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Research article

Acumulative effect of sodium nitrate on some parameters of broiler chickens

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Abstract

This study aims to clarify the accumulative effects of Sodium Nitrate at level of 200 mg/lit that added to drinking water on broiler chicks. 120 of one-day-old broiler chicks (Hubbard Classic) were used. They were divided into two equal groups using 3 replicates 20 chicks for each replicate in 49 days. Group one (control) received just plain tap water. Group two received 200 mg/lit Sodium Nitrate. Weights were taken weekly. Feed conversion ratio, dressing percentage, Liver weight, humoral immunity against Newcastle disease and liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured couple of times during the experiment. These two tests done at 35 and 49 days old. At the end of the experimental period (7 weeks old), liver and bursa of fabricious were removed after slaughter for histological examination. Results show no significant differences of feed conversion ratio and dressing percentage in both times. Weekly body weight of sodium nitrate group was significantly decreased at ($p \leq 0.05$) in the sixth and seventh weeks. Rate of liver weights and antibody titers of the second sets significantly decreased ($p \leq 0.05$) comparing with the control group at the 35 days test. The levels antibody titers, AST and ALT of sodium nitrate group are significantly higher in 49 days old broiler chickens comparing with the 35 days old. On the other hand, ALT of the second sets is significantly higher ($p > 0.05$) comparing with the control group at the 49 days test. Sodium Nitrate led to numerous histological lesions in liver and bursa of fabricious in 49 days old broiler chickens. In brief, Sodium nitrate consumption has a negative correlation with body weights, liver enzymes, and histological change.

Key words: Sodium Nitrate, Body Weights, Liver Enzymes, Antibody Titers.

Introduction

Water pollution by nitrate is a problem in both surface and ground waters of many farmland areas (1). The application of inappropriate or excessive concentrations of nitrogen fertilizers in the livestock and agriculture are the most important sources of nitrate in our environment (2). Nitrates and nitrites are pollutants present in water and food. They may contribute to failure of immunity and the etiology of kidney and liver diseases in domestic fowls (3). Chronic exposure to low level of sodium nitrate contaminated water may result in lymphoid organ hypertrophy and affected body weights during the growing and final periods (4). In other experiment, differences were not observed in feed conversion, body weight, Histology of liver tissues and liver sizes among levels of 0, 111, and 427 ppm. However, decreases in body weight and histological changes were observed with the 2033-ppm concentration (5). The safe level of nitrate in drinking water is 10 mg/lit, and 25 to 45 mg/lit is the maximum acceptable level **(**6**).**

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Comparing the values of nitrate in main water supplies and drinker's water clarified higher significant values in water of drinkers. Moreover, investigation of examined water samples in relation to its source revealed higher nitrite and nitrate in ground water than treated surface water (7). In a study to assess the quality of water in poultry farms in different regions of Sulaimania Province, result of nitrate ranged between 1.4-20 mg/lit (8). Moreover, In a study to estimate nitrate levels in the underground water of some wells in the south part of Iraq, recorded values were 15–100 mg/lit nitrate and most values were more than the allowed values, which indicates that ground water is nitrate polluted (9). Conversion of nitrate to nitrite in poultry has been reported but after feeding high nitrate dietary (4.2 *g/kg* of diet) because the methaemoglobinaemia induced by nitrite (3). This experiment sought to study the effect of accumulative of 200mg/lit nitrate level in drinking water on broiler growth performance, physiological, histological and immune parameters.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 360

One hundred and twenty of one day old broiler chickens (Hubbard Classic) were used in this experiment. These chicks were raised in a poultry field that belongs to the college of Agriculture / University of Al-Qadisiyah. Chicks were randomly distributed into 2 equal treatments (60 chicks for each treatment) at three replicates and 20 chicks per each. They were fed on starting, growing and finishing rations. These rations were composed according to NRC 1994 (Table 1), (10). They were vaccinated by Newcastle vaccine (Lassota strain) twice at 20 and 30 days old by spraying method. Treatments of the experiment were arranged as follow: control treatment was the first which was given just plain tap water; the second treatment was given water contains concentration of 200 mg/lit sodium nitrate (Nitrate solutions were premixed in the laboratory using city tap water). sodium nitrate that used in the experiment, was produced by Span. It was obtained from college of Agriculture / University of Al-Qadisiyah. Body weight weekly taken. At 35 and 49 days old measurements of: Feed conversion ratio, dressing percentage, liver weight, humoral immunity (antibody titers) against Newcastle virus using ELISA test, ALT and AST concentrations were done. Liver and bursa of fabricious were removed after slaughter for histological examination at the end. Collected tissue specimens of the liver and bursa immediately placed into a 10% neutral buffered formalin solution. They were processed and sectioned at 5 µm thickness with a microtome and stained with eosin and haematoxylin for histopathological examination (11). Later on, stained sections were subjected to visual examination using the light microscopy. Biotic 8800 XL was used to measure antibody titers, and Reflotron Plus was used to measure liver enzymes. Blood samples were taken during the slaughtering process and placed in 5 ml test tubes that were free from anticoagulant. After that, Serum isolated by using 2 ml special sealed test tubes. These serum samples were preserved under 20 Celsius till laboratory analysis. Statistical analysis:- Results of the study were analyzed statistically by using both one way and two way ANOVAs to determine statistically significant variance between the different groups. Significance between the two groups was calculated by using least significant difference (LSD) with SPSS program version 17. Data are expressed as means ± standard error of mean. Differences were considered significant at a P- value of less than 0.05 (12).

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Table (1) proximate analysis of experimental diet

Ingredients (kg)	Starter Period from 1 to 21 days age	Growing Period from 22 to 42 days age	Finishing Period from 43 to 49 days age
Yellow corn	45	55	58
Soya bean meal	38	36	32.5
Wheat	9	-	-
Premix*	2.8	2.8	2.8
Oil	3	4.5	5
Limestone	2	1.5	1.5
Salt	0.2	0.2	0.2
Total	100	100	100
Calculated Nutrient			
Crude Protein (%)	22.6	21.2	20
Energy (kcal)	2910	3050	3120
Lysine			
Methionine + Cysteine			
Calcium	%85	0.7 %	0. 7 %
Phosphorus	0.47 %	0.5 %	0.5 %

*** Premix containing minerals (Zinc, Copper, Iron, Manganese, Cobalt, Iodine, Selenium); vitamins (B1, B2, B6, B12, Pantothenic acid, Biotin, Choline, Folic acid, A, D3, E, K) and Amino acid (Methionine).**

Results

Results of weekly weight showed that no significant differences between the experimental groups until the fifth week. Nevertheless, there are significant decreases of the group of sodium nitrate in the sixth and the seventh week as illustrated in Table (2).There are no significant differences for feed conversion ratio trait between sodium nitrate group and control group at both 35 and 49 days old. However, there are statistical higher in G2 as compared with G1 at the 35 and 49 days old checks as illustrated in Table (3). According to the first test, the rate of liver weight decreased significantly in sodium nitrate group as compared to the first group. On the other hand, there are no significant differences between sodium nitrate group and the control group at 49 days old Table (4). The data of dressing percentage showed no significant differences between NO3 group and the control group at both 35 and 49 days old Table (5). Aantibody titers decreased significantly in the first test and statistically at the second test in sodium nitrate group as compared to the control. On the other hand, antibody titers increased significantly at the second test as compared with the first test in sodium nitrate group Table (6). Liver enzymes of ALT and ALP check in table (7) show clear effect of sodium nitrate at 49 days old test. It increased significantly in the ALT test, but was statistically higher in AST test in sodium nitrate group as compared to the control. Both of AST and ALT were significantly higher at 49 days old test comparing with 35 days old test in nitrate group. Histological examinations of tissues (liver and bursa of fabricious) on chicks that received plain tap water (control Group) showed no abnormalities (Figures 1: A and B). In the liver tissues of chicks that received 200 mg/lit sodium nitrate for the whole of the studying period had cellular abnormalities Figure (2): C and D). Necrosis of hepatocytes forming spaces filled with necrotic hepatocytes debris in liver parenchyma led to loose of hepatic normal architecture. The congestion in central vein was observed. Microscopic examination of the bursa of fabricious tissues

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from chicks consuming 200 mg/lit sodium nitrate showing atrophy to absence of cortex areas of lymphatic follicles due to necrosis in lymphocytes in cortex. Absence of undifferentiated epithelial layer in medulla periphery and the absence if cortex and undifferentiated epithelial layer led also to general atrophy in lymphatic follicles. Thickness of pseudo stratified epithelial layer of bursa was observed (Figure (2): E and F).

Table (2) Effects of adding sodium nitrate to the drinking water on rate of weekly weight

Groups	Second Week (g)	Third Week (g)	Fourth Week (g)	Fifth Week (g)	Sixth Week (g)	Seventh Week (g)
First	358.33±10 A	781.66±11.51 A	1328.61±29.63 A	1776.66±47.35 A	2237.66±76.71 A	2950.66±78.03 A
Second	374.72±10.96 A	768.05±24.06 A	1206.11±47.46 A	1624.66±55.12 A	1920±97.94 B	2511.33±120.23 B

Similar letters denote to non-significant differences at p<0.05.
Different letters denote to significant differences at p<0.05.

Table (3) Effects of adding sodium nitrate to the drinking water on feed conversion ratio

Groups	Feed conversion ratio	
	35 Days old	49 Days old
First	1.60±0.02^{Aa}	1.80±0.06^{Aa}
Second	1.65±0.04^{Aa}	1.90±0.13^{Aa}

Similar letters denote to non-significant differences at p<0.05
Capital letters means vertical statistical reading, Small letters means horizontal statistical reading.

Table (4) Effects of adding sodium nitrate to the drinking water on rate of liver weight

Groups	Liver weight (g) at the first test (35days)	Liver weight (g) at the second test (49 days)
First	49.63±2.27^{A}	58.74±4.98^{A}
Second	38.63±2.56^{B}	59.49±3.92^{A}

Similar letters denote to non-significant differences at p<0.05
Different letters denote to significant differences at p<0.05.

Table (5) Effects of adding sodium nitrate to the drinking water on dressing percentage

Groups	Dressing Percentage at the first test (35)	Dressing Percentage at the second test (49)
First	70.33±0.35^{Aa}	70±0.61^{Aa}
Second	69.66±0.26^{Aa}	69.2±0.62^{Aa}

Similar letters denote to non-significant differences at p<0.05
Capital letters means vertical statistical reading, Small letters means horizontal statistical reading.

Table (6) Effects of adding sodium nitrate to the drinking water according to antibody titers of Newcastle disease using ELISA test.

Groups	antibody titers at the first test (35 days)	antibody titers at the second test (49 days)
First	5025.66±337.72^{Aa}	5553.5±856.05^{Aa}
Second	2985.66±251.03^{Bb}	5144.83±468.51^{Aa}

Similar letters denote to non-significant differences at p<0.05
Different letters denote to significant differences at p<0.05
Capital letters means vertical statistical reading, Small letters means horizontal statistical reading

Table (7) Effects of adding sodium nitrate to the drinking water on enzymes, AST and ALT

Groups	AST (U.I/l)		ALT (U.I/l)	
	the first test (35 days)	the second test (49days)	the first test (35days)	the second test (49days)
First	537.16±14.64^{Aa}	529.16±27.83^{Aa}	11.95±0.24^{Aa}	10.03±0.77^{Ba}
Second	514.16±7.27^{Ab}	594±35.46^{Aa}	11.05±0.35^{Ab}	13.2±0.28^{Aa}

Similar letters denote to non-significant differences at p<0.05
Different letters denote to significant differences at p<0.05
Capital letters means vertical statistical reading, Small letters means horizontal statistical reading.

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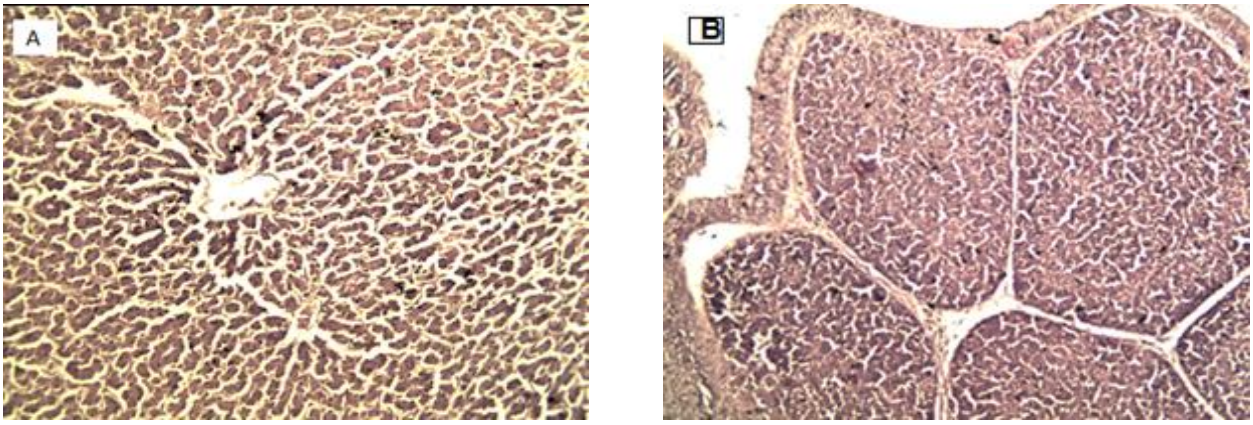

Figure (1): Photomicrograph of liver and bursa fabricious of control group chicken.

(A)Liver of control group chicken. Normal hepatic architecture is characterized by radially arrangement of hepatocytes around normal central vein. Sinusoids and small bile ducts are normal. (B) Bursa fabricious of control group chicken. Normal bursa fabricious architecture is characterized by small uniform lymphocytes in cortex area of lymphatic follicles, vary in size under maturation lymphocytes in medulla area with presence of undifferentiated epithelial layer on periphery of medulla, and normal thickness and structure inter follicular septa. Normal pseudostratified epithelial layer lining bursa fabricious follicles.

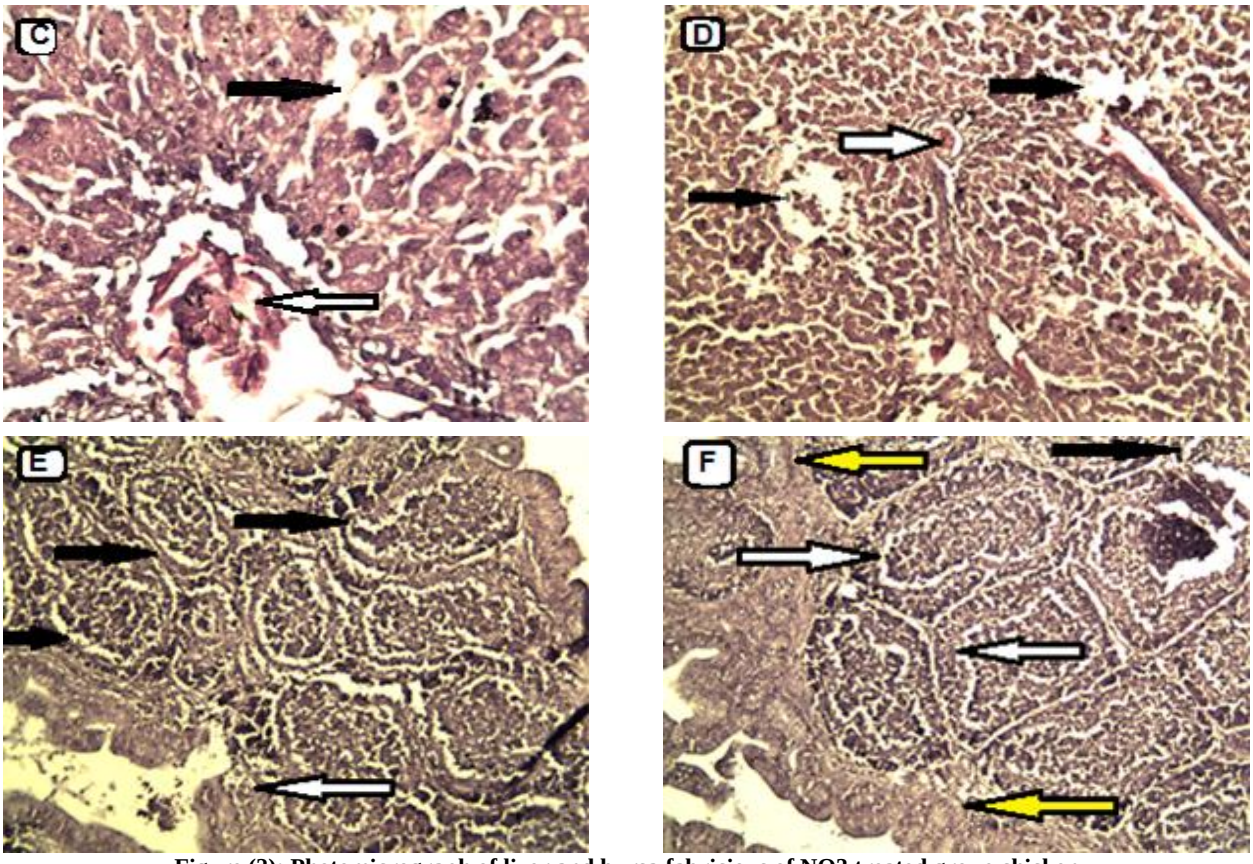

Figure (2): Photomicrograph of liver and bursa fabricious of NO3 treated group chicken.

C and D/ Liver of NO3 treated group chicken. Necrosis of hepatocytes forming spaces filled with necrotic hepatocytes debris (black arrows) in liver parenchyma led to loose of hepatic normal architecture. The congestion in central vein was observed (white arrows).
E and F/ Bursa fabricious of NO3 treated chicken. Atrophy to absence of cortex areas (black arrows) of lymphatic follicles due to necrosis in lymphocytes in cortex, also absence of undifferentiated epithelial layer (white arrows) in medulla periphery and the absence if cortex and undifferentiated epithelial layer led to general atrophy in lymphatic follicles. Thickness of pseudostratified epithelial layer of bursa fabricious (yellow arrows) observed.

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Discussion

In the present study, sodium nitrate causes a significant decrease in weekly weight only in the sixth and the seventh week of the nitrate group comparing with birds that given no sodium Nitrate. This observation supports the finding of (13), They found no main sodium nitrate effects **(**5 ,10 and 20 mg/lit**)** during the first 4 weeks of the experiment, but broilers exposed to sodium nitrate were significantly lighter at the week sixth comparing with control group. These results also agree with the findings of (5)**,** who reported no significant in body weights were observed among broiler chicks that consumed levels of zero, 111 and 427 ppm NO3. On the other hand, a decrease (P<.05) in body weight was observed with the 2033 ppm at the 4th week of age. It agrees also with (14) whom found body weight gain (42 days) in control group comparative to another groups that received different levels of water nitrate in which significantly increased. Different levels of nitrate had no effect on body weight gain in 21 days. Moreover, water sodium nitrate as a main effect depressed **(**p<0.05**)** body weights at 4th week of age (4). The significant decrease of the group of sodium nitrate in the sixth and seventh weeks may due to the effect of accumulative nitrate (NO3) from sodium nitrate consumed per bird (mg) from 1 day old to six or seven weeks. According to the obtained results, there are no significant differences between sodium nitrate group and control group at both 35 and 49 days old for feed conversion ratio trait. This observation supports the findings of (5), who found weekly feed conversions were not significantly different between nitrate levels. However, no consistent with the finding of (14), who pointed out that received 100 mg nitrate in drinking water caused a significant increase of feed conversion ratio in 1-42 days. Liver weights average in the first test decreased significantly in the sodium nitrate group. This does not agree with what (5) found, which when chicks are treated for period of 4 weeks with different levels of nitrate (NO3) from sodium nitrate, liver weights shows no differences at (P> .05) among treatments as compared with control group. On the other hand, there are no significant differences between sodium nitrate group and control group at 49 days old. The result that agrees with the work of (4). The result of immune status revealed that antibody titers decreased significantly in the first test in sodium nitrate group as compared to the control group. On the other hand, antibody titers increased significantly at the second test as compared with the first test in sodium nitrate group. This may due to histological lesion on burse especially atrophy to absence of cortex areas of lymphatic follicles due to necrosis in lymphocytes in cortex (Figure (2): E and F) or may due to Sodium nitrate that decreases total antioxidant capacity (15) This is consistent with the finding of (3), which is significant differences between control group and anti-SRBC hemagglutinin titers (log2) in cockerels fed on diets containing nitrate was observed. In addition, with conclusion (4), chronic exposure to nitrate-contaminated water sources may result in lymphoid organ hypertrophy and compromised broiler immune response. Other researchers also found group that got minimum nitrate water had minimum weight of burse of fabricious (14). Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been associated with hepatocellular damage. ALT increased significantly in sodium nitrate group compared to control group, but was statistically higher in AST at 49 days old test. On the other hand, both of AST and ALT were significantly higher at 49 days old test comparing with 35 days old test in nitrate group. This result may due to the accumulative effect of nitrate (NO3) from sodium nitrate that consumed per bird (mg) from 1day old to 49

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days old. However, AST (SGOT) levels were higher **(**p>0.05**)** in broiler chicks that consumed water containing 1000 ppm NO3 than chicks that consumed water containing 0, 50, or 200 ppm NO3 from Ca (NO3)2 (5). None of the blood ALT and AST enzymes were affected by dietary consumption of sodium nitrate in native hens at week 32 of age (p>0.05) hens were used from week 25 to 32 of age (15). ALT (SGPT) activity also was not increased significantly in nitrate-fed birds for 4 weeks (3). Other revealed result of oral intubation of 30mg/kg. B.W adult male rats on sodium nitrate for 84 days caused hepatic damage manifested by significant increase (p<0.05) in serum ALT and ALP activities (16). These differences in results could be because: age, type of animal, and source (dietary or water) of sodium nitrate. Result of this experimental study noticed microscopic change in both (liver and bursa of fabricious tissues) of chicks received 200 mg/lit sodium nitrate for the whole of studying period of 49 days as compared to the control. This is partly in line with what (5) agree on, which were showed abnormalities in histological examinations of liver tissues from chicks at three weeks of age consuming 2033 ppm, NO3 caused proliferation of the endothelial cells inside the lumen and an excess of stromal cells and lymphocytes outside the lumen creating a closure of the venule. This suggests that broiler chicks that consumed 2033-ppm NO3 water had hepatocellular damage. The liver of rats that were administered 50 mg/kg NaNO3 treated daily for 8 weeks caused hydropic degenerated hepatocytes. Dilated sinusoids and necrotic areas infiltrated with a number of inflammatory cells caused as well (17).

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