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

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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/liter for 30 day and third group laying hens received one liter of water contains 8mg nitrite/liter 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 thickness, 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...
(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 until boiling then evaporated all water and remaining 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 1\textsuperscript{st} 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).


c. Measurement of phagocytic activity of Peripheral Blood Monocyte (PBM) using Candida albicans was performed (Anthony et al., 1985 and Chu and Dietert, 1989).


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 severe 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 than 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. Urinephric 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 reticuloendothelial linings. (Fig.1 E, F)

Hens received one liter of water contains 4mg sodium nitrite /liter show: Liver dilated, congestedportal 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 showed normal nephron, including tubular structures, urinephric duct and avian glomeruli. 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, 25um.

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 C, D). 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).

<table>
<thead>
<tr>
<th>Centers</th>
<th>Zagazig</th>
<th>Kafr Sakr</th>
<th>El Salhia</th>
<th>Diarp Negm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>9.85±0.87</td>
<td>8.46±0.98</td>
<td>8.69±0.79</td>
<td>8.28±0.83</td>
</tr>
<tr>
<td>Total dissolved</td>
<td>1764.33±3.18</td>
<td>207.25±2.37</td>
<td>722.5±3.94</td>
<td>608±2.58</td>
</tr>
<tr>
<td>Nitrite(ppm)</td>
<td>0.34±0.05</td>
<td>0.05±0.004</td>
<td>0.63±0.12</td>
<td>0.013±0.006</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>1mg nitrite</th>
<th>4mg nitrite</th>
<th>8mg nitrite</th>
</tr>
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<tbody>
<tr>
<td>Erythrogram</td>
<td></td>
<td></td>
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<tr>
<td>RBCs(×10^6)</td>
<td>4.20±0.65a</td>
<td>3.90±0.65a</td>
<td>2.52±0.65b</td>
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<tr>
<td>Hb(g/dl)</td>
<td>9.40±0.92a</td>
<td>8.60±0.65a</td>
<td>6.00±0.92b</td>
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<tr>
<td>PCV (%)</td>
<td>27.78±2.14a</td>
<td>26.74±0.65a</td>
<td>19.24±2.14b</td>
</tr>
<tr>
<td>MCHC(%)</td>
<td>35.94±1.21a</td>
<td>35.57±0.65a</td>
<td>32.77±1.21b</td>
</tr>
<tr>
<td>MCH(Pg)</td>
<td>27.63±1.20b</td>
<td>28.48±0.65b</td>
<td>30.53±1.20a</td>
</tr>
<tr>
<td>MCV(FL)</td>
<td>31.85±1.12b</td>
<td>33.22±0.65b</td>
<td>36.22±0.65</td>
</tr>
<tr>
<td>Platelet (103/cmm)</td>
<td>268.23±2.14b</td>
<td>269.45±0.65b</td>
<td>284.18±2.14a</td>
</tr>
<tr>
<td>Total WBCs count 10^4/cm</td>
<td>9.21±0.41a</td>
<td>8.51±0.23a</td>
<td>9.21±0.43a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>leukogram</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterophils</td>
<td>28.03±0.39b</td>
<td>51.03±0.26a</td>
<td>39.23±0.21a</td>
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<tr>
<td>Lymphocyte</td>
<td>52.12±0.23a</td>
<td>51.03±0.26a</td>
<td>48.06±0.42b</td>
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<td>Eosinophils</td>
<td>3.11±0.19a</td>
<td>2.15±0.21a</td>
<td>2.03±0.11a</td>
</tr>
<tr>
<td>Basophils</td>
<td>3.20±0.16a</td>
<td>2.03±0.26a</td>
<td>1.07±0.27b</td>
</tr>
<tr>
<td>Monocyte</td>
<td>13.41±0.21b</td>
<td>13.41±0.21b</td>
<td>8.08±0.18a</td>
</tr>
<tr>
<td>Phagocytosis (%)</td>
<td>22.32±0.33a</td>
<td>21.42±0.40a</td>
<td>19.61±0.34b</td>
</tr>
</tbody>
</table>
Table (3): Effect of sodium nitrite on liver and kidney functions in laying hens (n=5)

<table>
<thead>
<tr>
<th>Liver enzymes (u/l)</th>
<th>Groups</th>
<th>1mg nitrite/liter</th>
<th>4mg nitrite/liter</th>
<th>8mg nitrite/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td></td>
<td>18.34±0.89b</td>
<td>19.18±0.95b</td>
<td>25.09±0.89a</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td>53.18±0.78b</td>
<td>55.34±0.98b</td>
<td>59.18±0.78a</td>
</tr>
<tr>
<td>ALP</td>
<td></td>
<td>31.21±0.88b</td>
<td>31.21±0.88b</td>
<td>37.26±0.98a</td>
</tr>
<tr>
<td>Protein picture (gm/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. protein</td>
<td></td>
<td>5.54±0.42a</td>
<td>5.08±0.21a</td>
<td>3.69±0.52b</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td>2.99±0.45a</td>
<td>2.78±0.33a</td>
<td>2.15±0.28b</td>
</tr>
<tr>
<td>Globulin</td>
<td></td>
<td>2.55±0.32a</td>
<td>2.30±0.40a</td>
<td>1.54±0.21b</td>
</tr>
<tr>
<td>A/G ratio</td>
<td></td>
<td>1.17±0.28b</td>
<td>1.21±0.38ab</td>
<td>1.40±0.23a</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>1mg nitrite/liter</th>
<th>4mg nitrite/liter</th>
<th>8mg nitrite/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>00</td>
<td>00</td>
<td>30.18±1.59</td>
</tr>
<tr>
<td>Kidney</td>
<td>00</td>
<td>0.59±0.17</td>
<td>48.43±1.40</td>
</tr>
</tbody>
</table>

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 an 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), urin-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.
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 um, 25 um.

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

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 um, 25 um.

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 heterophil,. 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, heterophils (table, 2 and 3). This observation was previously recorded by Kundsden 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 nitrates 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 nitrate 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 nitrate/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 Daneshyiar (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 though 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 modert to sever 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 sinuso-ids, degenerative changes in periaciner 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 shrunked 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.

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تأتي إلى ترلي انتاج البيض وتغيير في صورة الدم وبعض الوظائف الهيئاتيوبتوبتيكية

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