Chapter 3

The effect of elevated levels of $NaNO_3$ in the drinking water of layers and broilers.

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Introduction

Nitrates in feedstuffs occur 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. Nitrate forms N-nitroso compounds, many of which are known animal carcinogens. Biochemical studies in humans have shown that nitrate in water combines with amino acids to form these compounds (Whitehead, 1956).

The presence of nitrates in the soil is largely as a result of natural biological processes associated with the decomposition of plant residues and organic matter. Nitrogen becomes a concern to water quality when nitrogen in the soil is converted to the nitrate (NO_3) form. It is a concern because nitrate is very mobile and easily moves with water in the soil. Its inclusion in groundwater is a cause for concern. However, nitrates can also enter surface waters such as ponds, streams and rivers. Nitrates also occur in rainwater, animal manure and nitrogen fertilizers. Whether or not nitrates actually enter groundwater depends on underlying soil and/or bedrock conditions, as well as the depth to groundwater. If depth to groundwater is shallow and the underlying soil is sandy, the potential for nitrates to enter groundwater is relatively high. However, if depth to groundwater is deep and the underlying soil is heavy clay, groundwater contamination from nitrates is not likely (Killpack and Buchholz, 1993).

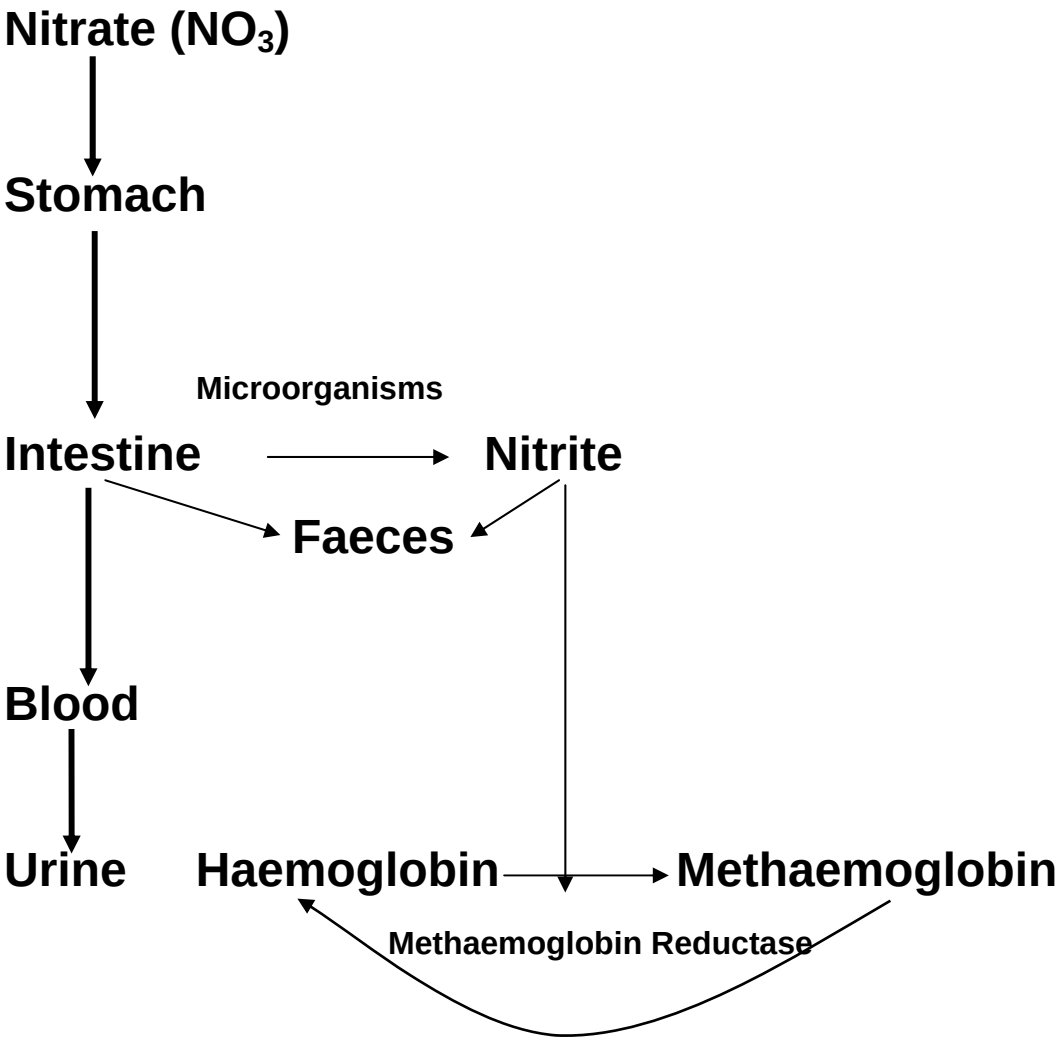
Nitrate is relatively nontoxic. The primary health hazard from drinking water with nitrate-nitrogen occurs when bacteria in the digestive system transform nitrate to nitrite. When it is reduced to nitrite its toxicity increases greatly.

Nitrite is readily absorbed into the bloodstream. (Mommers et al., 1997). The nitrite then oxidizes iron in the hemoglobin of red blood cells to form methemoglobin, which lacks the oxygen-carrying ability of hemoglobin. This creates the condition known as methemoglobinemia (sometimes referred to as "blue baby syndrome") (Skipton and Hay, 1998). Nitrite binds to this oxidized haem and is capable of oxidizing haem. The exact mechanism is not well defined. Normally, oxygenation of hemoglobin causes a partial transfer of one electron from the iron to the bound oxygen. Iron in this state resembles ferric iron (Fe3+) and oxygen resembles super oxide (O2-). Deoxygenation returns the electron to the iron, with the release of oxygen. Methemoglobin is formed when an electron is not returned. Methemoglobin is incapable of

binding oxygen, contrary to hemoglobin. This results in problems with oxygen transportation. The conversion of hemoglobin into methemoglobin occurs naturally, but the level is normally maintained below 2 % by methemoglobin-reducing enzymes. A clinical cyanosis will arise if the concentration of methemoglobin reaches 10 % of total hemoglobin amount (Mommers et al., 1997).

In monogastric animals such as swine and poultry, there is no fermentation vat similar to the rumen to aid in the digestion of roughage and to change nitrate to nitrite. In contrast monogastric animals convert nitrate to nitrite in the intestine, closer to the end of the digestive tract (Figure 3.1), where there is less opportunity for the nitrites to be absorbed by the blood. It is this difference in the site of conversion that makes nitrate poisoning a significantly smaller concern in monogastric animals (Yaremcio, 2000)

Figure 3.1: A simplified pathway for nitrates in swine and poultry. (Bergsrud & Linn, 1990)



It is difficult to determine the toxicity of nitrate in animals since it depends on the rate that the substance is ingested. A few hundred milligrams of nitrate may cause poisoning if ingested in a few hours. But, spread over a whole day, 1000 mg nitrate may cause no signs of toxicity. Common symptoms include abdominal pain, diarrhea, muscular weakness or poor coordination. Affected animals will have blood that is a chocolate- brown color. If the problem is diagnosed in time, they can fully recover with a treatment of methylene blue. Pregnant animals may abort within a few days. Nitrate also exists in animal feeds and fodder. Drought stressed forage plants commonly have high nitrate levels. These plants can have a cumulative effect when consumed with high levels of nitrate in the drinking water (Self and Waskom, 1992)

The toxicity of nitrates to poultry varies with the age of the birds, older birds being more tolerant. Levels in excess of 50 mg/l for chickens and 75 mg/l for turkeys have proven harmful in laboratory trials. Carter and Sneed, (1996) suspected that levels above 3mg/l were likely to affect egg production in layers and growth in broilers. Nitrites are toxic at much lower levels than nitrates; concentrations as low as 1 mg/l can be toxic.

The potential health hazard for poultry depends on the individual's reaction to nitrate-nitrogen and the total ingestion of nitrate-nitrogen and nitrates from all sources. The clinical signs of acute nitrate toxicity vary according to species. In general, ruminant animals develop methemoglobinemia while monogastric animals exhibit severe gastritis. Nitrate ingestion has also been linked to impairment of thyroid function, decreased feed consumption and interference with vitamin A and E metabolism.

Hematological changes seen with chronic high nitrate exposure include both compensatory increases in red blood cells and anemia, along with increased neutrophils and eosinophils. Unlike nitrate, nitrite is capable of inducing methemoglobinemia in a wide range of species, i.e. cattle, sheep, swine, dogs, guinea pigs, rats, chickens and turkeys (Bruning – Fann and Kaneene, 1993).

A potential cancer risk from nitrate (and nitrite) in water and food has been reported. A possibility exists that nitrate can react with amines or amides in the body to form nitrosamine, which is known to cause cancer. Nitrate must be converted to nitrite before nitrosamine can be formed. The magnitude of the cancer risk from nitrate in drinking water is not known (Jasa et al., 1998).

Consuming water from a source containing 10 or less mg/l nitrate-nitrogen provides assurance that methemoglobinemia should not result from drinking water.

Although nitrate occurs naturally in some groundwater, higher levels are thought to be the result of human activities in most cases.

Nitrate is easily dissolved in water, which means that it is difficult to remove. Three water treatment systems that remove nitrate are distillation, reverse osmosis and ion exchange. The distillation process boils water, then catches and condenses the steam while nitrate and other minerals remain in the boiling

tank. Reverse osmosis forces water under pressure through a membrane to filter out contaminants. Ion exchange introduces another substance, normally chloride, to "trade places" with nitrate in water (Jennings and Sneed, 1996).

Nitrate in drinking water is measured either in terms of the amount of nitrogen present or in terms of both nitrogen and oxygen. In 1962, the U.S. Public Health Service adopted drinking water standards and set the recommended limit for nitrate-nitrogen at 10 mg/l. This drinking water standard was established to protect the health of infants and was based on the best knowledge available at that time. The Environmental Protection Agency (EPA) has since adopted the 10 mg/l standard as the maximum contaminant level (MCL) for nitrate-nitrogen in public water systems. The South African standard for nitrate and nitrite levels in livestock drinking water has been set at 100 mg/l for nitrate and 10 mg/l for nitrite (DWAF, 1996).

Subsequent reviews of this standard have not resulted in any changes. However, it is difficult to establish an exact level at which nitrogen concentrations in water are safe or unsafe.

Adams *et al*. (1966) administered various levels of either sodium nitrate or nitrite continuously in the drinking water of day-old chicks or poults and laying hens. The stock was maintained in otherwise standard conditions and fed practical diets containing 9500, 14 000, and 9850 I.U. of vitamin A activity/kg, respectively. Up to 200 and 300 mg/l of nitrite and nitrate nitrogen, respectively, had very little effect on blood methemoglobin, mortality and feed and water consumption of chicks. A reduction in growth and liver vitamin A was observed with 200 mg/l nitrite nitrogen. Lloyd (1977), found that levels of up to 1867 mg/l of nitrate-nitrogen had such high mortality and morbidity levels, that the treatment was discontinued at the end of one week and the chicks were given nitrate free water. These chicks recovered quickly but remained lighter in weight than chicks on lower treatments. Chicks at all other levels appeared healthy. Growth retardation at the 466 and 933 mg/l level was significant but not severe. Water consumption increased with each addition of nitrate up to 933 mg/l in the drinking water.

Contradicting the above, Arendz (1967), found that sodium nitrate supplying 675 mg/l of nitrate in the drinking water of turkey poults during the first 4 weeks, caused increased weight gains at subsequent periods. Males were affected more than females. Sodium nitrate in the feed at 1000 mg/l nitrate for the first four weeks did not promote growth at subsequent ages. There was no effect from sodium nitrate in drinking water upon spleen, adrenal and thyroid sizes or thyroid activity as measured by I^{-131} trapping rate at either four or twenty-four weeks of age. No differences were found in haematocrit or blood glucose level at twenty-four weeks of age. A relatively large difference was noted in testes size at 24 weeks of age. The sodium nitrate treated birds' testes were about half the weight of the controls. These differences in testes size and body weights indicate that sodium nitrate may be upsetting normal gonadal hormone metabolism. A hypothesis is postulated that this apparent hormone imbalance may cause increased retention.

Marrett and Sunde (1968) reported that chicks up to five weeks old were more tolerant to nitrate and nitrite in the feed than poults. They also found that mortality and rate of respiration were increased and growth

reduced when high levels of nitrate and nitrite were fed in the presence of marginal levels of vitamin A.

Experimental evidence has been presented showing that dietary nitrate accelerates the depletion of vitamin A from body stores of ruminants (Hatfield *et al*., 1961) and that dietary nitrite or nitrate exerts similar effects on the rat (Smith *et al*., 1961). Roberts and Sell (1963) found that vitamin A is destroyed in the presence of nitrite in the ventriculus area of the digestive tract, where the pH is approximately 4 and that the nitrite depressed growth primarily by reducing feed consumption of chicks not receiving supplemental vitamin A.
Bloomfield and Welsh (1961) found it conceivable that the vitamin A deficiency that occurs when excess nitrate is found in the drinking water, is an indirect result of abnormal thyroid function induced by the nitrate.

Adams, (1974) reported that chickens and turkeys were tolerant to levels of nitrate commonly found in water (up to 1320 and 1485 mg/l nitrate for chickens and turkeys respectively). In commercial poultry production in the year 2001, nitrate inclusions as high as this will not be tolerated by the hen. Commercial meat- and egg-type chickens are exposed to high levels of environmental stress (stocking densities of up to 22 birds/m^2) and metabolic stresses. They need optimal nutritional conditions to achieve their genetic potential. Water Quality Guidelines for poultry in South Africa should therefore not be based on the amount of a constituent that a bird can tolerate, but rather on the maximum inclusion of the constituent without compromising production.

The aim of this study:
Firstly, to establish the effect of high levels of nitrate on the growth, physiology and production of layers (Experiment 1) and broilers (Experiment 2) and the alleviating effect of vitamin A on them.

Materials and Methods

Experiment 1: Layers

The same type of birds, water administration, housing and temperature control, vaccination, feeding regime and lighting schedule were used as discussed in Chapter 2.

Six levels of $NaNO_3$ (0, 25, 100, 150, 200, 300 mg/l) were administered to 720 Hy-line layers (Table 3.2). Each pen was stocked with 20 hens in a 6 X 3 factorial experiment (360 birds). These six treatments were repeated, with the repeat group receiving 8000 mg/l of additional vitamin A administered through the drinking water. Pretoria Municipal Water was used and the nitrates present in the water were taken into account when formulating the inclusion levels. The sodium content of the water was 4.1 mg/l.

Alberta Agriculture Food and Rural Department (1996) developed a guideline for nitrate inclusion levels in livestock watering (Table 3.1). This was used as a guideline for the inclusion levels in this experiment.

Table 3.1. A Guide to the use of waters containing nitrate for livestock

Nitrate content* (mg/l nitrate nitrogen)	Comments
Less than 100'*	Experimental evidence to date indicates that this water should not harm livestock or poultry.
100 to 300**	This water should not in itself harm poultry. When feeds contain nitrates, this water could add greatly to the nitrate intake to make it dangerous. This could be of some concern in the case of cattle or sheep when circumstances cause nitrates to accumulate in the plant; e.g., frost, hail, drought, and especially if the animals are given water containing levels of nitrates that approach the upper limits.
Over 300***	This water could cause typical nitrate poisoning in cattle and sheep, and its use for these animals is not recommended. Because this level of nitrate contributes significantly to salinity and also because experimental work with levels of nitrate nitrogen in excess of this are meager, the use of this water for swine, horses, or poultry should also be avoided.

* Includes nitrite nitrogen.
** Less than 443 mg/l of nitrate or less than 607 mg/l of $NaNO_3$
***Over 1329 mg/l of nitrate or over 1821 mg/l of $NaNO_3$

Table 3.2. Inclusion levels of nitrates:

Treatment Group	Nitrate inclusion level (mg/l)
1	0
2	25
3	100
4	150
5	200
6	300

Table 3.3. The vitamin A levels present in the feed:

Feed	Vitamin A level
Starter	12 000
Grower/Finisher	10 000
Layer	8 000

Water intake, feed intake, body weight, egg production and egg weight and temperature were measured weekly. Mortalities were recorded and post mortems conducted on them. The trial ended after 12 weeks. Eggshell thickness of a representative sample of eggs of each treatment was measured. A representative sample of hens from each treatment group was slaughtered at the end of the trial period according to The Slaughter of Poultry (Humane Conditions) Regulations (Amendment) 1990. Kidney, liver, spleen and pancreatic samples were examined histopathologically and the nitrite content of the colon and caecum contents were determined. Blood samples were taken from each slaughtered bird. Pathologists

established the methemoglobin contents of each blood sample by means of a blood gas analyses done on a co-oximeter. The Vitamin A levels in the feed are shown in Table 3.3.

Statistical analysis:

Statistical analysis was conducted using the PC - SAS Version 6.08 commercial software. Several measurements taken on the same experimental unit tend to be correlated with each other. The correlation of measurements of qualitatively different parameters such as weight, length, and width, is taken into account using multivariate methods of analyses. Measurements considered to be responses to levels of an experimental factor of interest, such as time, treatment, or dose, are analyzed using a repeated measures analysis of variance.

PROC GLM provides both univariate and multivariate tests for repeated measures for one response (Winer (1971)). The multivariate approach is covered in Cole and Grizzle (1966). LaTour and Miniard (1983) discussed the relative merits of the two approaches. Means for parameters measured were analysed, using analysis of variance - PROC GLM methods. Main factors were treatment, vitamin inclusion, organ where sample was taken and interactions between these factors. These factors were analysed with week being the predictor. The significance of differences between treatments were determined with Bonveroni test at a $P < 0.05$ significance level.

Results

Differences in nitrite levels observed in the caecum and colon, were not significantly different ($P = 0.2167$), (Table 3.4), although numerically less nitrite was found in the colon than caecum and more nitrite occurred in hens, receiving 8000 IU Vitamin A. No significant interactions occurred between nitrate levels, addition of Vitamin A or the organ sampled.

Table 3.4. Nitrite levels in the caecum and colon (mg/kg) ($P = 0.2167$), (SD ± 7.224)

Nitrate (mg/l)	Caecum with Vitamin A	Caecum without Vitamin A	Colon with Vitamin A	Colon without Vitamin A

0	1.650	0.743	2.867	3.183
25	1.383	1.113	2.647	1.973
100	0.923	6.397	7.4000	2.610
150	0.567	33.633	2.240	4.113
200	28.140	4.243	35.773	2.670
300	5.753	3.633	3.067	2.927

No methemoglobin was found in the blood (Table 3.5).

Table 3.5. Percentage of methemoglobin in blood

Treatment	Methemoglobin level without Vitamin A	Methemoglobin level with Vitamin A
1	-1.700	-1.800
2	-1.833	-1.733
3	-2.233	-1.967
4	-1.900	-1.933
5	-1.733	-1.633
6	-1.500	-1.733

The addition of 8000 mg/l of Vitamin A had a significant positive influence on egg production (Table 3.8) (P=0.0305) during weeks 21, 22 and 23. Within nitrate treatments, the groups receiving the added Vitamin A produced more eggs than the treatments without the added Vitamin A. This implies that the onset of lay was earlier and quicker in treatments receiving the Vitamin A. Later on the treatments without the Vitamin A caught up with treatments with the Vitamin A and the initial spurt was equalised. Egg weight was not influenced by added nitrate levels. Hens receiving 300 mg/l of sodium nitrate without Vitamin A had an egg production percentage (Table 3.9) of 85% versus the 82.62 % of the control in week 23. Elevated nitrate levels did not significantly influence egg weights.

The addition of Vitamin A had no significant influence on food intake (Table 3.10). The 300 mg/l nitrate addition group of hens receiving no added Vitamin A however had markedly lower food intakes than the 25 and 100 mg/l treatment groups in the 22, 25, 27, 28 and 29th weeks.

The addition of Vitamin A to the drinking water had a significant positive effect on body weights in all the treatments over all the weeks (Table 3.12). Hens receiving elevated nitrate levels without added Vitamin A had significant higher body weights than the controls, in weeks 22 and 28. In week 32 however, the control had significantly higher bodyweights than all the other treatments without the added Vitamin A.

Water intakes (Table 3.11) were not influenced by nitrate treatment. The addition of Vitamin A to the water however had a significant influence on water intakes in week 32.
Mortalities were not linked to nitrate administration.

Histopathology:

Hearts

Treatment 5 without Vitamin A showed a few microscopical foci of round cell infiltration (mainly lymphocytes = lymphoid foci) in the myocardium. These were also present, but milder, in Treatment 1 with Vitamin A and Treatment 5 with Vitamin A.

Kidneys

A number of kidneys showed scattered foci of lymphocytic cell infiltration (lymphoid foci). The treatment and the number of occurrences in each treatment are shown in the Table 3.6. below.

Table 3.6. Number of lymphoid foci in the kidneys.

Treatment	No Vitamin A	With Vitamin A
1	1	1
2	2	1
3	2	1
4	1	1
5	2	1
6	1	0

Intestines and pancreas

A number of sections showed evidence of chronic serositis (inflammation of the serous membrane covering the outside of the intestine). This was mild, subacute, and multifocal in Treatments 1, without Vitamin A, Treatment 2 with Vitamin A, Treatment 3 with Vitamin A and Treatment 5 with Vitamin A. The inflammatory reaction was more chronic in Treatments 2 without Vitamin A, Treatment 1 with Vitamin A, Treatment 2 with Vitamin A and Treatment 5 with Vitamin A. In a few of these cases, but especially in Treatment 2 with Vitamin A, droplets of egg yolk were observed. This indicates that the serositis is part of a syndrome k own as “egg yolk peritonitis”. This is a common but un-important finding in laying birds (Table 3.7).

Table 3.7. Number of serositis in the intestines and pancreas.

Treatment	No Vitamin A	With Vitamin A
1	1	1
2	1	2
3	0	1

4	0	0
5	0	2
6	0	0

Livers

Lymphoid foci were present in all the livers, to varying degrees.

Table 3.8. Weekly egg production (eggs/hen/week) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A

		Egg Production			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
1	21	1.000	0.397	1.450	0.409
1	22	3.500	0.218	3.800	0.180
1	23	5.500	0.879	5.780	0.584
1	24	6.330	0.355	6.530	0.256
1	25	6.420	0.176	6.460	0.275
1	26	6.650	0.132	6.670	0.161
1	27	6.670	0.076	6.550	0.180
1	28	6.780	0.293	6.920	0.029
1	29	6.630	0.029	6.630	0.153
1	30	6.500	0.218	6.850	0.050
1	31	6.830	0.104	6.870	0.284
1	32	6.630	0.252	6.730	0.029
2	21	0.617	0.247	1.167	0.764
2	22	2.920	0.247	3.420	0.553
2	23	4.980	0.535	6.950	0.050
2	24	6.080	1.052	6.630	0.225
2	25	6.360	0.242	6.270	0.104
2	26	6.680	0.157	6.700	0.132
2	27	6.810	0.065	6.520	0.104
2	28	6.880	0.027	6.770	0.189
2	29	6.600	0.246	6.580	0.160
2	30	6.590	0.227	6.770	0.126
2	31	6.950	0.088	6.930	0.126
2	32	6.540	0.076	6.780	0.202
3	21	0.730	0.850	0.930	0.058
3	22	3.230	0.759	3.420	0.275
3	23	4.620	0.275	5.350	0.409
3	24	6.180	0.454	6.050	0.654
3	25	5.930	0.153	6.000	0.436
3	26	6.480	0.275	6.620	0.058
3	27	6.500	0.180	6.500	0.265
3	28	6.630	0.293	6.630	0.301
3	29	6.430	0.144	6.500	0.087
3	30	6.750	0.050	6.770	0.076
3	31	6.750	0.132	6.800	0.250

Table 3.8. Weekly egg production (eggs/hen/week) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued).

		Egg Production			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
3	32	6.450	0.100	6.600	0.173
4	21	0.770	0.416	1.180	0.465
4	22	3.133	0.850	3.780	0.723
4	23	4.988	0.868	5.700	0.229
4	24	6.670	0.765	6.900	0.050
4	25	6.320	0.158	6.550	0.132
4	26	6.820	0.176	6.960	0.076
4	27	6.490	0.039	6.500	0.100
4	28	6.760	0.150	6.850	0.132
4	29	6.600	0.225	6.510	0.029
4	30	6.820	0.346	6.800	0.087
4	31	6.920	0.117	6.720	0.301
4	32	6.730	0.200	6.420	0.189
5	21	1.230	0.104	1.380	0.275
5	22	3.570	0.425	4.070	0.379
5	23	5.780	0.225	5.550	0.361
5	24	6.950	0.100	6.320	0.404
5	25	6.370	0.126	6.500	0.436
5	26	6.850	0.173	6.910	0.080
5	27	6.530	0.161	6.660	0.223
5	28	6.680	0.104	6.680	0.071
5	29	6.630	0.161	6.460	0.115
5	30	6.600	0.132	6.600	0.451
5	31	6.600	0.087	6.730	0.501
5	32	6.730	0.301	6.640	0.166
6	21	1.250	0.436	1.350	0.050
6	22	3.900	0.218	4.020	0.404
6	23	5.570	0.236	5.950	0.173
6	24	6.700	0.050	6.780	0.355
6	25	6.070	0.184	6.520	0.369
6	26	6.570	0.247	6.920	0.375
6	27	6.650	0.100	6.780	0.104
6	28	6.720	0.355	6.820	0.153
6	29	6.680	0.029	6.500	0.132
6	30	6.800	0.100	6.820	0.029
6	31	6.730	0.419	6.800	0.087
6	32	6.750	0.180	6.530	0.144

Table 3.9. Weekly egg weight of eggs of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A

		Egg Weight			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
1	21	37.159	0.842	39.849	1.961
1	22	41.990	0.725	43.020	0.402
1	23	46.532	1.081	44.773	0.883
1	24	48.622	0.922	47.767	1.062
1	25	50.280	0.983	51.071	1.577
1	26	52.135	0.689	51.332	1.086
1	27	53.420	1.142	51.968	1.174
1	28	53.734	0.616	53.111	1.904
1	29	54.380	0.578	54.175	1.287
1	30	55.449	0.658	55.282	1.372
1	31	56.274	0.760	55.544	1.205
1	32	56.641	0.809	55.456	2.324
2	21	39.984	1.459	38.235	0.885
2	22	41.567	0.245	41.832	1.286
2	23	45.622	0.863	44.718	0.844
2	24	53.209	9.788	47.598	0.585
2	25	49.906	0.805	49.634	0.614
2	26	51.710	0.462	51.074	0.997
2	27	52.505	0.980	51.989	0.945
2	28	53.447	1.371	52.649	0.687
2	29	54.024	0.650	54.156	0.452
2	30	54.532	1.296	54.983	0.312
2	31	55.416	1.412	55.405	0.219
2	32	55.632	1.374	55.693	0.143
3	21	40.985	1.654	37.963	2.784
3	22	42.827	1.108	42.471	1.436
3	23	47.420	2.762	45.975	0.462
3	24	48.111	0.808	52.498	7.412
3	25	50.132	0.760	50.131	0.629
3	26	52.121	0.966	51.813	0.530
3	27	53.039	0.828	52.922	0.524
3	28	53.303	0.636	53.624	1.348
3	29	54.113	0.220	54.037	0.282
3	30	55.219	0.295	54.377	1.223

Table 3.9. **Weekly egg weight of eggs of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued).**

	Week	No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
3	31	55.212	0.498	55.505	0.390
3	32	55.454	0.315	56.102	0.524
4	21	37.532	0.994	38.356	0.685
4	22	41.034	1.700	41.667	0.512
4	23	45.385	1.024	44.809	0.295
4	24	47.099	0.476	47.487	0.863
4	25	49.084	1.092	49.380	1.117
4	26	51.203	0.499	51.055	1.685
4	27	52.547	1.020	52.273	1.094
4	28	52.389	0.987	52.860	1.551
4	29	53.755	1.004	53.468	1.409
4	30	54.505	1.068	54.137	1.130
4	31	54.644	0.852	54.627	1.288
4	32	55.326	0.522	54.974	1.555
5	21	39.806	0.194	38.343	1.162
5	22	42.232	0.305	42.800	0.463
5	23	45.811	0.529	45.622	1.047
5	24	47.691	1.032	48.603	0.907
5	25	50.124	0.052	50.299	1.038
5	26	51.339	0.822	52.356	0.525
5	27	52.322	1.023	53.033	0.547
5	28	54.114	2.481	54.031	0.379
5	29	53.974	1.108	53.984	0.403
5	30	54.927	1.171	54.619	0.448
5	31	54.955	1.180	55.096	0.161
5	32	55.612	0.969	55.803	0.485
6	21	39.158	1.332	38.426	0.880
6	22	42.472	0.715	42.485	0.254
6	23	45.509	0.321	45.694	0.650
6	24	47.993	0.481	50.045	2.915
6	25	55.379	9.143	50.738	0.418
6	26	50.993	0.455	51.015	1.892
6	27	52.701	0.374	53.390	0.473
6	28	53.012	0.803	54.098	0.506
6	29	53.787	0.790	54.657	0.567
6	30	54.874	0.898	54.979	0.119
6	31	55.110	0.772	55.506	0.350
6	32	55.222	0.589	56.063	0.349

Table 3.10. **Daily food intake (g) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A**

Treatment	Week	Feed intake			
		No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
1	21	98.2	5.8	96.1	5.0
1	22	121.5	1.5	121.6	13.1
1	23	123.2	5.4	129.1	9.7
1	24	132.2	3.3	131.9	8.9
1	25	140.7	2.0	141.8	12.3
1	26	138.2	14.5	142.3	10.1
1	27	138.7	5.5	146.2	10.4
1	28	149.3	7.0	147.5	13.9
1	29	140.7	7.8	152.4	13.7
1	30	155.2	9.4	161.1	18.0
1	31	163.1	13.2	161.4	18.4
1	32	160.5	5.7	167.8	18.2
2	21	109.0	6.4	99.0	3.0
2	22	131.6	15.6	123.0	4.0
2	23	138.2	20.1	126.0	7.0
2	24	151.6	22.8	132.0	6.0
2	25	158.6	22.7	143.0	5.0
2	26	164.4	24.1	143.0	2.0
2	27	158.7	17.0	146.0	7.0
2	28	168.7	11.8	148.0	3.0
2	29	163.1	11.1	150.0	6.0
2	30	168.8	7.4	162.0	2.0
2	31	189.4	5.6	158.0	1.0
2	32	177.3	5.8	169.0	8.0
3	21	101.1	15.0	91.0	6.0
3	22	114.0	10.0	113.0	4.0
3	23	118.0	9.0	115.0	2.0
3	24	131.0	7.0	123.0	2.0
3	25	137.0	5.0	134.0	6.0
3	26	144.0	5.0	139.0	6.0
3	27	148.0	2.0	142.0	9.0
3	28	147.0	4.0	148.0	4.0
3	29	151.0	9.0	146.0	4.0
3	30	156.0	8.0	162.0	7.0
3	31	158.0	7.0	153.0	3.0

Table 3.10. **Daily food intake (g) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued).**

		Feed intake			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
3	32	164.0	9.0	162.0	2.0
4	21	90.2	3.1	104.5	4.6
4	22	107.3	4.6	121.8	4.3
4	23	116.2	11.7	128.0	4.7
4	24	122.1	18.1	136.3	8.4
4	25	135.2	17.4	141.6	2.8
4	26	133.3	11.5	144.1	4.5
4	27	135.3	8.0	143.9	15.4
4	28	141.1	4.3	152.2	3.8
4	29	143.3	16.8	146.1	6.1
4	30	154.4	16.5	155.8	4.5
4	31	156.1	20.4	158.4	3.3
4	32	161.6	23.7	164.1	3.6
5	21	90.5	4.6	95.1	9.1
5	22	107.8	5.8	114.3	12.2
5	23	119.0	6.9	125.2	10.1
5	24	127.2	6.2	127.6	10.9
5	25	129.7	4.6	132.1	10.9
5	26	133.7	4.9	139.0	13.6
5	27	131.2	6.4	143.2	19.2
5	28	141.3	1.6	144.8	18.6
5	29	137.1	5.9	137.7	12.5
5	30	150.5	2.6	155.8	8.9
5	31	148.8	4.0	156.0	19.5
5	32	158.3	4.5	163.8	18.0
6	21	91.4	4.4	99.2	6.1
6	22	106.8	6.3	119.4	10.6
6	23	123.2	4.5	124.3	1.9
6	24	124.1	4.3	138.7	4.9
6	25	136.6	2.6	144.9	4.9
6	26	134.4	7.4	137.0	2.1
6	27	137.1	6.2	144.1	2.1
6	28	142.2	7.1	146.3	2.0
6	29	140.6	4.3	146.4	4.8
6	30	154.4	7.4	154.3	5.9
6	31	152.9	9.3	156.5	4.8
6	32	156.8	11.0	161.4	7.8

Table 3.11. **Daily water intake (ml) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A**

Treatment	Week	Water intake			
		No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
1	20	156.4	5.7	150.5	3.2
1	21	202.3	11.4	198.5	7.9
1	22	219.9	3.4	204.6	8.2
1	23	216.5	4.4	205.6	8.1
1	24	216.2	4.6	217.1	2.0
1	25	214.6	4.7	209.3	10.7
1	26	221.7	6.9	203.9	5.7
1	27	214.0	7.4	210.4	20.1
1	28	240.0	9.3	223.3	6.4
1	29	243.8	3.2	237.6	6.1
1	30	240.2	4.5	230.6	11.6
1	31	236.3	3.4	223.5	8.9
2	20	152.5	6.5	159.4	6.6
2	21	193.7	18.7	215.6	5.4
2	22	216.9	15.1	216.1	5.4
2	23	215.4	6.6	218.9	12.3
2	24	216.1	8.1	221.3	7.2
2	25	223.7	17.3	218.1	11.9
2	26	229.7	10.5	222.3	12.0
2	27	217.5	10.1	211.5	3.0
2	28	238.3	10.3	232.9	9.0
2	29	245.2	5.6	238.9	2.0
2	30	244.7	12.8	233.7	11.1
2	31	242.4	13.9	231.7	10.6
3	20	161.5	7.4	156.9	3.4
3	21	215.8	17.2	207.1	3.6
3	22	213.5	15.2	212.7	12.5
3	23	221.2	14.8	211.0	9.4
3	24	223.1	15.9	214.4	10.9
3	25	222.3	8.1	224.5	16.4
3	26	231.4	9.7	224.3	14.6
3	27	214.6	10.6	216.8	13.7
3	28	240.0	13.1	234.8	16.3
3	29	248.1	6.9	243.7	4.8
3	30	236.4	2.2	234.0	5.5

Table 3.11. **Daily water intake (ml) of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued).**

		Water intake			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
3	31	243.8	2.4	232.7	12.2
4	20	162.6	4.6	160.2	1.8
4	21	213.2	5.3	206.3	2.9
4	22	222.4	7.4	213.5	3.2
4	23	227.4	6.5	211.5	3.5
4	24	220.7	7.7	214.3	6.5
4	25	228.7	14.3	212.5	4.8
4	26	231.6	10.0	216.5	5.4
4	27	217.1	9.9	215.0	4.1
4	28	244.0	14.2	231.1	4.1
4	29	244.4	6.2	240.5	6.1
4	30	238.0	6.2	227.5	6.8
4	31	242.8	5.2	232.1	8.1
5	20	159.2	4.0	164.0	4.0
5	21	215.7	3.9	213.7	7.8
5	22	230.8	9.0	228.7	10.4
5	23	200.8	42.2	220.0	12.8
5	24	224.4	6.6	221.5	7.0
5	25	223.7	12.6	215.8	11.6
5	26	221.8	11.2	223.9	10.4
5	27	212.6	14.6	222.9	7.5
5	28	235.2	15.8	244.4	13.4
5	29	241.5	11.6	243.0	5.1
5	30	236.8	16.9	248.8	5.0
5	31	238.2	9.8	245.0	9.4
6	20	157.7	5.7	159.2	3.2
6	21	211.0	15.0	209.6	8.1
6	22	226.9	16.0	230.7	8.7
6	23	217.4	18.6	221.2	5.9
6	24	216.0	12.7	193.8	48.2
6	25	218.3	14.9	212.3	4.3
6	26	213.7	11.7	223.5	3.4
6	27	215.1	6.5	211.9	4.2
6	28	238.2	9.3	236.4	4.6
6	29	245.8	2.2	241.5	5.2
6	30	237.1	5.9	244.5	2.9
6	31	241.1	9.0	236.9	3.0

Table 3.12. **Weekly body weights of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A**

Treatment	Body Weight

	Week	No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
1	20	1.575	0.042	1.611	0.055
1	21	1.635	0.027	1.682	0.034
1	22	1.507	0.061	1.708	0.032
1	23	1.722	0.046	1.749	0.007
1	24	1.738	0.037	1.743	0.007
1	25	1.783	0.045	1.773	0.015
1	26	1.777	0.046	1.795	0.014
1	27	1.792	0.033	1.814	0.007
1	28	1.810	0.031	1.816	0.012
1	29	1.813	0.038	1.836	0.008
1	30	1.800	0.040	1.835	0.011
1	31	1.819	0.033	1.844	0.002
1	32	1.846	0.039	1.853	0.017
2	20	1.575	0.026	1.595	0.044
2	21	1.654	0.049	1.675	0.016
2	22	1.685	0.058	1.739	0.004
2	23	1.759	0.078	1.767	0.010
2	24	1.715	0.039	1.756	0.018
2	25	1.724	0.036	1.751	0.039
2	26	1.700	0.034	1.802	0.014
2	27	1.735	0.034	1.786	0.038
2	28	1.766	0.039	1.851	0.053
2	29	1.769	0.031	1.842	0.021
2	30	1.754	0.038	1.828	0.043
2	31	1.754	0.038	1.842	0.048
2	32	1.794	0.043	1.858	0.021
3	20	1.568	0.023	1.601	0.015
3	21	1.662	0.030	1.673	0.020
3	22	1.714	0.045	1.722	0.012
3	23	1.782	0.071	1.746	0.024
3	24	1.729	0.033	1.729	0.009
3	25	1.753	0.034	1.744	0.025
3	26	1.771	0.031	1.784	0.027
3	27	1.773	0.022	1.741	0.084
3	28	1.793	0.024	1.817	0.017
3	29	1.752	0.085	1.789	0.018
3	30	1.790	0.027	1.823	0.033

Table 3.12. Weekly body weights of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued)

		Body Weight			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD

3	31	1.812	0.025	1.833	0.038
3	32	1.835	0.018	1.880	0.027
4	20	1.523	0.051	1.590	0.045
4	21	1.623	0.036	1.686	0.022
4	22	1.668	0.011	1.715	0.032
4	23	1.682	0.058	1.732	0.034
4	24	1.680	0.047	1.734	0.028
4	25	1.708	0.021	1.761	0.026
4	26	1.728	0.022	1.788	0.046
4	27	1.740	0.022	1.772	0.036
4	28	1.756	0.013	1.797	0.019
4	29	1.773	0.031	1.803	0.029
4	30	1.763	0.031	1.787	0.033
4	31	1.783	0.041	1.801	0.021
4	32	1.809	0.043	1.786	0.049
5	20	1.577	0.086	1.597	0.034
5	21	1.647	0.068	1.702	0.041
5	22	1.691	0.042	1.730	0.036
5	23	1.728	0.062	1.756	0.042
5	24	1.643	0.025	1.761	0.047
5	25	1.732	0.051	1.783	0.040
5	26	1.739	0.055	1.786	0.041
5	27	1.740	0.063	1.786	0.042
5	28	1.765	0.061	1.810	0.014
5	29	1.770	0.063	1.837	0.048
5	30	1.776	0.052	1.818	0.050
5	31	1.775	0.051	1.829	0.035
5	32	1.807	0.059	1.863	0.038
6	20	1.653	0.033	1.622	0.024
6	21	1.708	0.032	1.696	0.015
6	22	1.722	0.028	1.733	0.020
6	23	1.749	0.021	1.766	0.017
6	24	1.736	0.032	1.772	0.011
6	25	1.732	0.014	1.794	0.015
6	26	1.763	0.030	1.809	0.017
6	27	1.771	0.033	1.809	0.015

Table 3.12. Weekly body weights of layers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued)

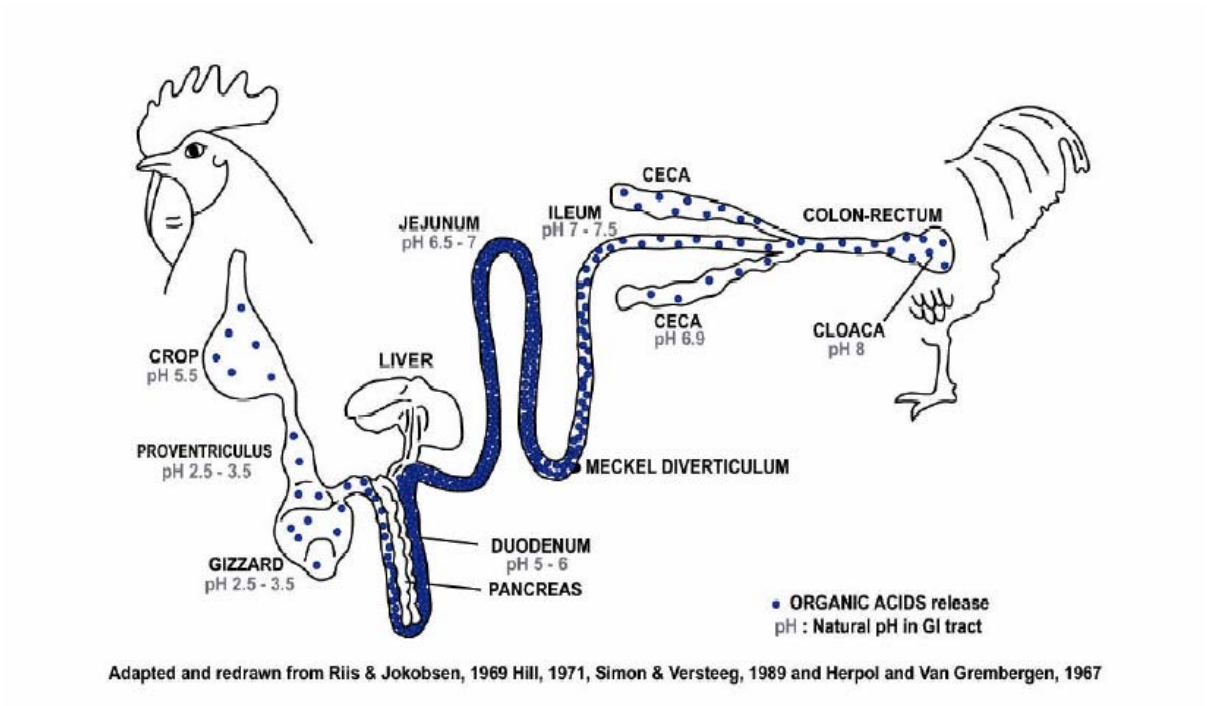
		Body Weight			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
6	28	1.825	0.031	1.837	0.017
6	29	1.769	0.054	1.842	0.012
6	30	1.796	0.034	1.837	0.004
6	31	1.796	0.026	1.825	0.018
6	32	1.827	0.025	1.865	0.024

Discussion

Nitrate poisoning usually occurs subsequent to reduction to nitrite (Bruning-Fann and Kaneene, 1993). Acute nitrate poisoning, though common in ruminants, is rare in monogastric animals. Nitrite is approximately 2.5 times more toxic for ruminants and 10 times more toxic for monogastrics than nitrate (Emerick, 1974).

Pugh et al.; (1962) presented evidence that Vitamin A destruction in the presence of nitrite is dependent on pH. In Figure 3.2 below the natural pH of the GI tract of the chicken is shown.

Figure 3.2. pH of gastro intestinal tract of the chicken (Pugh et al; 1962)



Adapted and redrawn from Riis & Jokobsen, 1969 Hill, 1971, Simon & Versteeg, 1989 and Herpol and Van Grembergen, 1967

Roberts and Sell, (1963) found that rapid Vitamin A destruction took place in the ventriculus where the pH was below 4, and not in the crop or small and large intestines. The fact that no significant differences in nitrite levels measured in the caecum and colon in this experiment occurred support the fact that the nitrite effect reported by Roberts and Sell, (1963) indeed takes place in the ventriculus of birds before they were

killed.

Supporting the findings of Adams *et al*, (1966) who found no consistent relationship in the rate of egg production, egg weight or shell thickness in chickens consuming up to 300 ppm of nitrate, the nitrate levels administered to the water in this experiment had no significant effects on the egg parameters monitored. The addition of Vitamin A however significantly increased egg production.

The decrease in feed intake observed by birds receiving the higher levels of nitrate in the water coincides with work reported by Adams *et al*., (1966).

Vough *et al*. (2000) wrote in a report on nitrate poisoning in livestock that as with feed, frequent intake of water appears to increase the total amount of nitrate that can be consumed daily without harmful effects. Conversely, water consumption limited to only once daily will reduce the level of tolerable nitrates in water before poisoning symptoms appear. This and the fact that monogastrics are less prone to nitrate poisoning would explain why no significant effects on production parameters in the layers were observed.

Conclusion

This experiment showed increased body weights in some weeks with the addition of nitrate to the water. The addition of Vitamin A to the nitrate treated water further increased body weights of hens. The increase in body weight was however not due to increased food intakes, as food intakes decreased in hens receiving elevated levels of nitrate in the drinking water. This could either be due to better feed utilization or experimental error. The addition of 8000 IU of Vitamin A had no significant influence on food intake or water intake. The hens receiving up to 300 mg/l of nitrate in the drinking water showed no significant differences in egg production or egg weight over a 12 week period.

This experiment therefore shows that relatively high concentrations of NaNO3 in drinking water are required before reductions in growth and egg production are observed in poultry.

Experiment 2: Broilers
Materials and Methods

972 Ross male day old chicks were subjected to different levels of Sodium nitrate through the drinking water. The trial design was six levels of sodium nitrate (Table 3.13) with three repetitions and 27 birds per replicate. This was then repeated with the addition of 8000 mg/l vitamin A supplemented to the water. The water from the Pretoria Municipal Source was used and the nitrates present in the water was taken into account when formulating the inclusion levels. All groups received the same commercial diet, and the prescribed vaccination program was followed. Water intake, feed intake, body weight and temperature was measured weekly. Mortalities with accompanying *post mortem* reports were acquired. After 6 weeks the trial was terminated. A representative sample of each group was sacrificed. Liver vitamin A, liver weights, thyroid weights, blood haemoglobin and methemoglobin levels were determined.

Table 3.13. Inclusion levels of nitrates:

Treatments	LEVEL (MG/L)
1	0
2	25

3	100
4	150
5	200
6	300

Statistical Analysis

Statistical analysis was done or performed using the PC - SAS Version 6.08 commercial software. Repeated measures were determined as described in Experiment 1. Means for parameters measured were analysed, using analysis of variance - PROC GLM methods. Main factors were treatment, vitamin inclusion, organ where sample was taken and interactions between these factors. These factors were analysed with week being the predictor. The significance of differences between treatments were determined with Bonveroni test at a $P < 0.05$ significance level.

Method for the determination of the Vitamin A content of the chicken livers (University of Pretoria)

Defrost livers (which have been stored at –70°C) and take a sample of approximately 2 g from each of the 2 prominent lobes of the livers. Cut into small pieces and wash with saline solution containing 0.5mg/ml EDTA and 0.5 mg/ml Vitamin C. Dry samples on filter paper, determine weight of sample and homogenise with equal amounts of saline water using an Ultra-turax.

Measure 100 µl liver homogenate into a 2ml Eppendorf micro centrifugal tube. Add 200 µl saline, 400 µl ethanol, 200 µl KOH (100%). Heat the tube for 30 minutes at 70 °C on a hotplate. Remove from plate, to cool to room temperature.

Dilute the mixture 20 times and add 100 µl in a 2 ml Eppendorf tube. Add 1 ml hexane and vortex vigorously for 50 seconds. Retain a 800 µl supernatant and put in a 1.5 ml Eppendorf tube and leave to dry making use of liquid nitrogen.

Add 50 µl methanol to the samples and vortex for 20 seconds. Put contents in a HPLC tube and chromatography.

Results

Histopathology results:

No histological lesions were evident in the gizzards, spleens, intestines and pancreas or the Bursa of Fabricius.

Hearts

Lymphoid foci were found in the treatments tabulated below. In Treatment 3 with the Vitamin A addition there was severe, chronic epicarditis (inflammation of the outside covering of the heart). The cause of the epicarditis could not be established (Table 3.14).

Table 3.14. Number of lymphoid foci found in the hearts

Treatment	No Vitamin A	With Vitamin A
1	0	2
2	2	0
3	0	1
4	2	2
5	0	1
6	0	2

Kidneys

Most kidney sections had one or more lymphoid foci present in the renal parenchyma.

They were judged as mild or moderate as shown in the Table 3.15 below.

Table 3.15. Treatments where mild and moderate lymphoid foci were observed.

Treatment	No Vitamin A	With Vitamin A
Mild		
1	2	2
2	2	2
3	1	2
4	2	2
5	1	1
6	0	1
Moderate		
Treatment	**No Vitamin A**	**With Vitamin A**
1	1	0
2	0	1
3	1	1
4	0	0
5	0	0
6	1	0

The lymphoid foci found in various organs are indicative of an immune response to a persistent antigen. Most likely the antigen is a virus or mycoplasma of low pathogenicity, i.e. one that does not cause overt clinically recognizable disease.

Table 3.16. LS Means of daily food intake (g) of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A.

		Feed intake			
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	± SD	Mean	± SD
1	1	13.0	0.3	14.0	0.0
1	2	44.0	2.0	44.0	1.0
1	3	96.0	3.0	94.0	5.0
1	4	120.0	4.0	117.0	3.0
1	5	136.0	3.0	137.0	5.0
1	6	151.0	6.0	151.0	0.5
2	1	14.0	0.0	14.0	0.2
2	2	45.0	3.0	42.0	0.8
2	3	97.0	5.0	89.0	1.0
2	4	123.0	1.0	113.0	3.0
2	5	139.0	1.0	134.0	3.0
2	6	144.0	7.0	146.0	2.0
3	1	14.0	0.1	14.0	0.2
3	2	44.0	3.0	42.0	1.0
3	3	98.0	2.0	93.0	0.4
3	4	122.0	2.0	115.0	0.7
3	5	141.0	6.0	135.0	4.0
3	6	140.0	3.0	150.0	3.0
4	1	14.0	0.2	14.0	0.4
4	2	44.0	0.4	42.0	1.0
4	3	98.0	4.0	91.0	1.0
4	4	125.0	5.0	113.0	1.0
4	5	134.0	2.0	129.0	3.0
4	6	145.0	8.0	144.0	5.0
5	1	14.0	0.7	13.0	0.4
5	2	44.0	1.0	41.0	1.1
5	3	96.0	5.0	86.0	5.0
5	4	120.0	2.0	110.0	4.0
5	5	137.0	4.0	129.0	3.0
5	6	147.0	2.0	144.0	3.0
6	1	14.0	0.3	14.0	0.3
6	2	42.0	1.5	43.0	1.0
6	3	93.0	3.0	91.0	2.0
6	4	115.0	2.0	114.0	5.0
6	5	133.0	3.0	135.0	6.0
6	6	143.0	3.0	144.0	1.0

Table 3.17. LS Means of daily water intake (ml) broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A

Treatment	WATER INTAKE				
	Week	No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
1	1	53.0	2.0	44.5	8.0
1	2	82.0	1.9	84.0	3.0
1	3	150.0	31.5	139.0	1.0
1	4	180.0	5.2	175.0	5.0
1	5	252.0	6.8	257.0	19.7
1	6	285.0	10.8	287.0	26.0
2	1	49.6	1.7	40.7	5.0
2	2	86.8	2.0	79.0	2.6
2	3	147.0	2.9	132.9	2.4
2	4	186.0	2.5	166.5	2.9
2	5	268.6	1.7	254.4	8.0
2	6	275.6	4.5	270.0	4.9
3	1	49.9	3.0	40.0	10.2
3	2	85.5	0.3	81.8	1.7
3	3	147.0	1.0	139.0	1.5
3	4	190.5	1.5	174.0	4.7
3	5	278.0	8.0	269.8	4.2
3	6	279.0	2.6	281.6	10.2
4	1	52.6	1.6	44.9	4.6
4	2	87.0	2.6	82.9	2.3
4	3	148.0	4.6	140.6	3.6
4	4	190.9	3.4	177.2	2.0
4	5	280.0	6.9	266.9	15.5
4	6	292.0	1.5	280.3	6.0
5	1	50.8	3.0	44.7	4.0
5	2	86.0	1.0	82.0	0.7
5	3	149.8	2.0	137.0	1.4
5	4	188.5	7.0	174.0	4.2
5	5	283.7	9.0	252.0	11.3
5	6	303.0	3.0	288.0	17.2
6	1	44.7	0.9	41.0	1.0
6	2	81.7	1.7	77.8	3.6
6	3	145.5	3.7	143.0	1.2
6	4	185.1	3.2	182.6	6.8
6	5	286.7	15.3	270.0	10.3
6	6	294.0	3.8	298.0	6.0

Table 3.18. LS Means of weekly body weights of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A

Treatment	*Body Weight*

	Week	No Vitamin A		With Vitamin A	
		Mean	± SD	Mean	± SD
1	1	0.039	0.000	0.039	0.000
1	2	0.118	0.012	0.109	0.005
1	3	0.330	0.004	0.318	0.014
1	4	0.712	0.019	0.677	0.030
1	5	1.213	0.210	1.177	0.050
1	6	1.714	0.002	1.650	0.064
1	7	2.210	0.036	2.195	0.064
2	1	0.038	0.001	0.038	0.000
2	2	0.114	0.002	0.107	0.006
2	3	0.338	0.006	0.313	0.013
2	4	0.712	0.127	0.666	0.024
2	5	1.238	0.022	1.165	0.047
2	6	1.720	0.012	1.637	0.059
2	7	2.205	0.056	2.166	0.015
3	1	0.039	0.001	0.039	0.001
3	2	0.118	0.004	0.107	0.004
3	3	0.337	0.006	0.318	0.005
3	4	0.714	0.015	0.684	0.009
3	5	1.232	0.033	1.185	0.015
3	6	1.733	0.036	1.581	0.061
3	7	2.181	0.031	2.151	0.004
4	1	0.040	0.000	0.039	0.001
4	2	0.123	0.007	0.103	0.005
4	3	0.348	0.004	0.305	0.012
4	4	0.731	0.009	0.666	0.019
4	5	1.1.246	0.020	1.153	0.013
4	6	1.716	0.045	1.588	0.020
4	7	2.196	0.065	2.093	0.014
5	1	0.040	0.001	0.039	0.001
5	2	0.123	0.008	0.108	0.006
5	3	0.340	0.003	0.304	0.009
5	4	0.714	0.008	0.647	0.017
5	5	1.236	0.009	1.122	0.008
5	6	1.727	0.027	1.588	0.029
5	7	2.412	0.008	2.083	0.047

Table 3.18. LS Means of weekly body weights of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (continued).

	Body Weight				
		No Vitamin A		With Vitamin A	
Treatment	**Week**	**Mean**	**± SD**	**Mean**	**± SD**
6	1	0.038	0.002	0.039	0.000
6	2	0.117	0.003	0.111	0.007
6	3	0.323	0.013	0.326	0.015
6	4	0.679	0.011	0.699	0.025
6	5	1.181	0.023	1.204	0.050
6	6	1.671	0.067	1.704	0.089
6	7	2.161	0.028	2.224	0.088

Table 3.19. LS Means of weekly feed conversion ratios of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A

	FEED CONVERSION RATIO				
		No Vitamin A		With Vitamin A	
Treatment	Week	Mean	Std Dev	Mean	Std Dev
1	1	1.20	0.19	1.38	0.10
1	2	1.45	0.14	1.46	0.07
1	3	1.75	0.03	1.83	0.08
1	4	1.68	0.08	1.64	0.04
1	5	1.90	0.04	2.03	0.13
1	6	2.13	0.08	1.95	0.01
2	1	1.29	0.02	1.38	0.09
2	2	1.43	0.12	1.43	0.04
2	3	1.80	0.08	1.77	0.07
2	4	1.63	0.02	1.59	0.03
2	5	2.02	0.04	1.99	0.09
2	6	2.09	0.11	1.94	0.03
3	1	1.23	0.06	1.43	0.10
3	2	1.41	0.09	1.40	0.07
3	3	1.83	0.13	1.78	0.06
3	4	1.65	0.03	1.61	0.02
3	5	1.98	0.05	2.42	0.44
3	6	2.18	0.08	1.85	0.18
4	1	5.00	0.08	1.51	0.15
4	2	1.38	0.05	1.45	0.13
4	3	1.79	0.08	1.76	0.10
4	4	1.70	0.15	1.62	0.02
4	5	2.00	0.16	2.08	0.06
4	6	2.12	0.09	2.00	0.04
5	1	1.19	0.10	1.36	0.09
5	2	1.40	0.08	1.44	0.03
5	3	1.81	0.07	1.76	0.11
5	4	1.61	0.03	1.62	0.03
5	5	1.96	0.08	1.94	0.06
5	6	2.00	0.09	2.03	0.07
6	1	1.22	0.06	1.32	0.10
6	2	1.44	0.02	1.40	0.00
6	3	1.82	0.08	1.70	0.02
6	4	1.60	0.02	1.58	0.05
6	5	1.92	0.13	1.89	0.10
6	6	2.05	0.18	1.93	0.03

Table 3.20. LS Means of methemoglobin content (%) in blood (P = 0.8335) (SD±0.2476)

Treatment	Methemoglobin level without Vitamin A	Methemoglobin level with Vitamin A
1	-1.700	-1.800
2	-1.833	-1.733
3	-2.233	-1.967
4	-1.900	-1.933
5	-1.733	-1.633
6	-1.500	-1.733

Table 3.21. LS Means of liver weights (g) of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (P = 0.2200) (SD±4.0644)

Treatment	Mean liver weights of chicks without Vitamin A in drinking water	Mean liver weights of chicks receiving 8000 IU of Vitamin A in drinking water
1	45.5	38.3
2	39.6	43.333
3	38.433	46.467
4	42.2	42.367
5	37.4	44.433
6	49.333	53.767

Table 3.22. LS Means of thyroid weights (g) of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (P = 0.7564) (SD±1.1905)

Treatment	Thyroid weight of chicks with no Vitamin A addition to drinking water	Thyroid weight of chicks receiving 8000 IU of Vitamin A in the drinking water
1	7.867	7.133
2	8.233	7.400
3	6.033	8.200
4	8.700	8.800
5	8.300	9.100
6	8.133	6.233

Table 3.23. LS Means of liver Vitamin A concentration (mg/100g wet liver) of broilers receiving 6 different levels of sodium nitrate, each treatment with and without the addition of 8000 mg/l of Vitamin A (P = 0.0001)

Treatment	Mean liver Vitamin A concentration of chicks without Vitamin A in drinking water	SD	Mean liver Vitamin A concentration of chicks receiving 8000 IU of Vitamin A in drinking water	SD
1	18.83	±1.75	72.17	±12.93
2	17.30	±2.02	111.93	±42.48
3	14.83	±3.88	77.00	±17.61
4	19.53	±6.13	101.83	±33.12
5	25.03	±4.31	70.67	±21.31
6	19.23	±2.52	71.1	±30.27

Discussion

Sell and Roberts (1963), compared the effects of added Vitamin A to the diet versus Vitamin A administered by intramuscular injection. They reported that chicks receiving Vitamin A by injection did not utilize the vitamin as well in terms of liver storage as chicks receiving Vitamin A as part of the ration. In this experiment the Vitamin A was therefore added to the diet (water administration).

Carver and Pfander, (1973) reported a tendency for dietary nitrate administration to decrease thyroid activity. The thyroid is important in the conversion of carotene to Vitamin A (Johnson and Bauman, 1947). Since the thyroid is important in transforming carotene to Vitamin A, anything, which alters thyroid activity, should also affect Vitamin A status. Thyroid weights were therefore measured and were found not to be influenced by nitrate administration or Vitamin A addition (Table 3.22).

The Vitamin A concentrations in the livers (Table 3.23) clearly indicate that the added Vitamin A was ingested and stored in the liver.

No methemoglobin was found in the blood (Table 3.20) of any treatment group and the liver weights were not significantly influenced in any treatment (Table 3.21)

Bruning-Fann and Kaneene (1993) found that nitrite toxicity syndrome could be reproduced with potassium nitrite but not with sodium nitrate. They concluded that the weight of evidence points to the reduction of nitrate to nitrite in the plants prior to consumption by the chickens and not in vivo. This therefore supports the findings of this study that nitrate levels of up to 300mg/l of sodium nitrate have no negative influence on the food intake (Table 3.16), water intake (Table 3.18), body weights (Table 3.17) and feed conversion ratios (Table 3.19) of broilers.

Conclusion

In this experiment no negative effects on broiler production and growth were observed. However, there is a suspicion among many broiler producers that the minerals in natural sources of drinking water may affect broiler performance. This has been examined in a large-scale survey of the effect of well water on broiler

performance in Arkansas (Barton, 1989). This survey used 100 broiler farms from each of three integrated poultry companies. By separating the best and poorest producers in each company, attempts were made to define the factors affecting broiler growth, food conversion, liveability and condemnation. The only mineral ion to show a significant effect on performance was nitrate, with lower nitrate concentrations in well water being associated with better performance (Balnave, 1998).