AN ABSTRACT OF THE THESIS OF

Julie A. Reeder for the degree of Masters of Science in Animal Science presented on May 10, 1996.

Title: The Effects On The Performance of Broilers Consuming Calcium, Potassium, and Sodium Nitrates and Nitrites From the Drinking Water.

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Four experiments were carried out with broiler chicks from day-old to three or four weeks of age to assess the effects of nitrates and nitrites from calcium, potassium, and sodium salts in the drinking water of broilers on growth, body weights, feed utilization, blood chemistries, liver tissues, and lipid oxidation of breast and thigh tissues. Body weights were lower ($P<.05$) among chicks consuming calcium nitrate than those of chicks consuming sodium nitrate or the control. Chicks consuming calcium nitrate ($Ca(NO_3)_2$) at 1000 ppm had lower ($P<.05$) body weights than those drinking $Ca(NO_3)_2$ at 0, 50, or 200 ppm. Broilers receiving 1000 ppm of calcium nitrite ($Ca(NO_2)_2$) had depressed body weights when compared to chicks consuming 0, 50, or 200 ppm of $Ca(NO_2)_2$. Consumption of sodium nitrate ($NaNO_3$) at 2033 ppm reduced ($P<.05$) broiler weights in comparison to broilers ingesting 0, 111, or 427 ppm of $NaNO_3$. Feed utilization was less efficient ($P<.05$) by chicks ingesting $Ca(NO_3)_2$ and calcium, potassium, or sodium salts of nitrite when compared with the control.

Water consumption was reduced ($P<.05$) among chicks consuming 2033 ppm $NaNO_3$ when contrasted to those consuming 0, 111, or 427 ppm $NaNO_3$.

Blood glucose levels were reduced ($P<.05$) among chicks consuming 2033 ppm $NaNO_3$, and chicks consuming calcium nitrite when compared to chicks consuming water with no additional nitrate or nitrite. Concentrations of blood sodium was increased ($P<.05$) and potassium was decreased ($P<.05$) in chicks consuming 2033 ppm when compared to the control. Chicks consuming 1000 ppm $Ca(NO_3)_2$ had higher ($P<.05$) levels of aspartate amino transferase when compared to chicks consuming 0, 50, or 200 ppm $Ca(NO_3)_2$. Uric acid levels were lower ($P<.05$) in chicks consuming 50, 200, or 1000 ppm of $Ca(NO_2)_2$ than for the control.

No differences ($P>.05$) in liver weights were observed between the salts or levels of nitrate or nitrite. Microscopic examination of the liver tissues from chicks receiving 2033 ppm NO_3 from $NaNO_3$ had cellular abnormalities. The lumen of the liver venule showed an excess of endothelial cells on the inside, and a proliferation of stromal cells on the outside causing a closure of the venule.

Analyses of oxidative rancidity of breast and thigh tissues from the first experiment found no difference ($P>.05$) between tissues from chicks consuming $NaNO_3$ compared to the control. Chicks consuming $NaNO_3$, $Ca(NO_3)_2$, or KNO_3 had a reduced ($P<.05$) level of rancidity when compared to the control.

According to these studies, chicks consuming 2033 ppm NO_3 from $NaNO_3$ had cellular abnormalities. Calcium nitrate or nitrite at a level of 1000 ppm will experience reduced body weight, feed utilization, and reduced levels of blood glucose, uric acid, and aspartate amino transferase.

The Effects on the Performance of Broilers Consuming Calcium, Potassium, and Sodium Nitrates and Nitrites from the Drinking Water

by

Julie A. Reeder

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Julie A. Reeder, Author

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CHAPTER 1

INTRODUCTION

The broiler industry occupies a vital niche in the agricultural market in the 1990's. Per capita consumption of chicken and chicken by-products has continued to increase steadily and has now overcome per capita consumption of all other meat products in the United States. In 1994, per capita consumption of broilers was 69.8 lbs, vs. 68 lbs of beef, 55 lbs of pork, 15.2 lbs of fish, and 1.5 lbs of lamb (USDA, 1995). To meet the steadily increasing demand of consumers for a healthy, low-fat product, the poultry industry has changed dramatically in the last half of this century. No longer are chickens raised in small backyard flocks. Instead, a billion dollar industry, based on scientific research and high-tech machinery, produce the broiler of today. Millions of hours and dollars have been spent studying and improving the environment, genetics, and nutrition of today's commercial broiler. In general, the chicken's nutritional requirements are well documented. However, among the thousands of papers on nutrition, very few have chosen to focus on the most essential nutrient, one which when deficient will lead to mortality within a few days. This vital, yet overlooked nutrient is water. A one-week-old chick has a body water content of about 85%. This gradually decreases with age, dropping to a level of about 55% at 42 weeks of age (Scott *et al.*, 1976) A chicken consumes twice as much water as it does feed (Scott *et al.*, 1976). Water is needed to carry out many important functions in the body, including temperature regulation. It also acts as a universal solvent, transporting hormones and nutrients to the cells, and picking up and dissolving waste products such as urea and sulfates. Water serves as a lubricant making movement of the eyes, tongue, and joints possible. In addition, it plays an active role in metabolism, in reactions requiring hydrolysis, hydration, and dehydration (NRC, 1989). Without a good, clean, quality supply of water, chick mortality will be high, and therefore, grower profits will be low. Water quality is not easy to define objectively. It can be assessed on the basis of taste, odor, color, turbidity, and content of pollutants. Water of good quality is defined as water which has an acceptable taste, odor, and color, and does not contain any substances that may be detrimental to the health of those who consume it. The definition is quite general and open to subjective interpretation.

Federal and state laws have been created to specify requirements for water quality. The Clean Water Act, which is a federal law created in 1971 defines many of the water quality standards and lists goals for improving water quality in the future. The act has been reviewed and amended several times in the past two decades. In the 1995 legislative session of the U.S. Congress, the act has again come up for review, and may be weakened to decrease the financial burden on businesses created by trying to meet water quality requirements. This attempt to rewrite the Clean Water Act has raised much concern from those who feel that water quality should not be compromised, and heated debate has begun among people about whether economics are more important than the health of the environment. But, no matter what the outcome of the legislative session, water quality has become a key concern of many people in our nation, and will continue to be so for many decades to come.

Due to the renewed public interest in water quality, and the lack of research papers focusing on the importance of water as a nutrient vital to the success of raising broilers, this research was carried out to determine the relationship between water quality and broiler performance. Of the many potential pollutants to be explored, nitrates and nitrites were chosen due to a recent focus on their potential danger. In addition, several water quality surveys done on poultry farms have found nitrate and nitrite present on some of the sites. Therefore, the objectives of these experiments were to determine if nitrate and nitrite in the drinking water of broilers would cause detrimental effects on performance and health of the birds, and to determine if specific chemical forms of nitrate and nitrite were more detrimental than others.

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CHAPTER 2

REVIEW OF LITERATURE

Water quality is of concern to poultry producers because a good, clean water supply is vital for chick survival. Of all the possible contaminants of the water supply, nitrate is one that has received considerable attention in the past few years. Nitrates are present in soils, water, plant materials, and meats. A small concentration is also found in the air due to pollution. Nitrites, on the other hand, are produced by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates. Normally, concentrations of nitrites in plants and water are very low (World Health Organization, 1978).

The nitrogen cycle is continually occurring, causing nitrogen to be synthesized and degraded. The main factors affecting the production of the cycle are climatic conditions, the type and density of animal and plant populations, agricultural practices, and animal husbandry practices. The nitrogen cycle begins with atmospheric dinitrogen. Part of the dinitrogen undergoes nitrogen fixation, which transforms it by microbial action and then allows its incorporation into living organisms. Each year 150 million tons of nitrogen becomes fixed (World Health Organization, 1978). A portion of the nitrogen is assimilated by plants, some leaches into rivers and ground water, the rest undergoes denitrification which degrades nitrates to nitrogen or nitrous oxides, which are released back to the atmosphere (World Health Organization, 1978)

Industrial nitrogen fixation accounts for one-fourth of the total world production of fixed nitrogen (World Health Organization, 1978). In this process, atmospheric nitrogen is joined with hydrogen at high temperatures and pressures in the presence of an appropriate metal catalyst to produce ammonia. Biological fixation occurs through the root nodules of legumes. In each process, ammonia is produced and is converted to nitrite and nitrate by the process of nitrification. Nitrates from natural fixation are used for synthesis of biological molecules, particularly proteins. In the end, plant and animal wastes and dead tissues will return nitrogen to the soil, where part of it will be recycled into new molecules, and part will return to the atmosphere (World Health Organization, 1978).

Agricultural nitrogen enters surface waters by direct surface run-off and soil erosion, or by ground water discharges from unconfined aquifers into streams. Data from the U.S. Geological Service (USGS) show that 20 states with a notably higher percentage of wells with nitrate levels exceeding maximum safety recommendations (10 mg/l) are also the same 20 states with the largest agricultural production (USDA, 1991). Between 1974 and 1981, the USGS found the nitrogen concentration in surface water increased at 116 stations, while decreasing at 27 stations. The rising level of nitrogen in surface water may be attributed a four fold increase in the use of nitrogen fertilizer from 1960-1981. Since 1981, fertilization has been decreased to a level of 10.5 million tons in 1988 (USDA, 1991).

Primary sources of nitrate and nitrite exposure to humans and livestock occur from plant and water consumption (Osweiler *et al.*, 1985). In normal healthy adult animals, nitrates and nitrites are rapidly absorbed from the gastrointestinal tract (GIT). Absorbed nitrite reacts with hemoglobin to form methemoglobin, which in adults is rapidly converted to oxyhemoglobin by enzymatic systems such as nicotinamide adenine dinucleotide (NADH)-methemoglobin reductase. A part of ingested nitrates are readily absorbed by the gastric mucosa, and a part is metabolized by microflora in the GIT. Health risks occur when infants ingest water containing nitrates. Microorganisms present in the GIT of very young animals can convert nitrates to nitrites (World Health Organization, 1978). The nitrites bind to hemoglobin causing it to be converted to methemoglobin. When this occurs, oxygen can no longer bind to red blood cells. Clinical signs include cyanosis and anoxia due to defective transport of oxygen by high levels of circulating methemoglobin, which is an oxidation product of hemoglobin with the oxidized ferrous iron (ferric form) (World Health Organization, 1978). Methemoglobinemia is the resulting condition, and there were 2000 cases reported in North America and Europe between 1945 and 1971 (National Academy Press, 1981). Infants under three months of age are at greatest risk for developing methemoglobinemia due to three reasons. First, infant hemoglobin is much more readily oxidized than in adults. Secondly, infants have a transitory deficiency of methemoglobin reductase or its cofactor NADH, which are necessary to maintain iron in its reduced state. Finally, the stomach of an infant is less acidic than in an adult, which favors an overgrowth of nitrate reducing organisms in the GIT (World Health Organization, 1978). When 10% of the hemoglobin has been converted to methemoglobin, clinical cyanosis occurs. At a

20% conversion, cerebral anoxia occurs. Finally at a 60% conversion, stupor, coma, and death occur. In most cases, the condition can be reversed with oral doses of ascorbic acid given 3 times daily. In more extreme cases, an immediate intravenous injection of methylene blue is given (National Academy Press, 1981).

Due to the risk of infants developing methemoglobinemia, the Environmental Protection Agency has set the maximum safe level for nitrate in drinking water at 10 mg/l. Even with the guidelines being tested and enforced, it is estimated that out of 219 million people using public drinking water supplies, approximately 1.7 million are exposed to nitrate levels above the maximum. Of the 1.7 million at risk, 1.6% are infants, meaning that 27,000 infants per year are exposed to water with a nitrate level higher than 10 mg/ml (World Health Organization, 1978). The nitrite ion is 10 times more toxic than the nitrate ion. Nonruminant animals are 10 times more susceptible to oral nitrite than to nitrate, where as ruminants are only 2 to 3 times more sensitive to nitrite than nitrate (Osweiler *et al.*, 1985). Acute toxicity with nitrates and nitrites has been proven to occur at various levels according to age, size, and species. Chronic toxic effects have yet to be proven. Field observations of chronic nitrate poisoning in ruminants and monogastrics have shown poor growth rate, abortion, infertility, vitamin A deficiency, goiter, and increased susceptibility to infection in various species (Osweiler *et al.*, 1985). In some cases, outward symptoms may be the result of the conversion of a borderline nutrient deficiency to a gross one (National Academy of Sciences, 1972). However, it is known that nitrate and nitrite do affect certain organs.

Nitrate can act as an antithyroid substance. It increases the level of iodine needed for proper thyroid function (National Academy of Sciences, 1972). Nitrate has also been linked to liver damage and a drop in vitamins A and E. Vitamin A deficiency may be caused by the destruction of carotene or by interfering with the utilization of vitamin A (National Academy of Sciences, 1972).

The effects of oat hay containing nitrates on dairy cows was reported by Murdock *et al.* (1972). Cows fed higher levels of nitrate did not have a higher level of methemoglobin than cows fed a lower level. Davison *et al.* (1964) also explored the role of nitrate in reproduction, growth, and lactation of dairy heifers. Heifers consuming the highest level of nitrate (660 mg/kg) had a lower conception rate and a higher incidence of abortion. No significant differences between treatment groups were discovered for growth, vitamin A status, birth weight, and health of calves. Mitchell, *et al.* (1967) fed potassium nitrate to steers at a level of 1% of the ration and found no indication that nitrate increased vitamin A destruction.

Studies have been reported on the chronic effects of nitrate and nitrite ingested by farm animals. Sell and Roberts (1963) fed 0.4% dietary nitrite to Single Comb White Leghorn cockerels from day-old to 4 weeks of age, and found a decrease in growth rate, and a reduction in vitamin A liver stores.

Barton *et al.* (1986) surveyed 300 large broiler operations in Arkansas, noting production performance, and sampling water supplies. By running a series of correlations, a positive correlation between nitrate and carcass condemnation, and negative correlations between nitrate and growth, livability, and body weight were found.

Hermes and Holleman (1992) surveyed Oregon broilers farms and found the nitrate levels ranged from 0 to 37.5 ppm, with an average of 2.05 ppm nitrate. The average level of 2.05 ppm is well below the limits set by the Environmental Protection Agency of 10 mg nitrate/ml, and higher than the National Academy of Sciences (1991) average level of nitrate in surface water of 1.3 ppm.

Segars (1995) surveyed water wells throughout the Georgia and found that wells used for livestock production contained higher levels of nitrate than those used only for human consumption. The level of nitrate found on 257 poultry farms surveyed showed the average level of nitrate of 3.69 ppm.

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CHAPTER 3

EFFECTS OF NITRATE FROM SODIUM, POTASSIUM, AND CALCIUM NITRATE IN DRINKING WATER ON BROILERS

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ABSTRACT

Water quality is of concern to broiler producers because poor water quality has been linked to inferior production performance. No specific information on the dose-effect of nitrate (NO_3) in broilers has been reported in the literature. Therefore, two experiment were conducted with broiler chicks to quantify the level of NO_3 in drinking water with physiological effects. In Experiment one, thirty-two straight-run commercial Arbor Acres x Peterson broiler chicks were used from day-old to four wk of age, with four chicks assigned to each pen. The four levels of NO_3 were 0, 111, 411, and 2033 ppm from sodium nitrate ($NaNO_3$). Each treatment level was provided to duplicate pens. In Experiment two, two-hundred and forty Arbor Acres x Peterson feather-sexed broiler chicks were randomly assigned to ten treatment groups from day-old to three wk of age. The chicks consumed nitrate (NO_3) from calcium, potassium, or sodium salts, at a level of 0, 50, 200 or 1000 ppm. Each treatment consisted of three replicate pens of four male and four female chicks. In both experiments, feed and water consumptions on a per pen basis, and individual body weights were measured weekly. At the end of each experiment, broilers were sacrificed and blood, liver, breast and thigh tissues were sampled.

In Experiment one, differences were not observed ($P>.05$) in body weight and feed conversion between 0, 111, and 427 ppm. However, a decrease ($P<.05$) in body weight was observed with the 2033 ppm. No differences were found in total water consumption at either wk two or four. Liver sizes (g/100 g body wt) were not different among the treatments. Blood sodium and protein levels were higher

($P<.05$) and levels of potassium and glucose were lower with chicks consuming 2033 ppm NO_3 than with the 0, 111, or 427 ppm NO_3. Histology of liver tissues showed no differences with the 0, 111, or 427 ppm of NO_3. However at 2033 ppm, NO_3 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.

In Experiment two, no interactions between the salts and the levels of nitrate were observed. No differences ($P>.05$) in blood parameters were observed between chicks consuming the three NO_3 salts and the control. No differences between the nitrate levels within each salt was observed of the parameters measured except for SGOT. Aspartate amino transferase (SGOT) levels were higher ($P<.05$) in chicks consuming water containing 1000 ppm NO_3 than chicks consuming water containing 0, 50, or 200 ppm NO_3 from $Ca(NO_3)_2$. Chicks consuming $Ca(NO_3)_2$ had lower ($P<.05$) three wk body weights than chicks receiving the control or $NaNO_3$ and a less efficient feed conversion than chicks consuming water with no additional nitrate. Histological examination of liver tissues showed no abnormalities. Oxidative rancidity was higher in the breast and thigh tissues from the broilers consuming water without nitrate than from broilers consuming water with nitrate.

Under the conditions of these experiments, NO_3 from $NaNO_3$ in drinking water of broilers equal to or exceeding 2033 ppm will cause a reduction in performance and cause liver pathology. $Ca(NO_3)_2$ affected performance by lowering three wk body weights and decreasing efficiency of feed utilization, and at 1000 ppm increasing aspartate amino transferase levels which are associated with hepatocellular damage.

INTRODUCTION

Water is an essential nutrient for chick survival. Many contaminants may cause a decrease in the quality of the water supply. One such contaminant that has recently been focused on by the media and industry publications is nitrate. Nitrate which is converted to nitrite in the digestive tract can cause methemoglobinemia in human infants, and is suspected of being carcinogenic (World Health Organization, 1978). Due to the cases of methemoglobinemia seen earlier in the century, the Environmental Protection Agency has established the safe level of nitrate in drinking water as 45 ppm NO_3 Surveys of water quality throughout the nation report that most sources of drinking water contain levels of nitrate greatly below the recommended safe level. However in certain areas, particularly in areas of high agricultural production, nitrate levels have been reported to reach 200 ppm (National Academy of Sciences, 1981).

The potential risks of nitrate, and the lack of data on the effects of chicks consuming nitrate from the drinking water, these experiments were conducted to determine the effects on performance of broilers consuming nitrate from calcium, potassium, and sodium salts and to compare the effect of the nitrate salts in the drinking water.

MATERIALS AND METHODS

Two experiments were conducted to determine the effects of nitrate, from potassium, sodium, and calcium salts. In both experiments, chicks were housed in an enclosed room in Petersime battery cages, with raised wire floors. Lighting was provided 24 h/day throughout the experiment. In Experiment 1, thirty-two Arbor Acre x Peterson broiler chicks of mixed sex were randomly assigned to one of the four different treatments from day-old to four wks of age. Chicks were studied only for four weeks rather than for the entire seven week production life-time because younger chicks would be more susceptible to the effects of nitrates. The chicks were randomly assigned four to a pen, with each treatment level provided to duplicate pens. The levels of nitrate from sodium nitrate were 0, 111, 427, and 2033 ppm (analyzed values).

In Experiment 2, two-hundred and forty feather-sexed Arbor Acres x Peterson broiler chicks were divided into 10 treatment groups. Each treatment had three replicate pens consisting of four male and four female chicks. The control group received water with no additional nitrate. The remaining groups received nitrate from either sodium, calcium, or potassium nitrate at levels of 50, 200, or 1000 ppm in the drinking water. The management procedure was the same as in Experiment one.

The nitrate solutions were premixed in the laboratory using city tap water. Granular sodium, potassium, or calcium nitrates were weighed, placed in 2 L volumetric flasks, and then filled with tap water to the meniscus. This was repeated until the 19 L plastic jugs assigned to each treatment were filled. The jugs of water were stored in a cool dark place to avoid possible chemical changes in the solutions. Samples of the solution were taken after the initial mixing, and each mixing thereafter. Analyses for nitrate content were performed by Dr. Lee Tsai using the method of Heckenberg *et al.*,(1989).

Feed and water consumptions, and individual body weights were measured each week. Measured amounts of water were added daily to the trough of each pen. Losses from evaporation were accounted for each week by having an open water trough with similar surface area as other water troughs. These evaporation losses were used to correct the amount of water consumed for each pen.

In Experiment one, all 32 birds were sacrificed at four wk of age by cervical dislocation. Blood samples (1-2 cc) were drawn from the wing vein in Vacutainer® tubes coated with lithium heparin, using a 22 gage needle. In Experiment 2, one male chick and one female chick from each pen were selected at the end of the three wk period for blood, liver and tissue samples. A total of 60 birds (30 males, 30 females) were sacrificed by cervical dislocation. Blood samples were taken after excising the jugular vein of each bird and collected in Vacutainer®[1] tubes with lithium heparin coating. The blood parameters analyzed are those that are done in a standard avian blood screening. Blood samples were analyzed by a commercial laboratory. Analyses for electrolytes were performed using an ion specific

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[1]Becton Dickinson, Rutherford, New Jesey, 07070

electrode[2]. Slide were prepared to determine the counts of white blood cells, heterophils and lymphocytes. Protein, uric acid, glucose, albumin and aspartate amino transferase values were measured with a Ciba-Corning 550 Express Random Access Chemistry Analyzer[2].

In both experiments, each broiler chick was weighed prior to sacrifice and then the liver was excised and weighed. Portions of the liver were sliced and immediately placed into a 10% formalin solution. The samples were prepared by a commercial laboratory using H and E stain (Armed Forces Institute of Pathology 1960).

The breast and both thigh muscles were removed from each bird, immediately dipped into liquid nitrogen, bagged, and then stored in a -80 C freezer. Analyses of lipid oxidation were performed by Dr. Annie King and Dennis Fitzpatrick (Dept. of Avian Sciences, UC Davis) using the modified extraction 2-thiobarbituric acid method (Salih *et al.*, 1987). The TBA values are expressed as mg malonaldehyde/kg tissue.

Statistical analyses of Experiment one were done with Statgraphics, (Manugistic Inc. 1992) using one-way analysis of variance and least significant difference (Steele and Torrie, 1980). The experimental unit was each pen, and a probability level of .05 was chosen to determine significance.

Experiment 2 was a 3 X 4 factorial design. The individual pen was considered the experimental unit. Statistical analyses were performed on SAS 6.11 (General Linear Models) (SAS®, 1995). Two-way ANOVA were performed on the data with

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[2]Ciba-Corning Diagnostic Corp. Oberlin, Ohio 44074 USA

NO_3 salts and NO_3 levels serving as the main effects (Steele and Torrie, 1980). Significant differences of the data were separated by LSD (Steele and Torrie, 1980) with probability level of $P=.05$.

RESULTS AND DISCUSSION

Experiment 1

No differences in body weights were observed among broiler chicks for the first two weeks (Table 3.1). Chicks consuming nitrate at 2033 ppm had lower ($P<.05$) mean body weights in comparison to chicks consuming 0, 111, and 411 ppm NO_3 at 4 wk of age (Table 3.1). This observation supports the findings of Barton, *et al* (1986) who found a negative correlation between nitrate consumption and body weights and growth. However, weekly feed conversions were not significantly different between the nitrate levels (Table 3.1). These findings are contrary to Barton *et al.* (1986) who found negative correlations between nitrate consumption and feed conversion.

Water consumption was not affected by the levels of nitrate for the first two weeks (Table 3.2). However by the third week, water consumption was lower ($P<.05$) in chicks consuming nitrate at 111 or 2033 ppm NO_3 than the 0 or 427 ppm levels. The decrease in water consumption by the group receiving 111 ppm was caused by a housing problem that was quickly corrected. By the fourth week, only chicks consuming 2033 ppm NO_3 had a lower ($P<.05$) water consumption than 0, 111, or 427 ppm NO_3 (Table 3.2).

The weekly amount of nitrate consumed by each chick is displayed in Table 3.3. According to these data, broiler chicks consuming an accumulative nitrate level somewhere between 962 mg to 3863 mg will display physiological changes.

Table 3.1. Effect of nitrate (NO_3) from sodium nitrate at levels of 0, 111, 427, and 2033 ppm in drinking water on body weight and feed conversion at two and four weeks of age (Expt. 1)

Analyzed NO_3 Level (ppm)	Weeks of age: 2 — Body weight (g)	2 — Feed conversion ratio	4 — Body weight (g)	4 — Feed conversion ratio
0	337^{a}	1.34^{a}	963^{a}	1.66^{a}
111	325^{a}	1.47^{a}	992^{a}	1.44^{a}
427	330^{a}	1.11^{a}	1003^{a}	1.67^{a}
2033	328^{a}	1.19^{a}	735^{b}	2.19^{a}
SEM	0.01	0.16	.02	0.15

a/b indicates significant difference ($P<.05$) for each column

Table 3.2. Influence of nitrate (NO_3) from sodium nitrate on weekly water consumption (Expt. 1)

Analyzed NO_3 Level	Weeks			
(ppm)	1 (ml)	2 (ml)	3 (ml)	4 (ml)
0	117[a]	290[a]	739[a]	1425[a]
111	87[a]	265[a]	486[b]	1063[a]
427	74[a]	275[a]	654[a]	1401[a]
2033	100[a]	381[a]	578[b]	870[b]
SEM	11	19	23	136

a/b indicates significant difference ($P<.05$) for each column

Table 3.3. Accumulative nitrate (NO_3) from sodium nitrate consumed per bird (mg) from day-old to two wks and from day-old to four wks of age (Expt. 1)

Analyzed NO_3 Level (ppm)	0-2 Weeks (mg/bird)	0-4 Weeks (mg/bird)
111	35	190
427	99	921
2033	962	3863

Chicks ingesting 2033 ppm NO_3 had higher ($P<.05$) plasma levels of sodium, and lower ($P<.05$) levels of potassium than the chicks consuming 0, 111, or 427 ppm NO_3 (Table 3.4). The level of salt intake was highest amongst this group; therefore, the rise in plasma sodium level is not surprising. Since sodium is the major extracellular cation, and potassium is the major intracellular cation, it is natural that when sodium levels increase, potassium levels would decrease. Broiler chicks drinking water containing 111 ppm NO_3 had a lower ($P<.05$) concentration of plasma calcium than chicks consuming 0, 411, and 2033 ppm NO_3 (Table 3.4). Chicks consuming 111 and 2033 ppm had lower ($P<.05$) blood glucose levels than the control (Table 3.4). The normal range of plasma glucose for avian species is from 200 to 450 mg/dl; therefore, none of the values obtained were outside of the normal range (Coles 1986). However, chicks who consumed the highest levels of nitrate also had increased plasma protein. The increased amount of nitrogen consumed by these chicks may have caused the body to utilize energy derived from glucose to fuel the removal of the excess nitrogen, thus causing a decreased glucose level.

No differences ($P>.05$) in liver weights were observed between the levels of nitrate (Table 3.5).

Histological examinations of tissues from chicks consuming 111, or 427 ppm NO_3 showed no abnormalities. Examinations of the liver tissues from chicks consuming 2033 ppm NO_3 had cellular abnormalities (Figures 1 and 2). The lumen of the liver venule showed an excess of endothelial cells on the inside, and a proliferation of stromal cells and lymphocytes on the outside, causing a closure of the

Table 3.4. Effect of nitrate (NO_3) from sodium nitrate in the drinking water of broilers on blood plasma levels of sodium, potassium, glucose, protein, and calcium (Expt. 1)

Analyzed NO_3 Level (ppm)	Na (Meq/L)	K (Meq/L)	Glucose (mg/dl)	Protein (g/dl)	Ca (mg/dl)
0	153^{a}	6.76^{a}	297^{b}	3.33^{ab}	12.13^{a}
111	150^{a}	6.43^{a}	242^{a}	3.03^{a}	11.37^{b}
427	158^{a}	7.60^{a}	262^{ab}	3.45^{ab}	12.02^{a}
2033	165^{b}	5.83^{b}	252^{a}	3.97^{b}	12.03^{a}
SEM	1.40	0.15	3.74	0.10	0.09

a/b indicates significant difference ($P < .05$) for each column

Table 3.5. Influence of nitrate (NO_3) from sodium nitrate at levels of 0, 111, 427 and 2033 ppm on liver weights of chicks at four weeks of age (Expt. 1)

Analyzed NO_3 Level (ppm)	gm/100 g BW
0	2.36[a]
111	3.23[a]
427	2.36[a]
2033	2.41[a]
SEM	0.17

a/b indicates significant difference ($P < .05$) for each column

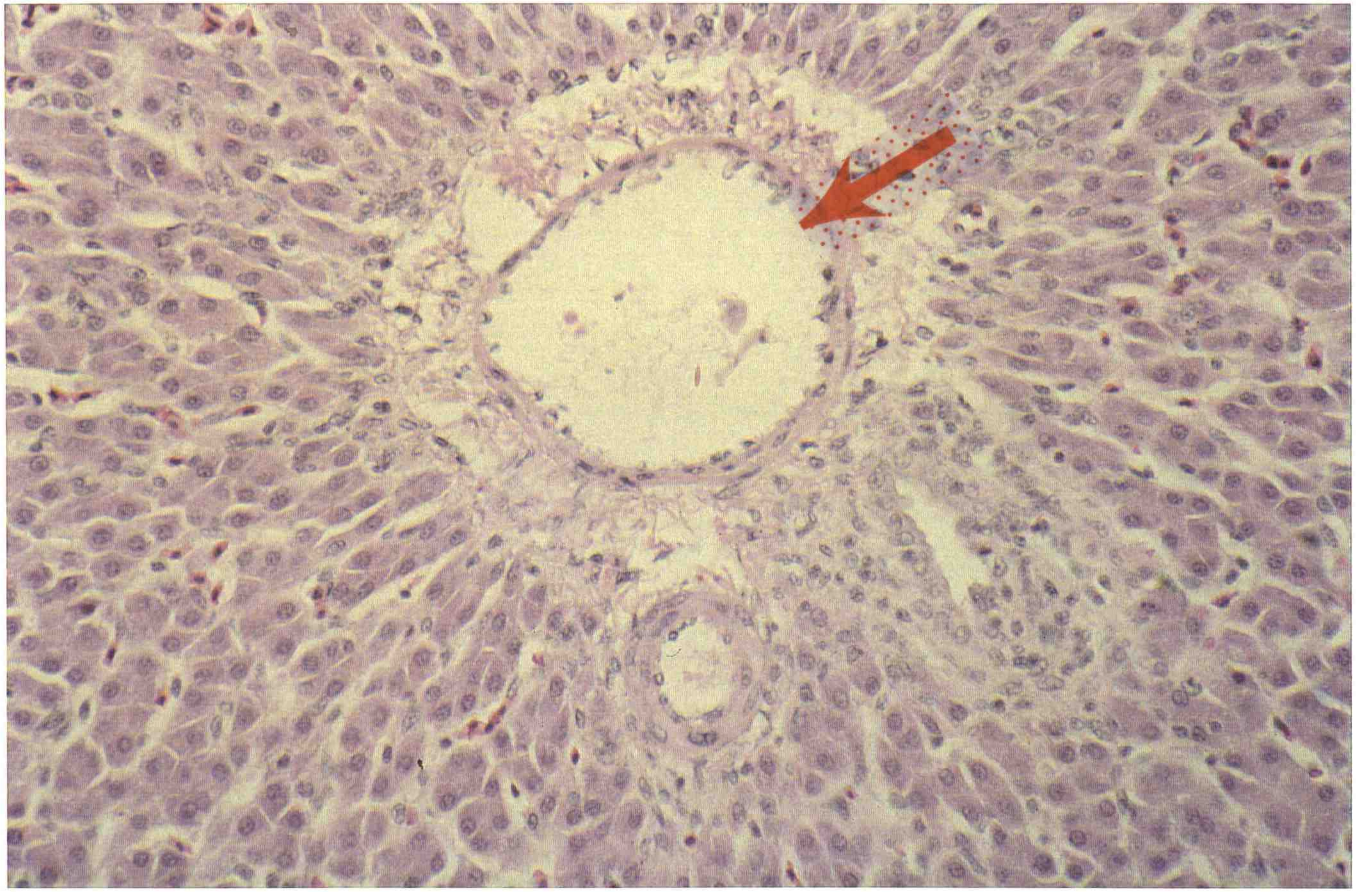

Figure 3.1. Cross section of liver tissue obtained from a broiler chick at three weeks of age consuming no additional nitrate in the drinking water. The venule is unobstructed (noted with arrow).

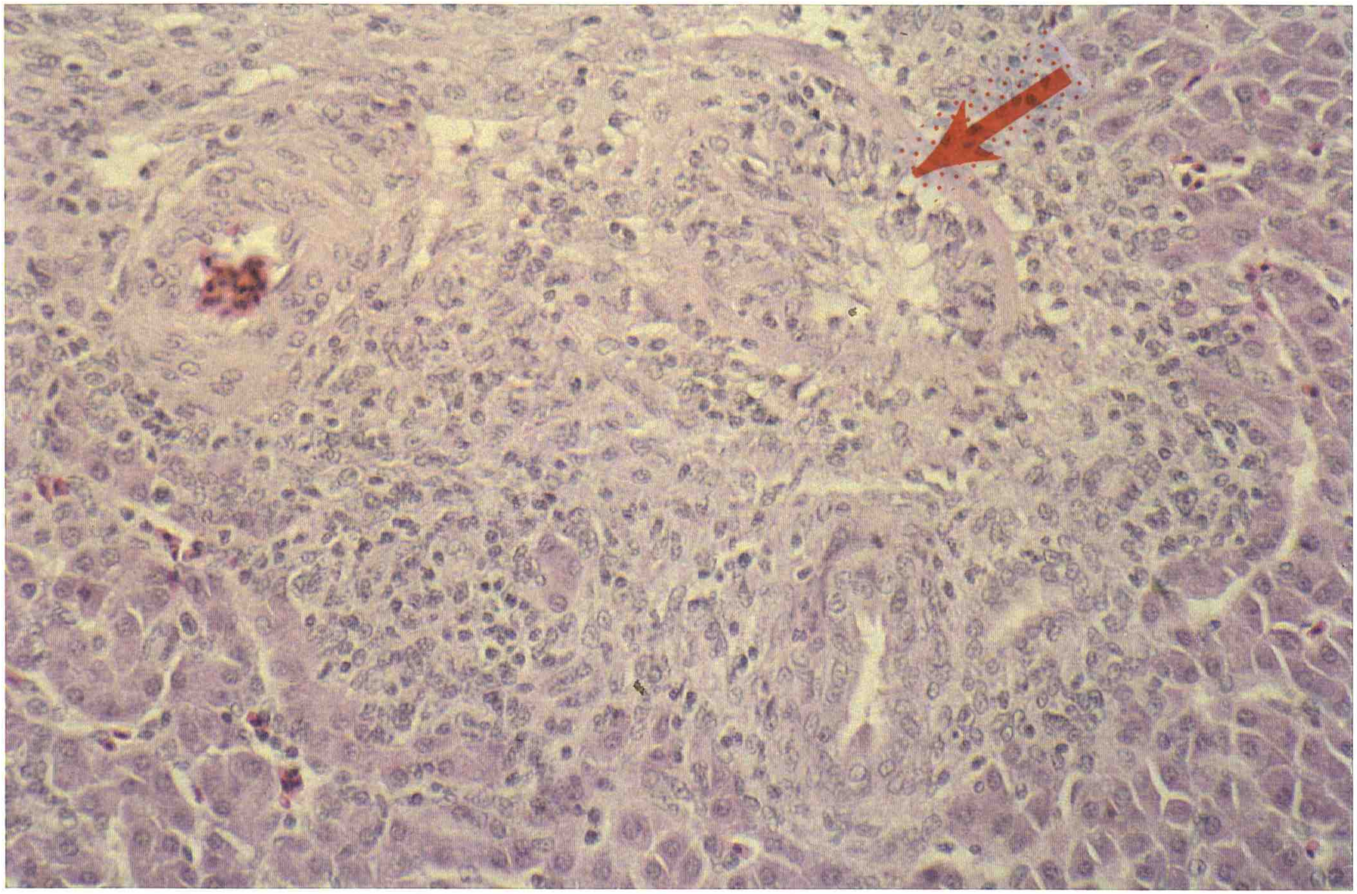

Figure 3.2. Cross section of liver tissue obtained from a broiler chick at three weeks of age consuming 2033 ppm NO_3 from sodium nitrate. The venule is obstructed due to an excess of endothelial cells in the lumen, and a proliferation of stromal cells and lymphocytes on the exterior (noted with arrow).

venule. This suggests that broiler chicks consuming 2033 ppm NO_3 water had hepatocellular damage.

Determination of oxidative rancidity of tissues showed no differences ($P<.05$) in thiobarbituric acid (TBA) values between chicks receiving nitrate and the control (Table 3.6). Therefore, addition of nitrate in the water does not appear to accumulate in the tissues where it might act as a deterrent against lipid oxidation.

Experiment 2

No salts x levels interactions were observed; therefore, salts and levels were analyzed separately. No differences in mean body weights for wk one or wk two were observed between the three NO_3 salts; however, mean body weights at wk three were lower ($P<.05$) in chicks consuming NO_3 from $Ca(NO_3)_2$ than for chicks consuming $NaNO_3$ or the control (Table 3.7). No differences in mean body weights were observed between the $Ca(NO_3)_2$ and KNO_3 at wk three. Weekly feed conversions were not different for the first two wks among the NO_3 salts. However, feed conversion at wk three was less efficient ($P<.05$) for chicks consuming the $Ca(NO_3)_2$ than for the control (Table 3.7).

Water consumption was lower ($P<.05$) for birds consuming the three NO_3 salts than no NO_3 during the first wk (Table 3.8). No differences in water consumption between the NO_3 salts were observed at wk two; however, water consumptions were lower ($P<.05$) for the three NO_3 salts than the no NO_3 group (Table 3.8). The difference in water consumption may have occurred as a period of

Table 3.6. Effect of nitrate from sodium nitrate on lipid oxidation of breast and thigh muscle tissues (Expt. 1)

Treatment	Breast TBA[1]	Thigh TBA[1]
Control	0.59[a] ± 0.08	1.4[a] ± 0.10
Nitrate	0.46[a] ± 0.05	1.3[a] ± 0.06

a/b indicates significant difference ($P<.05$) for each column
[1] mg malonaldehyde/kg tissues

Table 3.7. Effects of nitrate (NO_3) from sodium, calcium, and potassium nitrate on weekly mean body weights and feed conversions from day-old to three weeks of age (Expt. 2)

	Weeks on Test					
NO_3 Salts	1		2		3	
	Body weights (g)	Feed conversion ratio	Body weights (g)	Feed conversion ratio	Body weights (g)	Feed conversion ratio
Control	$147^{a} \pm 4.6$	$1.19^{a} \pm 0.01$	$355^{a} \pm 14.1$	$1.37^{a} \pm 0.01$	$702^{a} \pm 27.3$	$1.46^{b} \pm 0.01$
Sodium	$155^{a} \pm 2.6$	$1.21^{a} \pm 0.01$	$366^{a} \pm 8.2$	$1.49^{a} \pm 0.01$	$704^{a} \pm 15.8$	$1.56^{ab} \pm 0.01$
Calcium	$147^{a} \pm 2.6$	$1.21^{a} \pm 0.01$	$351^{a} \pm 8.2$	$1.45^{a} \pm 0.01$	$621^{b} \pm 15.8$	$1.78^{a} \pm 0.01$
Potassium	$149^{a} \pm 2.6$	$1.19^{a} \pm 0.01$	$363^{a} \pm 8.2$	$1.43^{a} \pm 0.01$	$657^{ab} \pm 15.8$	$1.63^{ab} \pm 0.01$

a/b indicates significant difference ($P < .05$) for each column

Table 3.8. Influence of sodium, calcium, and potassium nitrates (NO_3) on weekly water consumption of broilers (Expt.2)

	Weeks on test		
NO_3 Salts	1 (ml)	2 (ml)	3 (ml)
Control	$3258^{a} \pm 438$	$4385^{a} \pm 543$	$10{,}386^{a} \pm 1187$
Sodium	$2502^{b} \pm 253$	$4751^{a} \pm 313$	$8829^{b} \pm 685$
Calcium	$2641^{b} \pm 253$	$5127^{a} \pm 313$	$7778^{b} \pm 685$
Potassium	$2622^{b} \pm 253$	$5380^{a} \pm 313$	$8443^{b} \pm 685$

a/b indicates significant difference ($P < .05$) for each column

Table 3.9. Effect of consumption of nitrate from calcium nitrate $(CaNO_3)_2$ on blood aspartate amino transferase (SGOT) levels (Expt. 2)

Nitrate (ppm)	SGOT (IU/L)
0	165^{b}
50	180^{ab}
200	190^{ab}
1000	193^{a}
SEM	46

a/b indicates significant difference ($P<.05$)

adjustment to the taste of the added salts. At wk two, the chicks could have become adjusted to the taste. The decrease in consumption at wk three can be attributed to the effect of the nitrate, and the higher requirement for water by a larger bird.

No significant differences between the three nitrate salts on any of the blood parameters were observed. Intra-salt analyses of the data showed significant differences only among birds consuming NO_3 from $Ca(NO_3)_2$. Chicks ingesting 1000 ppm NO_3 from $Ca(NO_3)_2$ had higher ($P<.05$) levels of aspartate amino transferase when compared to chicks consuming no additional $Ca(NO_3)_2$ (Table 3.9). In chickens, aspartate amino transferase activity (AST, formerly known as SGOT) is highest in the heart and the liver (Coles 1986). Elevated levels of AST have been associated with hepatocellular damage, indicating that consumption of 1000 ppm NO_3 from $Ca(NO_3)_2$ causes liver pathology; however, histological examination of the liver tissues showed no signs of pathology.

Analysis of lipid oxidation of the tissues showed a higher degree of rancidity in the breast and thigh tissues from birds consuming no additional nitrate (Table 3.10). These data suggest that nitrates from calcium, potassium, or sodium salts accumulate in the tissues and act as an aid in deterring oxidative rancidity.

Under the conditions of these experiments, broiler chicks consuming 2033 ppm NO_3 from $NaNO_3$ experienced a reduction in performance and liver pathology. Calcium nitrate had the greatest effect on broiler chicks by lowering body weights, decreasing efficiency of feed conversion, and initiating hepatocellular damage.

Table 3.10. Effect of nitrate (NO_3) from sodium, calcium and potassium NO_3 in drinking water of broilers on lipid oxidation of breast and thigh tissues (Expt. 2)

NO_3 Salts	TBA Value[1]
Control	$8.4^{a} \pm 0.4$
Sodium	$4.6^{b} \pm 0.2$
Calcium	$4.4^{b} \pm 0.3$
Potassium	$4.5^{b} \pm 0.2$

a/b indicates significant difference ($P < .05$)
[1]mg malonaldehyde/kg tissue

CHAPTER 4

EFFECT OF NITRITE FROM SODIUM, POTASSIUM, AND CALCIUM NITRITE IN DRINKING WATER OF BROILERS

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ABSTRACT

Water quality is important for good chick performance. Nitrite is a water pollutant that may decrease the performance of young broiler chicks. Two-hundred and forty Arbor Acres x Peterson feather-sexed chicks were divided into 10 treatment groups. Each treatment consisted of 3 replicate pens of 4 male and 4 female chicks. The experiment was carried out from day-old to 3 weeks of age. Drinking water contained 0, 50, 200, or 1000 ppm of nitrite from sodium, potassium, and calcium nitrites. The broilers were housed in Petersime battery cages with raised wire floors in an enclosed room. The chicks had free access to feed and water. Weekly individual body weights, and feed and water consumptions on a per pen basis were recorded. At the end of the experiment, 1 chick of each sex from each pen was selected, and blood samples, and liver, breast and thigh tissue samples were taken for further analysis. Livers were examined for tissue damage at the cellular level.

No interactions were observed between the salts and the level, so data were analyzed for the differences between the salts and the levels of nitrites within each of the salts. No differences ($P > .05$) in body weights between the three NO_2 salts were observed.

The levels of nitrite of each nitrite salt were significant for chicks consuming NO_2 from of calcium nitrite ($Ca(NO_2)_2$). Chicks ingesting $Ca(NO_2)_2$ at 1000 ppm had lower ($P < .05$) second and third wk body weights compared to chicks receiving $Ca(NO_2)_2$ at 0, 50, or 200 ppm. Feed utilization during the third wk was less efficient ($P < .05$) among birds receiving nitrite in any of the three salts in comparison to the control.

Weekly water consumption was lower ($P < .05$) among chicks consuming the three nitrite salts compared to chicks consuming none of the nitrite salts. Analysis of blood parameters showed chicks consuming $Ca(NO_2)_2$ had lower ($P < .05$) levels of plasma glucose than chicks consuming no nitrite water. Chicks ingesting $NaNO_2$ had lower levels of plasma protein than the chicks consuming no nitrite water. Uric acid levels in the birds consuming no nitrite from the drinking water were higher ($P < .05$) than in those receiving nitrite from the three salts.

Significant differences were observed only in birds consuming the 50, 200, and 1000 ppm NO_2 from $CaNO_2$, and not among potassium or sodium nitrites. Chicks consuming $Ca(NO_2)_2$ at 50, 200, or 1000 ppm had lower ($P < .05$) plasma levels of glucose and uric acid compared to chicks consuming no nitrite water.

Histological examinations of the liver samples at the cellular level showed no abnormalities among the nitrite levels and nitrite salts.

Under the conditions of this experiment, calcium nitrite affected chick performance by lowering body weights and negatively influencing blood parameters.

INTRODUCTION

Nitrite is naturally found in minute quantities in vegetation and water. The levels of nitrite in drinking water rarely exceeds 1 mg/L (USDA, 1994). Even in small doses, nitrite can be deadly if ingested by human infants. Nitrite can bind to hemoglobin, causing it to be converted to methemoglobin. When this occurs, oxygen can no longer bind to red blood cells, and anoxia and death may follow if left untreated (World Health Organization, 1978). Other effects of nitrite ingestion include a decrease in growth and liver vitamin A levels in chicks (Sell and Roberts 1963), when Single Comb White Leghorn cockerels were fed 0.4% dietary KNO_2 from day-old to four wk of age. To further understand the physiological effects of nitrite in drinking water of broiler chicks, this experiment was conducted to determine the effects of nitrites from sodium, potassium, and calcium nitrites on the performance of broilers.

MATERIALS AND METHODS

Two-hundred and forty Arbor Acres x Peterson broiler chicks were housed in Petersime battery cages with raised wire floors in an enclosed room from day-old to three wk of age. Chicks were studied for the three wk period rather than the seven wk production life-time because younger chicks would be more susceptible to nitrite effects. Lighting was continuous throughout the experiment. Broiler chicks were feather-sexed at day-of-age and assigned to ten treatment groups. Four male and four female chicks were randomly assigned to each pen, and each treatment was replicated with three pens. The control group received tap water containing no supplemental nitrite. The remaining groups received nitrite in the drinking water at levels of 50, 200, or 1000 ppm from sodium nitrite ($NaNO_2$), calcium nitrite ($Ca(NO_2)_2$), and potassium nitrite (KNO_2).

The water containing nitrite was mixed in the laboratory prior to placement in the water troughs. The granular forms of the chemicals were weighed and then added to a 2-L volumetric flask, which was filled to the meniscus with tap water, shaken thoroughly, and then poured in a 19 L plastic jug for each level of nitrite.

Weekly individual body weights and feed and water consumptions on a per pen basis were recorded. Measured amounts of water were added to the troughs daily. Losses of water volume by evaporation from the open troughs were accounted for by choosing pens with no chicks and recording the weekly amount of water losses. These water loss values were subtracted from the water volume of the other troughs.

At the end of the experiment, one male and one female chick from each pen were selected for blood and liver and tissue samples. Six birds were sampled for each treatment level and weighed individual prior to sacrifice. Two blood samples were drawn from the wing vein of each bird with a 22 gage needle, and one was placed in Vacutainer®[3] tubes with lithium heparin coating, and the other in Vacutainer® tubes coated with EDTA. Blood samples were observed for a dark brown appearance which is an indication of methemoglobinemia. Livers were excised from each chick and weighed. Cross sections were taken of each bird's liver, and placed immediately into a 10% formalin solution. The samples were prepared by a commercial laboratory using H and E stain (Armed Forces Institute of Pathology 1960) for examination of liver pathology. Blood samples were analyzed by a commercial laboratory. Analyses for electrolytes were performed using an ion specific electrode[4]. Slides were prepared to determine the counts of white blood cells, heterophils and lymphocytes. Protein, uric acid, glucose, albumin and aspartate amino transferase values were measured with a Ciba-Corning 550 Express Random Access Chemistry Analyzer[5].

The experiment was a 3 X 4 factorial design. The individual pen was the experimental unit. Statistical analyses were performed on SAS® 6.11 (General Linear Models) (SAS, 1995). A two-way ANOVA was performed on the data with the NO_2 salts and NO_2 levels serving as the main effects. Significant differences of the data were separated by LSD (Steele and Torrie, 1980) with a probability level of P=.05.

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[3] Becton Dickinson Rutherford, New Jersey 07070 USA

[4] Ciba-Corning Diagnostic Corp. Oberlin, Ohio, 44074 USA

RESULTS AND DISCUSSION

No salt x level interactions were observed; therefore, salt and level were analyzed separately (Table 4.1). No differences in mean body weights were observed between the three nitrites and the control at wk one and three (Table 4.2); however, mean body weight was lower ($P<.05$) for the chicks ingesting the $NaNO_2$ than chicks consuming no nitrite in the drinking water at wk two.

Feed conversion ratios were better ($P<.05$) among chicks consuming the three nitrites sources than the chicks consuming no nitrite at wk three (Table 4.2). No differences were observed among the NO_2 sources and the no nitrite control at wk one and two.

Chicks ingesting $Ca(NO_2)_2$, KNO_2 and $NaNO_2$ at 1000 ppm had lower ($P<.05$) 2 wk body weights compared to those receiving 0, 50, or 200 ppm of the same salt (Table 4.3). No differences in mean body weights were observed at 0, 50, 200, or 1000 ppm at wk one or three.

Water consumption by the control chicks was greater ($P<.05$) during wk one than chicks consuming the three NO_2 salts; however, during wk three, only chicks consuming NO_2 from $NaNO_2$ had a lower ($P<.05$) water consumption compared to the no nitrite control (Table 4.4). This suggests that while chicks adjusted to the tastes of calcium and potassium salts, they may not do so for sodium.

No gross observations of blood samples for methemoglobinemia were noted. Normally, blood taken from an animal with methemoglobinemia will appear dark brown in appearance (Osweiler *et al.*, 1985).

Table 4.1. F-values of statistical analyses

Source of Variation	df	F value
Salts	3	2.11
Level	2	0.70
Salt X Level	4	0.50

Table 4.2. Effects of nitrite (NO_2) from sodium, calcium, and potassium NO_2 on weekly mean body weight and feed conversion from day-old to three weeks of age

	Wks of age					
NO_2 Salts	1		2		3	
	Body weight (g)	Feed conversion ratio	Body weight (g)	Feed conversion ratio	Body weight (g)	Feed conversion ratio
Control	$149^{a} \pm 3.7$	$1.37^{a} \pm 0.01$	$393^{a} \pm 11.6$	$1.35^{a} \pm 0.01$	$692^{a} \pm 23.2$	$2.08^{a} \pm 0.01$
Sodium	$143^{a} \pm 2.1$	$1.35^{a} \pm 0.01$	$361^{b} \pm 6.7$	$1.38^{a} \pm 0.01$	$686^{a} \pm 13.4$	$1.60^{b} \pm 0.01$
Calcium	$146^{a} \pm 2.1$	$1.33^{a} \pm 0.01$	$373^{ab} \pm 6.7$	$1.42^{a} \pm 0.01$	$711^{a} \pm 13.4$	$1.65^{b} \pm 0.01$
Potassium	$145^{a} \pm 2.1$	$2.31^{a} \pm 0.01$	$369^{ab} \pm 6.7$	$1.38^{a} \pm 0.01$	$689^{a} \pm 13.4$	$1.61^{b} \pm 0.01$

a/b indicates significant difference ($P<.05$) for each column

Table 4.3. Effect of 0, 50, 200, and 1000 ppm nitrite (NO_2) from sodium (Na), potassium (K) and calcium (Ca) nitrite on second week body weights of broilers

	Body Weight (g)		
NO_2 Levels	$NaNO_2$	KNO_2	$Ca(NO_2)_2$
0	393[a]	393[a]	393[a]
50	377[a]	382[ab]	397[a]
200	376[a]	379[ab]	384[a]
1000	330[b]	346[b]	337[b]
SEM	10	12	8

a/b indicates significant difference (P<.05) for each column

Table 4.4. Influence of nitrite (NO_2) from sodium, calcium, and potassium nitrite on water consumption in broilers from day-old to three weeks of age

	Weekly Water Consumption (ml)		
NO_2 Salts	1	2	3
Control	$2793^{a} \pm 59$	$3867^{a} \pm 349$	$8112^{a} \pm 175$
Sodium	$2514^{b} \pm 34$	$3380^{a} \pm 201$	$7559^{b} \pm 74$
Calcium	$2277^{c} \pm 34$	$3452^{a} \pm 201$	$7915^{ab} \pm 78$
Potassium	$2266^{c} \pm 34$	$2972^{a} \pm 201$	$7990^{ab} \pm 79$

a/b indicates significant difference ($P < .05$) for each column

Chicks consuming $Ca(NO_2)_2$ had lower ($P < .05$) concentrations of plasma glucose than the no nitrite control, but was not different from the $NaNO_2$ or KNO_2 (Table 4.5). The decrease in plasma glucose may be due to a protein-glucose interaction. Since protein is made of nitrogen, a high level of NO_2 consumption may cause the body to react as though there is an overabundance of protein. When an excess of protein occurs, the body must work to clear it from the body, using glucose to do this work. Therefore, the decrease in glucose levels in chicks consuming $Ca(NO_2)_2$ may be caused by the increased need of glucose derived energy for clearance of nitrogen from the blood. This decrease in plasma glucose may explain the lower body weights and reduced feed conversions of chicks consuming nitrite.

Plasma protein levels were less concentrated ($P < .05$) in chicks receiving $NaNO_2$ in comparison to the chicks consuming no nitrite (Table 4.5). Uric acid levels were higher ($P < .05$) in the group receiving no_2 water when compared to those who received NO_2 from any of the three salts (Table 4.5). The normal value for blood uric acid levels is from 2 to 15 mg/dl, with values over 20 mg/dl considered elevated (Coles, 1986). Elevated levels of uric acid in the blood are associated with renal damage. However, none of the uric acid values obtained from the chicks consuming NO_2 from $NaNO_2$, $Ca(NO_2)_2$, or KNO_2 in the drinking water were different from each other.

Analyses between the levels of nitrite showed significant differences in plasma uric acid and glucose only amongst those birds ingesting NO_2 from $Ca(NO_2)_2$.

Table 4.5. Effects of nitrite (NO_2)from sodium, calcium, and potassium salts on plasma levels of glucose, protein and uric acid in broilers consuming nitrite in the drinking water from day-old to three weeks of age

NO_2 Salts	Glucose (mg/dl)	Protein (g/dl)	Uric Acid (mg/dl)
Control	$302^{a} \pm 9.8$	$3.52^{a} \pm 0.14$	$10.3^{a} \pm 0.9$
Sodium	$289^{ab} \pm 5.7$	$3.18^{b} \pm 0.08$	$7.3^{b} \pm 0.5$
Calcium	$281^{b} \pm 5.7$	$3.25^{ab} \pm 0.08$	$6.9^{b} \pm 0.5$
Potassium	$295^{ab} \pm 5.7$	$3.26^{ab} \pm 0.08$	$7.5^{b} \pm 0.5$

a/b indicates significant difference ($P<.05$) for each column

Chicks that consumed $Ca(NO_2)_2$ at 50, 200, or 1000 ppm had lower ($P<.05$) concentrations of blood glucose and uric acid when compared to the no nitrite control (Table 4.6).

Histological examinations of the liver tissues showed no cellular abnormalities, due to either the NO_2 salts or the levels of each of the NO_2 sources.

Under the conditions of this experiment, 1000 ppm of nitrite from the three nitrite salts affected body weight and the three nitrite salts lowered blood uric acid levels. All levels of $Ca(NO_2)_2$ affect blood uric acid and glucose levels.

Table 4.6. Effect of different levels of nitrite from calcium nitrite on plasma uric acid and glucose levels in broilers from day-old to three weeks of age

Level (ppm)	Uric Acid (mg/dl)	Glucose (mg/dl)
0	10.3[a]	302[a]
50	6.6[b]	283[b]
200	7.2[b]	283[b]
1000	7.1[b]	278[b]
SEM	0.77	35

a/b indicates significant difference (P<.05) for each column

CHAPTER 5

EFFECT OF NITRATE AND NITRITE FROM A MIXTURE OF CALCIUM, SODIUM, AND POTASSIUM SALTS IN DRINKING WATER OF BROILERS

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ABSTRACT

Nitrates and nitrites occur naturally in water supplies and their content may be increased by improper application of nitrogen fertilizer or animal wastes on farmlands. Nitrates and nitrites may occur in the environment as calcium, potassium, and sodium salts. This experiment was designed to determine the effect of providing 0, 50, or 1000 ppm of either nitrate (NO_3) or nitrite (NO_2) from a mixture of calcium, potassium, and sodium salts. One-hundred and sixty-eight Arbor Acres x Peterson feather-sexed chicks were divided into seven treatment groups. The control group consumed water with no supplemental NO_3 or NO_2. The remaining groups consisted of 50 ppm and 1000 ppm of NO_3 from equal parts of NO_3 from $Ca(NO_3)_2$, $NaNO_3$, and KNO_3 for each level; 50 ppm and 1000 ppm NO_2 from of $NaNO_2$, KNO_2, and $Ca(NO_2)_2$ for each level; 25 ppm NO_3 and 25 ppm NO_2 or 500 ppm NO_3 and 500 ppm NO_2 with equal parts from $Ca(NO_3)_2$, $NaNO_3$, KNO_3; $Ca(NO_2)_2$, $NaNO_2$, and KNO_2, respectively, for each level.

Each treatment consisted of three replicate pens of four males and four females. The experiment was conducted in Petersime battery cages with raised wire floors in an enclosed room. The chicks had free access to food and water. Lighting was provided 24 h/day throughout the experiment. Weekly individual body weights and feed and water consumptions on a per pen basis were recorded.

Body weights among chicks consuming water containing both NO_3 and NO_2 were lower ($P<.05$) than for the control. No differences were observed between the levels of NO_3 and NO_2 for body weights.

Feed conversion ratios during the second week were less efficient ($P<.05$) among chicks consuming either the mixed salts of nitrate or the mixture of nitrite salts in comparison to the unsupplemented control. Broilers consuming either 1000 ppm NO_3 from equal parts of $Ca(NO_3)_2$, $NaNO_3$, and KNO_3 or 1000 ppm NO_2 from equal parts $NaNO_2$, KNO_2, and $Ca(NO_2)_2$ or the combination treatment of NO_3-NO_2 at 1000 ppm had less efficient ($P<.05$) feed conversion ratios than the unsupplemented control water. Water consumption was higher ($P<.05$) in chicks consuming additional amounts of nitrate or nitrite than the control.

Under the conditions of this experiment, a mixture of NO_3 and NO_2 from sodium, calcium, and potassium salts seem to affect broiler chicks more severely.

INTRODUCTION

Water quality is of concern to poultry producers because poor water quality has been linked to inferior production performance. Nitrates and nitrites are two contaminants of the water that may lead to decreased production. Sell and Roberts (1963) fed potassium nitrite to Single Comb White Leghorn cockerels and found decreased growth and decreased liver stores of Vitamin A. Barton *et al.*, (1986) surveyed water quality on Arkansas broiler farms and ran a series of correlations between performance and water quality. A positive correlation was found between nitrate and carcass condemnation, and negative correlations with growth, body weight, and livability. Nitrates and nitrites in drinking water can be derived from calcium, potassium, or sodium salts. Nitrates are found in much greater concentration in water supplies than nitrite, which is found in very small quantities, if at all. Since water contains nitrate and nitrite in mixed forms, this experiment was conducted to determine the effects on body weight, feed conversion, and water consumption of nitrate and nitrite in a form mimicking what would naturally occur in a drinking water source.

MATERIALS AND METHODS

One-hundred and sixty-eight Arbor Acres x Peterson broiler chicks were housed in an enclosed room in Petersime battery cages with raised wire floors from day-old to three wk of age. The chicks were feather sexed at day-of-age. Chicks were studied for three wks instead of their seven wk production life-time because younger chicks would be more susceptible to the effects of nitrates and nitrites. Four males and four females were randomly assigned to a pen, and each treatment was replicated by 3 pens. The chicks were given free access to food and water. Light was provided for 24 h/day throughout the experiment. The control group received tap water containing no additional nitrate (NO_3) or nitrite (NO_2). The remaining groups received nitrate or nitrite from mixtures of calcium (Ca), sodium (Na), and potassium (K) salts at levels of 50 or 1000 ppm (Table 5.1). The first treatment consisted 16.7 or 333.3 ppm $NaNO_3$, 16.7 or 333.3 ppm KNO_3, and 16.6 or 333.4 ppm $Ca(NO_3)_2$. The second consisted of 16.7 or 333.3 ppm $NaNO_2$, 16.7 or 333.3 ppm KNO_2, and 16.6 or 333.4 ppm $Ca(NO_2)_2$. The third was comprised of 8.35 or 166.7 ppm $NaNO_3$, 8.35 or 166.7ppm KNO_3, 8.35 or 166.6 ppm $Ca(NO_3)_2$, 8.35 or 166.7 ppm $NaNO_2$, 8.35 or 166.7 ppm KNO_2, and 8.35 or 166.7 ppm $Ca(NO_2)_2$ (Table 5.1).

The water containing nitrate and nitrite was mixed in the laboratory prior to placement in the water troughs. The granular forms of each of the chemicals were weighed, transferred to a 2-L volumetric flask, and filled with tap water to the meniscus.

Each flask was shaken thoroughly, and the mixed solution was poured into a 19 L plastic jug assigned to each level of nitrate and nitrite.

Weekly individual chicks weights and feed and water consumptions were recorded for each pen. Daily measured amounts of water were added to the troughs. Losses of water volume by evaporation in water troughs of similar surface area were measured each week by having an open trough with a known amount of water, and no birds in the pen. The water losses were used to correct water consumption of the individual pens.

Statistical analysis was performed on SAS® 6.11 (General Linear Models) (SAS, 1995). The individual pen was considered the experimental unit. A two-way ANOVA was performed on the data with the NO_3 and NO_2 salts and NO_3 and NO_2 levels serving as the main effects. Significant differences of the data were separated by LSD (Steele and Torrie, 1980) with a probability level of P=.05.

Table 5.1. Description of experimental treatments: Mixtures of nitrate (NO_3) and nitrite (NO_2) from sodium (Na), potassium (K), and calcium (Ca) salts

Trt No.	Total NO_3	Total NO_2	Nitrate (ppm) from:			Nitrite (ppm) from:		
	(ppm)	(ppm)	Calcium	Sodium	Potassium	Calcium	Potassium	Sodium
1	0	0	0	0	0	0	0	0
2	50	0	16.7	16.6	16.7	0	0	0
3	1000	0	333.3	333.3	333.4	0	0	0
4	0	50	0	0	0	16.6	16.7	16.7
5	0	1000	0	0	0	333.3	333.4	333.3
6	25	25	8.35	8.35	8.35	8.35	8.35	8.35
7	500	500	166.7	166.7	166.6	166.7	166.7	166.6

RESULTS AND DISCUSSION

No salts x levels interactions were observed; therefore, salts and levels were analyzed separately. No differences were observed between the treatments for body weights during the first and third wk (Table 5.2). Second wk body weights were lower ($P<.05$) among chicks consuming the combined nitrate-nitrite water compared to chicks consuming either NO_3, NO_2, or the control (Table 5.2). In our earlier studies calcium nitrate and calcium nitrite reduced mean body weights. Feed conversion was less efficient ($P<.05$) for the NO_3-NO_2 treatment than the control during the second week (Table 5.2).

No differences in mean body weights were observed between the levels of 0, 50, and 1000 ppm mixtures of NO_3 and NO_2 salts and the NO_3-NO_2 (Table 5.3). Feed was less efficiently utilized by broilers consuming 1000 ppm of either the mixture of the three salts of NO_2, the three NO_3 salts, or the mixture of the three NO_3 and NO_2 salts compared to chicks consuming these same salts at 0 or 50 ppm (Table 5.4).

Water consumption during the third wk was higher ($P<.05$) among chicks consuming NO_3 than among those drinking water with no additional nitrate or nitrite (Table 5.5).

Data from this experiment shows that nitrate and nitrite when combined in drinking water exert a greater effect on broiler performance. However, the effects were only observed during the second wk of production, and did not continue into the third wk. Mixtures of nitrate-nitrite in drinking water may require a longer adjustment period than for nitrate or nitrite consumed separately. In our studies calcium salts of nitrate and nitrite were observed to have a greater effect on chick perfomance. In this study when calcium salts of NO_3 and NO_2 are mixed with potassium and sodium salts, the same effect on bird performance was observed. Under the conditions of this experiment, NO_3-NO_2 mix had a greater effect on broiler chicks by lowering body weights and decreasing feed utilization. The effects did not persist beyond wk two, and feed conversion was compromised only among chicks consuming 1000 ppm of the mix of NO_3 salts or NO_2 salts. Since drinking water supplies contain nitrates and nitrites in mixed forms, and rarely exceed the recommended maximum safe level of 10 mg/L nitrogen-nitrate, nitrates and nitrites should not be a major cause of concern for decreasing broiler production.

Table 5.2. Effects of mixtures of equal parts nitrate salts or equal parts nitrite salts or equal parts of nitrate and nitrite salts in drinking water of broilers on weekly body weight and feed conversion

Salts	Week 1		Week 2		Week 3	
	Body weight (g)	Feed conversion ratio	Body weight (g)	Feed conversion ratio	Body weight (g)	Feed conversion ratio
Control	$152^{a} \pm 4$	$0.94^{a} \pm 0.03$	$389^{a} \pm 9$	$0.98^{a} \pm 0.01$	$678^{a} \pm 21$	$1.83^{a} \pm 0.1$
NO_3 mixture	$147^{a} \pm 3$	$0.91^{a} \pm 0.03$	$375^{a} \pm 7$	$0.95^{bc} \pm 0.01$	$660^{a} \pm 15$	$2.00^{a} \pm 0.1$
NO_2 mixture	$152^{a} \pm 3$	$0.94^{a} \pm 0.03$	$367^{a} \pm 7$	$0.93^{c} \pm 0.01$	$653^{a} \pm 15$	$1.77^{a} \pm 0.1$
NO_3-NO_2 mixture	$152^{a} \pm 3$	$0.94^{a} \pm 0.03$	$366^{b} \pm 7$	$0.96^{ab} \pm 0.01$	$651^{a} \pm 15$	$1.84^{a} \pm 0.1$

a/b indicates significant difference ($P<.05$) for each column

Table 5.3. Effect of 0, 50, and 1000 ppm of nitrate, nitrite, and nitrate-nitrite from mixed salts of calcium, sodium, and potassium on third wk body weights of broilers

Levels	Week 3 Body Weights (g)		
(ppm)	Nitrate Mixture	Nitrite Mixture	Nitrate + Nitrite Mixture
0	678^{a}	678^{a}	678^{a}
50	671^{a}	661^{a}	670^{a}
1000	650^{a}	646^{a}	632^{a}
SEM	18	17	23

a/b indicates significant difference ($P<.05$) for each column

Table 5.4. Effect on second week feed conversion by nitrate, nitrite and nitrate-nitrite at 0, 50, and 1000 ppm in drinking water of broilers

Levels	Second Week Feed Conversion (kg/kg)		
(ppm)	Nitrate	Nitrite	Nitrate-Nitrite
0	0.98^{a}	0.98^{a}	0.97^{a}
50	0.95^{ab}	0.94^{ab}	0.98^{a}
1000	0.94^{b}	0.91^{b}	0.93^{b}
SEM	0.08	0.01	0.03

a/b indicates significant difference ($P<.05$) for each column

Table 5.5. Effects of nitrate, nitrite and nitrate-nitrite salts on water consumption in broilers from day-old to three weeks of age

Treatments	Week 1	Week 2	Week 3
Control	$2916^{a} \pm 102$	$4958^{a} \pm 265$	$7667^{b} \pm 259$
NO_3 mixture	$2766^{a} \pm 51$	$5389^{a} \pm 132$	$8633^{a} \pm 130$
NO_2 mixture	$2675^{a} \pm 51$	$5150^{a} \pm 132$	$8425^{ab} \pm 130$
NO_3-NO_2 mixture	$2765^{a} \pm 51$	$5079^{a} \pm 132$	$8233^{ab} \pm 130$

a/b indicates significant difference ($P<.05$) for each column

CHAPTER 6

CONCLUSIONS

The recommended safe levels of nitrate and nitrite in drinking water were established due to cases of infant methemoglobinemia (Blue Baby Disease) in the earlier part of this century (World Health Organization, 1978). It was known these cases did not occur in areas where the nitrate content of the water supply was below 45 ppm NO_3. However, most occurred with water containing nitrate from 70 to 75 ppm. Therefore, the level set as being safe for human consumption was 45 ppm NO_3. Methemoglobinemia is most often seen in infants under three months of age because they have less acidic stomachs which are prime for growth of bacteria that can convert nitrate to nitrite. Infants are also deficient in methemoglobin reductase or its co-factor reduced nicotinamide adenine dinucleotide , which are necessary to maintain iron in its reduced state (World Health Organization 1978). It has ben assumed that young monogastric animals, although more physically advanced than a young human infant would suffer the same effects if nitrate and nitrite were present in the water. However, data from the four experiments conducted indicate that with levels as high as 2033 ppm NO_3 and 1000 ppm NO_2, methemoglobinemia does not occur in broiler chicks given nitrate or nitrite in the drinking water from day-old to three wk of age. Analyses of blood parameters showed pack cell volumes in the normal range for all chicks consuming nitrate or nitrite at 0, 50, 200, or 1000 ppm. The lack of occurrence of methemoglobinemia in young broiler chicks may be due to a greater acidity of the stomach, to the presence of methemoglobin reductase at an earlier age than in human infants, or to other factors yet to be determined.

Increased activity levels of aspartate amino transferase in chicks consuming the calcium salts of nitrate or nitrite indicated that liver damage may be occurring. Visual evidence of liver damage was observed among chicks consuming sodium nitrate at 2033 ppm. Although no direct link between nitrate and liver damage has been established, it is known that nitrites under certain acidic conditions can be converted to nitrosamines. Nitrosamines have been linked to liver damage (National Academy of Sciences, 1972). Additionally, it has been observed that there are enhancers and inhibitors of carcinogenicity. Agents that promote cell proliferation in the liver are enhancers of carcinogenicity (National Academy Press, 1981). Therefore, the proliferation of cells surrounding the liver venule in chicks consuming 2033 ppm of $NaNO_3$ may be an early indicator of liver cancer.

Calcium salts of nitrate and nitrite had a negative effect on body weights and blood parameters of chicks. An excess of calcium can interfere with the bioavailability of other minerals such as zinc, phosphorus, and magnesium (National Research Council, 1994). Zinc is a vital cofactor in many enzymes. Many of these enzymes are part of energy deriving cycles such as glycolysis, gluconeogenesis, and the citric acid cycle. If the availability of zinc is decreased, it may slow down that rate of energy production, which may account for the decreased growth and feed utilization among chicks consuming the calcium salts of nitrate and nitrite (NRC, 1989). Calcium has an antagonistic relationship to magnesium when in excess because they have shared channels for transport (NRC, 1989). Magnesium is required for the activation of over 300 enzymes. Interference with the bioavailability of magnesium may decrease energy production, and therefore, cause the reduction in body weights observed with chicks consuming nitrate and nitrite from calcium salts.

In conclusion, the data from the four experiments indicate that 2033 ppm NO_3 from $NaNO_3$ affect bird performance and cause liver pathology. Calcium nitrate had a greater effect that sodium or potassium nitrates at 1000 ppm. All three nitrite salts at 1000 ppm affected performance and $Ca(NO_2)_2$ initiated liver pathology. Mixtures of NO_3-NO_2 from calcium, sodium, and potassium salts had greater effect on performance of broilers than either the mixtures of NO_3 or NO_2.

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APPENDICES

APPENDICES

The following data provide more information regarding the studies described in the previous pages. The data represented in Table A.1 and A.2 are from Chapter 3. Tables A.5 and A.6 are from Chapter 4.

Table A.1. Effects of sodium, calcium, and potassium nitrates in drinking water on blood chemistries of broilers (Expt. 2)

NO_3 Salts	PCV[1] (%)	WBC[2] (n/ul)	Hetero[3] (n/ul)	Lymph[4] (n/ul)	Glucose (mg/dl)	Tot. Pro[5] (g/dl)
Control	$31.3^{a} \pm 0.9$	$36{,}250^{a} \pm 8102$	$10{,}725^{a} \pm 3892$	$25{,}525^{a} \pm 4880$	$251^{a} \pm 18$	$3.00^{a} \pm 0.12$
Sodium	$32.2^{a} \pm 0.6$	$45{,}188^{a} \pm 5053$	$14{,}708^{a} \pm 2427$	$30{,}400^{a} \pm 3043$	$269^{a} \pm 10$	$2.96^{a} \pm 0.07$
Calcium	$31.2^{a} \pm 0.6$	$43{,}833^{a} \pm 4678$	$14{,}815^{a} \pm 2247$	$28{,}510^{a} \pm 2817$	$243^{a} \pm 10$	$2.87^{a} \pm 0.07$
Potassium	$30.7^{a} \pm 0.6$	$44{,}167^{a} \pm 4678$	$18{,}135^{a} \pm 2247$	$25{,}911^{a} \pm 2817$	$261^{a} \pm 10$	$2.94^{a} \pm 0.07$

a/b indicates significant difference ($P < .05$) for each column

[1] packed cell volume

[2] white blood cells

[3] heterophils

[4] lymphocytes

[5] total protein

Table A.2. Effects of sodium, calcium, and potassium nitrates in drinking water on plasma levels of phosphorus, protein, uric acid, calcium, and albumin (Expt. 2)

NO_3 Salts	Calcium (mg/dl)	Phosphorus (mg/dl)	Protein (g/dl)	Uric Acid (mg/dl)	Albumin (g/dl)	SGOT[1] (IU/L)	Potassium (Meq/L)
Control	12.0[a] ± 0.4	7.1[a] ± 0.5	3.06[a] ± 0.09	5.8[a] ± 0.6	1.30[a] ± 5.35	165[a] ±64	4.75[a] ± 0.29
Sodium	11.6[a] ± 0.2	7.1[a] ± 0.3	3.16[a] ± 0.06	6.3[a] ± 0.4	1.32[a] ± 3.09	180[a] ±37	5.17[a] ± 0.17
Calcium	11.7[a] ± 0.2	6.9[a] ± 0.3	2.98[a] ± 0.06	5.8[a] ± 0.4	1.23[a] ± 3.09	186[a] ±37	4.84[a] ± 0.17
Potassium	11.7[a] ± 0.2	6.8[a] ± 0.3	3.08[a] ± 0.06	5.9[a] ± 0.4	6.95[a] ± 3.09	241[a] ±37	4.64[a] ± 0.17

a/b indicates significant difference ($P < .05$) for each column

[1] aspartate amino transferase

Table A.3. Effects of nitrite from sodium, calcium, and potassium salts on blood chemistries of broilers from day-old to three weeks of age (Expt. 3)

NO_2 Salts	PCV[1] (%)	WBC[2] (n/ul)	Hetero[3] (n/ul)	Lymph[4] (n/ul)	Glucose (mg/dl)	Tot. Pro[5] (g/dl)	Albumin (g/dl)
Control	$26.2^{a} \pm 1.2$	$8{,}000^{a} \pm 1820$	$8{,}715^{a} \pm 1470$	$9{,}285^{a} \pm 1405$	$302^{a} \pm 9.8$	$3.67^{a} \pm 0.17$	$1.07^{a} \pm 0.08$
Sodium	$26.5^{a} \pm 0.7$	$20{,}667^{a} \pm 1050$	$8{,}802^{a} \pm 849$	$11{,}065^{a} \pm 811$	$289^{ab} \pm 5.7$	$3.53^{a} \pm 0.09$	$1.06^{a} \pm 0.05$
Calcium	$26.5^{a} \pm 0.7$	$20{,}500^{a} \pm 1050$	$10{,}314^{a} \pm 849$	$10{,}118^{a} \pm 811$	$281^{b} \pm 5.7$	$3.58^{a} \pm 0.09$	$1.08^{a} \pm 0.05$
Potassium	$26.1^{a} \pm 0.7$	$18{,}000^{a} \pm 1050$	$7{,}782^{a} \pm 849$	$10{,}176^{a} \pm 811$	$295^{ab} \pm 5.7$	$3.47^{a} \pm 0.09$	$1.05^{a} \pm 0.05$

a/b indicates significant difference ($P < .05$)
[1] packed cell volume
[2] white blood cells
[3] heterophils
[4] lymphocytes
[5] total protein

Table A.4. Effects of nitrite from sodium, calcium, and potassium salts on plasma levels of calcium, phosphorus, protein, uric acid, potassium, and SGOT in broilers at three wk of age (Expt. 3)

NO_2 Salts	SGOT[1] (IU/L)	Potassium (Meq/L)	Calcium (mg/dl)	Phosphorus (mg/dl)	Protein (g/dl)	Uric Acid (mg/dl)
Control	$178^{a} \pm 11$	$7.03^{a} \pm 0.29$	$10.4^{a} \pm 0.6$	$8.3^{a} \pm 0.4$	$3.52^{a} \pm 0.14$	$10.3^{a} \pm 0.9$
Sodium	$169^{a} \pm 7$	$6.67^{a} \pm 0.17$	$9.9^{a} \pm 0.3$	$7.9^{a} \pm 0.2$	$3.18^{b} \pm 0.08$	$1.3^{b} \pm 0.5$
Calcium	$171^{a} \pm 7$	$6.90^{a} \pm 0.17$	$10.1^{a} \pm 0.3$	$8.0^{a} \pm 0.2$	$3.25^{ab} \pm 0.08$	$6.9^{b} \pm 0.5$
Potassium	$170^{a} \pm 7$	$7.06^{a} \pm 0.17$	$9.6^{a} \pm 0.3$	$7.8^{a} \pm 0.2$	$3.26^{ab} \pm 0.08$	$7.5^{b} \pm 0.5$

a/b indicates significant difference ($P < .05$) for each column

[1] aspartate amino transferase