol Biochem* 16:1-10
- Lee, S.; Helen, B.; Donald, A.; James, R. and Charles, E. (2018):* Secondary poisoning risk for encapsulated sodium nitrite, a new tool for possum control. *New Zealand Journal of Ecology* 42(1): 65-73
- Lundberg, J.; Weitzberg, E. and Gladwin, M. (2008):* the nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* 7:156-167.
- Ma, J.; Ding, Z.; Wel, N.; Zhao, H. and Huang, T. (2009):* Sources of water pollution and evolution of water quality in the Wuwei basin of Shiyang river, North-west China. *J. of Environmental Management* 90 (2): 1168 - 1177
- Mondal, D.; Pandey, N. and Charan, K. (1999):* Effects of prolonged low nitrite intoxication in goats. *Indian Vet. J.* 76: 800-803.
- McDonald, P.; Edwards, R.; Greenhalgh, J. and Morgan, C. (2002):* *Anim. Nutr.* 6th Ed. Pearson Prentice Hall, Harlow, England
- McNally, B.; Griffin, JL. and Roberts, LD. (2016):* Dietary inorganic nitrate: from villain to hero in metabolic disease? *Mol Nutr Food Res* 60(1):67-78.
- Mohammed, A.; Sarhan, A.; Shati, A. and Fahmy, G. and Elsaïda, B. (2014):* Biochemical and molecular studies on the possible influence of Brassica oleracea and Beta vulgaris extracts to mitigate the effect of food preservatives and food chemical colorants on albino rats. *Saudi J Biol Sci.*; 21(4): 342-354.
- Nagla A.; Ahmed, S; Mohamed, A. and Laila, A. (2020):* Effects of nitrite on hepatotoxicity and nephrotoxicity, histopathological change in adult rats. *Enviro Sci and Pollution Res.* 27, 4019-14032
- Ritman, S. and Frankle, S. (1957):* deterrmination of GOT and GPT activity. *Am. J. Clinic. Path.* 28:56.
- Safary, H. and Daneshyar, M. (2012):* Effect of dietary sodium nitrate consumption on egg production and quality characteristics and some blood indices in native hens of west Azarbaijan Province. *Asian-Australas J Anim Sci* 25(11)611-616
- Sadiq, M.; Al-Hiti, A.; Hussain, M. and Ammar, A. (2018):* Evaluation of histopathological changes in mice exposed to sodium nitrite for long term by using hematoxylin and eosin staining. *Inter. J. of Pharm. Sci. Res.* 9(2): 483-89.
- Salama, M; Abbas, A; Darweish, M. and Al-Gayyar, M. (2013):* Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. *Pharm Biol;* 51:1435-1443.
- Salem, M.; Eweida, A. and Azza, F. (2000):* Heavy metals in drinking water and their environmental impact on human health. *ICEHM* page 542- 556
- Sanaa, R. and Mahmoud, M. (2012):* protective effect of Vit A against sodium nitrate toxicity in liver and kidney of albino rats. *J Am Sci* 8(12):293-308
- Scott, M.; Nesheim, M. and Young, R. (1982):* *Nutrition of the Chicken.* 3rd Ed. Humphrey Press Inc., Geneva, New York.
- Sherif, O. and Al-Gayyar, M. (2015):* Cod liver oil in sodium nitrite induced hepatic injury: does it have a potential protective effect? *Redox Rep* 20(1): 11-16.
- SPSS (2004):* "Statistical and package for social sci., SPSS for windows release." Standard version, copyright SPSS Inc., 1989- 2004.
- Summers, J.; Grandhi, R. and Leeson, S. (1976):* Calcium and phosphorus requirem-ents of laying hens. *Poultry Sci.*, 55: 402-413.
- Talha, E.; Elfadil, A. and Omer, H. (2008):* water quality and its effects on productive performance of layers during winter season. *Inter. J. of Poultry Sci.* 7 (5) 37-40

Tan, Y.; Nambiar, R. and Yo, S. (1992): Prevalence of protein calorie malnutrition in general surgical patients. Ann. Acad. Med. Singapore, 21 (3): 334-338.

Wilkinson, P. (1976): "Recognition and response in mononuclear and granular phagocytes". Lin. Exp. Immunol. 25(3): 355-366.

تقييم تلوث الماء بالنيتريت على إنتاج البيض وبعض الوظائف الهيماتوبيوكيميائية بمزارع الدجاج البياض

ايمان اسماعيل محمد اسماعيل، أسامة السعيد رمضان ، ماجدة نعمت عبد الحميد ، محمد بدر عبد العظيم النيل

ناهد عبد الحفيظ قمورة ، سناء محمد سالم

E-mail: dr.emanismail201@gmail.com

Assiut University web-site: www.aun.edu.eg

الهدف من هذه الدراسة هو معرفة تأثير النيتريت في مياه الابار الجوفيه على انتاج البيض وتغيير في صورته الدم وبعض القياسات البيوكيميائية في مزارع الدواجن البياضة. تم تجميع عينات مياه الابار الجوفيه في مناطق مختلفة بمحافظة الشرقية (الزقازيق- كفر صقر- ديرب نجم- الصالحية). بتحليل عينات المياه وجد ان مياه الابار الجوفيه في تلك المناطق كانت ph ونسبة المواد الصلبة الذائبة والنيتريت في منطقة ديرب نجم ٨,٢٨- ٦٠٨- ٠,١٣ جزء في المليون وفي منطقة كفر صقر ٨,٤٦- ٢٠٧,٢٥- ٠,٠٥ جزء في المليون وفي منطقة الصالحية ٨,٦٩- ٧٢٢,٥- ٠,٦٣ وفي منطقة الزقازيق ٩,٨٥- ١٧٦٤,٣٣- ٠,٣٤ جزء في المليون على التوالي. في هذه الدراسة تم استخدام عدد ٦٠ دجاجة بياضة عمرها حوالي ٢٢ اسبوع تم تقسيمهم إلى ثلاث مجموعات متساوية يحتوى كلا منها على ٢٠ دجاجة. المجموعه الاولى دجاج يتم اعطائه ١ مجم نيتريت /لتر من مياه الشرب لمدة ٣٠ يوم متتاليه المجموعه الثانيه دجاج يتم اعطائه ٤مجم نيتريت /لتر من مياه الشرب لمدة ٣٠ يوم متتاليه بينما المجموعه الثالثه دجاج يتم اعطائه ٨ مجم نيتريت /لتر من مياه الشرب لمدة ٣٠ يوم متتاليه عند اليوم الاول من نهاية اعطاء النيتريت تم ذبح عدد ٥ دجاجة من كل مجموعة وتم أخذ ثلاث عينات دم من كل دجاجة لدراسة تأثير النيتريت على صورة الدم وبعض الوظائف البيوكيميائية وقوة اللتهام والقتل

وأظهرت النتائج أن النيتريت بجرعتي ١ , ٤ ملجم /لتر من مياه الشرب أدى إلى حدوث نقص غير معنوي في العدد الكلى لكرات الدم الحمراء, الهيموجلوبين, حجم كرات الدم المرصوصة, MCHC , الخلايا الليمفاويه, قوة اللتهام, البروتين الكلى, الزلال, الجلوبيولين انتاج البيض والكالسيوم مصحوبا بنقص معنوي في العدد الكلى لكرات الدم البيضاء وزيادة غير معنوية في الانين امينوترانسفيريز, الاسبرتيت امينوترانسفيريز, الفوسفاتيز القاعدى, الصفائح الدموية MCV, MCH , حمض اليوريك والكرياتينين بالمقارنه بالمجموعه الضابطة. ولكن النيتريت بجرعتي ٨ ملجم /لتر من مياه الشرب أدى إلى حدوث نقص معنوي في العدد الكلى لكرات الدم الحمراء, الهيموجلوبين, حجم كرات الدم المرصوصة, MCHC , العدد الكلى لكرات الدم البيضاء, الخلايا الليمفاويه, قوة اللتهام, البروتين الكلى, الزلال, الجلوبيولين انتاج البيض وزن البيضة سمك القشرة ووزن القشرة والكالسيوم مصحوبا بزيادة معنوية في الصفائح الدموية, MCV, MCH , الانين امينوترانسفيريز, اسبرتيت امينوترانسفيريز, الفوسفاتيز القاعدى, حمض اليوريك والكرياتينين . بقايا النيتريت كانت موجودة في الكليتين اكثر من الكبد وكانت نسبة وجود البقايا تبعا للجرعة.

النيترايت بجرعه ١ , ٤ مجم /لتر من مياه الشرب ادى الى عدم وجود اى تغيرات باثولوجية ولكن النيترايت بجرعه ٤مجم /لتر من مياه الشرب ادى الى وجود تغيرات باثولوجية متوسطة في الكبد والكلى والطحال ولكن النيتريت بجرعة ٨مجم /لتر من مياه الشرب ادى الى وجود تغيرات باثولوجية شديدة في الكبد والكلى والطحال.

وخلاصة هذه الدراسة أن استعمال المياه الجوفيه التى تحتوى على نسبة مرتفعه من النيتريت في مزارع الدواجن البياضة تؤدي الى تقليل انتاج البيض وتغيير في صورته الدم وبعض الوظائف الحيويه. لذلك من المهم عدم استخدام المياه الجوفيه في مزارع الدواجن البياضة الا بعد قياس نسبة النيتريت بها وتكون في الحدود المسموح بها.