

EFFECTS OF MODERATE LEVEL OF NITRATE OR NITRITE IN DRINKING WATER ON BROILER CHICKS PERFORMANCE

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SUMMARY

Total of 180 one day-old unsexed broiler chicks were used in this study to investigate the possible role of moderate dose of sodium nitrate or nitrite in drinking water on their performance and tissue residual of NO₃ or NO₂. Chicks were divided into three groups according to water supplemented with either 50 ppm NO₃ (a), 50 ppm NO₂ (b) or 0 supplement, control (c). Results indicated that body weights and body weight gains at the ages of 2 and 4 weeks were not affected by treatments, as they were almost similar to the control group ($P > 0.05$). However, final body weights and overall gains were significantly higher in both treated groups compared to the control ($P < 0.001$). The interaction between treatments and ages for body weights and body gain was highly significant ($P < 0.001$). Feed consumption and feed conversion were not affected by treatments. However, feed conversion was improved at the age of 4 weeks regardless of treatments ($P < 0.001$). Breast and thigh muscles concentrations of either NO₃ or NO₂ showed decreased levels of both elements in all treatments at older ages ($P < 0.01$). In conclusion, water contamination by either NO₃ or NO₂ (50 ppm) appeared to be below toxicity concern and chicks, might have possibly utilized them at older age through body metabolism.

Keywords: Broiler, nitrate, nitrite, gain, performance, tissues

INTRODUCTION

Unintentional exposure to high doses of nitrite or nitrate through drinking potable water may be harmful for human health (Morbidity and Mortality Weekly Report, 1997) as well as experimental animals (Borawska *et al.*, 1996).

Nitrite and nitrate ions are naturally occurring forms of nitrogen that can be present in ground and surface water (Osweiler *et al.*, 1985), in cereal grains

as it normally ranges from 0.5 to 18 mg/kg (Walker, 1990) and in vegetable at concentrations up to several grams per kilogram fresh weight (Vittozzi, 1992). Nitrate accumulation in feedstuffs occurs primarily in the leaves and stems of non-leguminous plants such as oats, corn, barley, wheat and sorghum (Whitehead, 1956). Since these plant materials make up a very small portion of modern poultry rations, it would appear that water represents the greatest potential nitrate hazard for poultry. In the lower part of the gastrointestinal tract of monogastric animals, nitrate can be reduced to the more toxic nitrite ion by micro-organisms (Swann, 1975; Mueller *et al.*, 1986; Bruning-Fann and Kaneene, 1993; and Polese *et al.*, 1993). While toxicological effects of nitrates and nitrites in different mammalian species are well documented, little is known about their effects in domestic fowls. Previous investigations in domestic fowls and other avian species were primarily concerned with the effects of nitrates and nitrites on growth and survival (Adams *et al.*, 1966 & 1969; Arends *et al.*, 1967; Marrett and Sunde, 1968; and Adams, 1974)

Other studies were concerned with the toxicity effects (Sell and Roberts, 1963; Sunde, 1964 and Marrett & Sunde, 1968; Osweiler *et al.*, 1985; and Walker, 1990). Also, excessive dietary nitrate and nitrite were reported to depress growth in swine (Tollet *et al.*, 1960); rats (Smith *et al.*, 1961) and cattle (Weichenthal *et al.*, 1963). Atef *et al.* (1991) confirmed that nitrate and nitrite were environmental pollutants, for food and water and might contribute to the etiology of liver and kidney diseases and problems related to failure of the immunity in domestic fowls.

Recent investigations, regarding pollution of the Nile water, found that the Nile has the highest dissolved salt content of any of the major African rivers (Martins & Probst, 1991 and Dekov *et al.*, 1997 and Komy & El-Samahy, 1995). In these reports, the concentration of NO₃ was found to be 1.2 (mg L), this was partially explained as a result of irrigation system and industrial activities (Kempe, 1983 & 1988). Generally speaking, one part per million (ppm) is well below the level which would have any effect on human health, the permissible level of NO₃ was reported to be 40 ppm. It cannot be expected that the pollution in the Nile will suddenly be alleviated. But consumers expect meat products of good quality that is free of any undesired toxic substances. Moreover, this can be achieved by attempting to eliminate cross contamination and minimize residues from meat prior to slaughter. Data reported herein describe the effects of nitrate and nitrite administered continuously in drinking water at a moderate dose on chicks performance and if any residual of these compounds might be present in the edible muscles that would be of great concern to public health.

MATERIALS AND METHODS

A total of 180 one-day old commercial meat-type unsexed chicks were used in a 6 weeks trial. At one-day old, chicks were wing banded, weighed individually and randomly allocated to 3 treatments with three replicate (20 chicks each). Birds were housed in pen floor brooders with heat controlled environment and a constant illumination (23L:1D). A basal corn-soybean diet that meets all nutrients requirement, as recommended by the NRC (1994), were fed to chicks for *ad. libitum* consumption in mash form and birds had free access to water (Table 1).

Table 1. Diet Composition.

Ingredients	gm / 100 gm	
	0- 3 week	3- 6 week
Yellow corn	57.75	65.75
Soybean meal (CP,44%)	29.25	21.00
Broiler conc. (CP., 52%) ¹	10.00	10.00
Bone meal	0.25	0.25
Corn oil	2.75	3.00
Calculated Analysis:		
Crude protein, %	23.16	20.15
ME, kcal / kg	3095	3206
Ca, %	0.97	1.02

¹broiler concentrate supplied per kilogram: vit. A, 125000 IU; vit.D 25000 IU; vit. E 100 IU; vit.B12 50 mcg; vit.K3 (MSB) 20 mg; vit.B2 50 mg; Pantothenic acid 110 mg; Niacin 250 mg; Thiamin 15 mg; Pyridoxine 15 mg; Biotin 1.5 mg; choline HCL 5000 mg; Manganese 640 mg; Zinc 450 mg; Iron 500 mg; Copper 40 mg; Iodine 7 mg; Cobalt 2.2 mg; Selenium 1.5 mg; Zinc bacitracin 150 mg; B.H.T. 1250 mg.

The source of nitrate and nitrite, used in this experiment, were reagent grade sodium nitrate and sodium nitrite (Fisher Scientific) dissolved in water to reach final concentration of 50 ppm of either NO₃ or NO₂ for treatment groups. The control group received tap water with no supplementation of nitrate or nitrite salts. The tap water was analyzed for total concentration of NO₃ and NO₂ at the beginning of the experiment and at the end; using modified method described by (Larson *et al.*, 1989). Values obtained for NO₃ and NO₂ were 6.2±1.73 and 0.0 ppm, respectively.

Body weights were recorded biweekly for all individuals and total feed consumption was calculated per pen for all treatments at 2,4 and 6 weeks old. Consequently, body gains (G1, G2, G3 and G4) were calculated by differences between body weight at at day-one from body weight at age 2 (G1), body weight at age 2 from age 4 (G2), body weight at age 4 from body weight at age 6 (G3) and body weight at one-day old from body weight at

age 6 (G4), respectively. Feed conversion for all treatments was calculated as total feed consumed per pen divided by total gain (g feed/ g gain) at the same periods. Also, mortality rate was recorded. Blood samples were obtained from 3 chicks for each replicate by heart puncture at biweekly intervals. The blood was collected in tubes for serum separation to measure total protein, albumin, globulin and total lipids concentrations using Diagnostic kits, BioMerieux, France. Chicks were then killed by cervical dislocation, breast and thigh muscles were exposed and 2g flesh was removed from each. Flesh samples were oven dried initially at 60C° for 24 hr. then at 105C° for three hrs. Samples from each replicate were pooled together, kept in vials and sealed tightly for later analysis for tissues residual NO₃ and NO₂ (Larsson *et al.*, 1989).

All data were subjected to analysis of variance, by the General Linear Model (GLM) procedure of SAS (SAS Institute, 1990). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with a 5% level of probability. The mathematical model used was:

$$Y_{ijk} = U + T_i + B_j + (T*B)_{ij} + e_{ijk}$$

Where:

Y_{ijk} : observation of individual birds in I treatments during different j ages

U : overall mean

T_i : treatments effect

B_j : ages effect

T_i*B_j : interaction between treatments and ages

e_{ijk} : residual error associated with observation Y_{ijk}

RESULTS AND DISCUSSION

Body weight data summarized in Table 2, gave some indication that the rate of growth of chicks received either NO₃ or NO₂ had no remarkable differences between treatments and the control group at both ages of 2 and 4 weeks. However, at 6 week-old, treated birds were superior in body weight than their corresponding control group (P<.05) by about 14.3 and 10.6%, respectively. Diaz *et al.* (1995) indicated that chickens that received 200 or 400 ppm of nitrite per kilogram diet tended to have higher body weights compared with control. Calculated body gain data (Table 3) confirmed the results of body weights, where, gain 3 (G3= weight at 6 week - weight at 4 week) and the overall gain (G4= weight at 6 week - weight at one- day old) for NO₃ and NO₂ treated chicks were significantly higher than the control group (p< .05). These results were in agreement with the data obtained by Marret and Sunde (1968) and Julian (1987). Julian (1987) explained the increase in body weight of chicks receiving 200 ppm NaNO₃ in drinking water as a result of increased water retention. His assumption was based on the amount of Na

provided from the nitrate salt to be .006%. The NRC recommended value of Na for starter or grower diets ranged from .11 to .20% (NRC, 1994), with some workers claiming better growth at levels up to 0.40%. Therefore, this value was far below the recommended levels and, hence irrelevant to the improvement that occurred in the body weights. Ruminants exposed continuously to urea developed a microflora adapted to the compound and were able to metabolize amounts of urea that would be toxic to unadapted animals (Osweiler *et al.*, 1985). This phenomena might be true in this experiment and that chick's gut microflora exposed to either NO₃ or NO₂ was adapted and was more efficient in utilizing both element at ages 4 and 6 week old. Other explanation by Bently *et al.* (1965) who claimed that 300 ppm NO₃ to water supply of laying hens was masked during cooler months which meant that the conversion of NO₃ to NO₂ was directly related to environmental temperature or possibly less water was consumed in cool weather.

Table 2. Average body weights (g) of broiler chicks receiving (0.0) or (50) ppm of either NO₃ or NO₂ in drinking water

TRT / AGE	2- Week	4- Week	6- Week
NO ₃	262 ± 30.34	742 ± 31.29	1593 ± 32.71
NO ₂	258 ± 30.34	647 ± 31.36	1542 ± 34.35
Control	252 ± 30.34	688 ± 31.63	1394 ± 32.34
	<u>Probability</u>		
TRT	P < .003		
AGE	P < .0001		
TRT* AGE	P < .007		

1 mean ± SE

Table 3. Average body gains (g) of broiler chicks receiving (0.0) or (50) ppm of either NO₃ or NO₂ in drinking water

TRT/ AGE	G1†	G2	G3	G4
NO3 (50ppm)	217 ± 32.6	556 ± 32.6	752 ± 33.7	1548 ± 33.7
NO2 (50ppm)	216 ± 32.6	516 ± 32.6	687 ± 35.4	1500 ± 35.4
Control (0.0 ppm)	210 ± 32.6	527 ± 32.2	592 ± 33.3	1352 ± 33.3
	<u>Probability</u>			
TRT	P < .0001			
AGE	P < .0001			
TRT*AGE	P < .06			

† G1= body weight at 2 wk (bw2) - body weight at 0 wk

G2= bw4 - bw2 , G3= bw6 - bw4 , G4= bw6 - bw0

1 mean ± SE

a,b Means with different superscripts within columns are significantly different (P<.05).

Feed conversion data is shown in Table 4 no significant differences were observed among treatments. But improved feed conversion was observed

only at 4 week old, regardless of treatments effect ($P < .05$). Mortality rate showed no differences, and the number of dead chicks were 1,3 and 1 out of 60 for each of NO_3 , NO_2 and the control group, respectively. Previous studies showed that excessive levels of either NO_3 or NO_2 in water or diet had no effect on feed conversion or mortality rate in quails (Adams, 1974), laying hens (Adams *et al.*, 1966) and chickens (Walker, 1990). High mortality and low feed conversion were recorded when nitrate supplementation was accompanied with low levels of vitamin A (Sell and Roberts, 1979).

Table 4. Feed conversion of broiler chicks receiving (0.0) or (50)ppm of either NO_3 or NO_2 in drinking water

TRT/ AGE	2- Week	4- Week	6- Week	0- 6Week
NO_3 (50ppm)	1.92 ± .106	1.56 ± .106	1.79 ± .106	1.76
NO_2 (50ppm)	2.01 ± .106	1.63 ± .106	1.93 ± .106	1.85
Control (0.0 ppm)	1.98 ± .106	1.59 ± .106	2.13 ± .106	1.90
X	1.97 ± .062 ^a	1.60 ± .062 ^b	1.95 ± .062 ^a	
	<u>Probability</u>			
TRT	N.S			
AGE	P < .001			
TRT*AGE	N.S			

a,b Means with different superscripts are significantly different ($P < .05$).

Figures 1, 2, 3 & 4 show tissue concentrations of NO_3 (mg/g) in both breast and thigh muscles. No significant differences were detected in nitrate concentration due to type of muscles ($P > .05$). Also, no treatment effect was observed on tissues concentrations of NO_3 ($P > .06$). In contrast, progress in age resulted in great reduction in NO_3 concentrations ($P < .002$) by about 77 and 88% at ages 4 and 6 weeks, respectively. This reduction in NO_3 concentration might be explained by two assumptions; first; that NO_3 was converted to NO_2 by the micro-organisms present in lower gastrointestinal tract of chicks (Swann, 1975; Mueller *et al.*, 1986; Bruning-Fann and Kaneene, 1993 and Polese *et al.*, 1993) Second; that chicks developed a microflora more adapted to NO_3 compound and were able to metabolize it (Osweiller *et al.*, 1985), hence better performance was found in growth and body gain. The concentration of NO_2 in breast and thigh muscles is shown in Figure 3 and 4. Again, type of meat had no effect on NO_2 concentration. Which was highly influenced by treatments and age. Higher concentration of NO_2 was obtained in group of chicks that received 50 ppm nitrate in drinking

Figure 1. NO3 concentrations in white muscles of broiler chicks

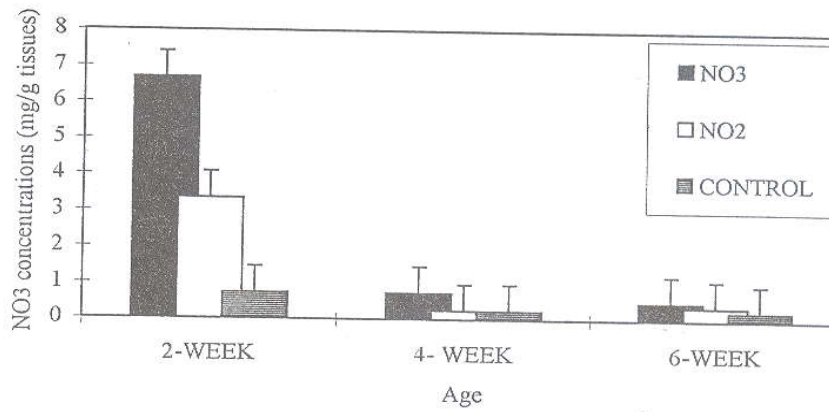


Figure 2. NO3 concentrations in red muscles of broiler chicks

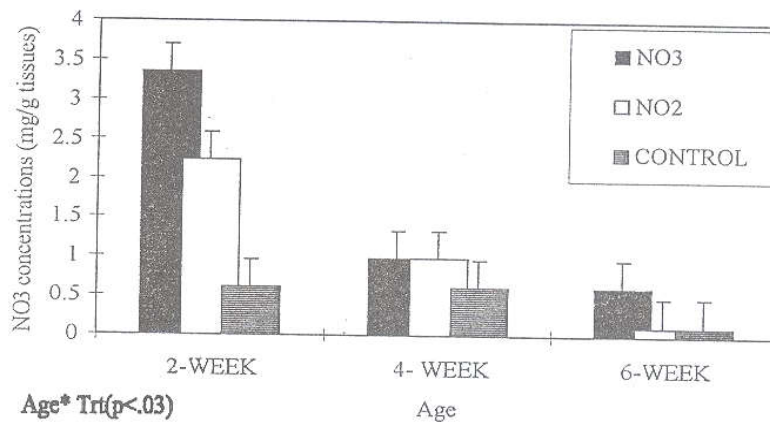
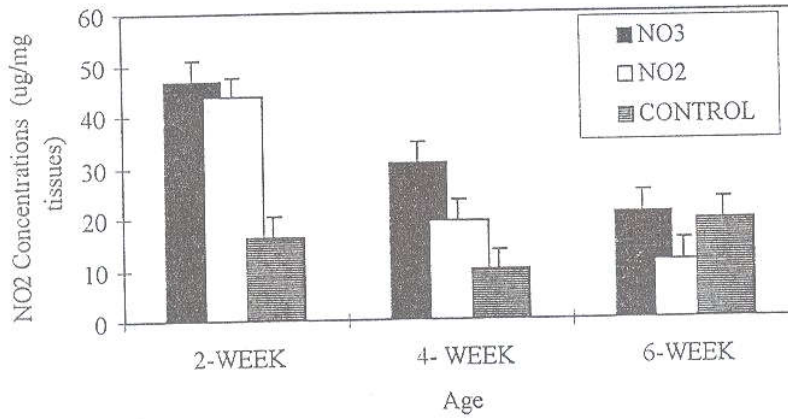
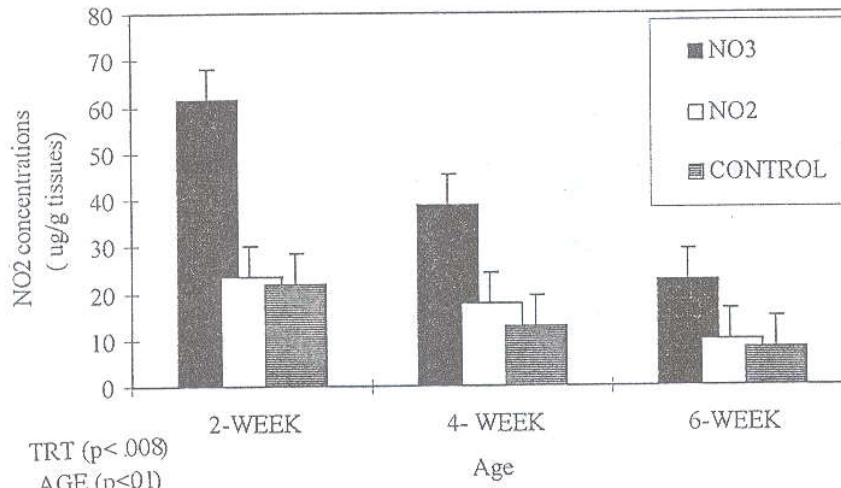


figure 3. NO2 concentrations in white muscles of broiler chicks



TRT (p<.008)
AGE (p<.01)

figure 4. NO2 concentrations in red muscles of broiler chicks



TRT (p<.008)
AGE (p<.01)

water than those receiving 50 ppm nitrite or the control ($P < .001$). The higher value was at 2 week-old, then declined sharply to the same values of the control and NO_2 treated chicks at ages 4 and 6 week ($P < .01$). These results confirm our first assumption that NO_3 was converted to NO_2 at early age then at older age birds were more adapted to the compound and body weight and body gain improved only after 4 weeks of age.

Serum concentrations of total protein, albumin, globulin and total lipids were analyzed and presented in Table 5. Results of total lipids and albumin were not affected by treatments. However, globulin was decreased ($P < .02$) at age 2 week. Coincided result of total protein was obtained ($P < .05$) at same age. But normal concentrations were found at ages 4 and 6 weeks.

Table 5. Serum concentrations of total protein, total albumin, globulin and total lipids in broiler chicks received 0.0 or 50 ppm of NO_3 or NO_2 in drinking water

TRT/ AGE	2- wks	4- wks	6- wks	overall mean
<u>Total protein, g/ dl</u>				
50ppm NO_3	3.8 ± .61 ^{a1}	2.2 ± .38 ^a	5.0 ± .41 ^a	3.7 ± .81
50ppm NO_2	2.3 ± .61 ^b	2.1 ± .38 ^a	3.6 ± .41 ^a	2.7 ± .47
Control	4.4 ± .61 ^a	3.3 ± .38 ^a	4.1 ± .41 ^a	3.9 ± .33
<u>Total albumin g/ dl</u>				
50ppm NO_3	1.2 ± .12	1.7 ± .12	1.5 ± .18	1.5 ± .33
50ppm NO_2	0.9 ± .12	1.9 ± .12	1.8 ± .18	1.5 ± .15
Control	1.3 ± .12	1.5 ± .12	1.2 ± .18	1.3 ± .09
<u>Total globulin g/ dl</u>				
50ppm NO_3	2.9 ± .64 ^a	0.8 ± .51 ^a	3.2 ± .32 ^a	2.3 ± .53
50ppm NO_2	1.2 ± .64 ^b	0.9 ± .51 ^a	2.1 ± .32 ^a	1.4 ± .93
Control	3.2 ± .64 ^a	1.8 ± .51 ^a	2.9 ± .32 ^a	2.6 ± .41
<u>Total lipids g/ l</u>				
50ppm NO_3	3.2 ± .16	3.5 ± .21	2.9 ± .18	3.2 ± .17
50ppm NO_2	3.4 ± .16	3.2 ± .25	3.2 ± .16	3.3 ± .06
Control	3.3 ± .16	3.5 ± .16	2.7 ± .37	3.2 ± .23

1 mean ± SE

a,b Means with different superscripts within columns are significantly different ($P < .05$).

In summary, 50 ppm of either nitrate or nitrite in drinking water was tolerated by chicks at age 4- week or older according to increased weight gain and normal viability among all treatments. No significant changes in blood parameters were found and tissues residual nitrate and nitrite were decreased when chicks progressed in age. The insignificant changes in serum albumin concentrations indicate that these levels of NO_3 and NO_2 did not affect liver

function. Broilers can tolerate well these levels of NO_3 and NO_2 with very slight residuals in both white and red meat (almost equal to the control group) especially at older ages, 4 and 6 weeks of age.

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تأثير مستويات متوسطة من النترات أو النيتريت فى ماء الشرب على أداء كتاكيت اللحم

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فى هذه الدراسة تم إستخدام ١٨٠ كتكوت عمر يوم من كتاكيت اللحم الغير مجنسة لدراسة تأثير إضافة ٥٠ جزء فى المليون من نترات أو نيتريت الصوديوم فى ماء الشرب على أداء الطيور والمتبقى فى العضلات.

أظهرت النتائج تماثل وزن الجسم فى المعاملات مع المجموعة المقارنة (الكونترول) عند عمر ٢، ٤ أسابيع. بينما كان الوزن النهائى للجسم عند عمر ٦ أسابيع للطيور أعلى فى المجموعة المعاملة مقارنة بالكونترول. كفاءة تحويل الغذاء لم تختلف معنويا بين المعاملات ولكن كان هناك تحسن ملحوظ عند عمر ٤ أسابيع. ارتفع تركيز النترات والنيتريت فى العضلات البيضاء والحمراء عند عمر أسبوعين ثم انخفض التركيز معنويا بالتقدم فى العمر. يتضح من هذه الدراسة أن مستوى ٥٠ جزء فى المليون من النترات أو النيتريت كانت فى قدرة تحمل الطائر خاصة عند الأعمار المتقدمة.