EFFECT OF BROMINE AND IODINE IN DRINKING WATER ON THE PHYSIOLOGICAL PARAMETERS OF BROILERS

By

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I declare that this thesis for the degree
MSc (Agric) Production Physiology
at the University of Pretoria,
has not been submitted by me
for a degree at any other university.

The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them ~ ***William Lawrence Bragg (1890 – 1971).***

ETHICS CLEARANCE

This project was approved by the Animal Use and Care Committee of the University of Pretoria.

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ABSTRACT

Assessment of a significant number of water samples across South Africa by Casey and Meyer over a number of years, revealed that high concentrations (30- 32 mg/L) of bromine (Br) occur naturally within groundwater in South Africa, hence a potentially hazardous chemical constituent (PHCC). Br, the only liquid non-metallic element, is ubiquitous and an abundant trace element, but it has not been conclusively shown to perform essential functions in plants, micro organisms or animals (NRC, 2005). The recommended limit (maximum limit for no risk) for Br in drinking water was set at 1 mg/L by Kempster *et al.* (1980); Casey and Meyer (2001) recommend the relevant safety guideline for Br to be 0.01 mg/L. The aim of this project was to establish the effect of Br and iodine (I) in drinking water on the physiological parameters of broilers. This will contribute to verifying and refining water quality guidelines. The effect of six treatments administered as sodium bromide (NaBr) and potassium iodide (KI): 0 mg Br/L and 0 mg I/L; 1 mg Br/L; 1mg Br/L and 0.7 mg I/L; 0.7 mg I/L; 3 mg Br/L; 3 mg Br/L and 0.7 mg I/L, in the drinking water of 540 mixed Ross broiler chickens was investigated. The trial ran over a 42-day growth period from Day 1 post-hatching where mortalities, water and feed intakes were recorded daily. Chickens were weighed weekly and slaughtered at 4 and 6 weeks of age. Blood samples were taken before slaughter and free T_3 and T_4 hormone levels were quantified. Thyroid gland, liver and kidney samples were analysed for Br and I quantity. The different treatments of Br, irrespective of I, significantly decreased water intake (P=0.0232) and feed intake (P=0.0035) over the 42 days. The overall interaction of Br and I had no significant effect on water (P=0.0928) and feed (P=0.9593) intakes thus I did have an effective ameliorating effect on Br. FCR, weight gain and mortalities were not significantly affected by Br intake. This was also found for free T_3 and T_4 hormone levels. Br had an overall effect on the thyroid gland (P=0.0457), liver (P=0.0025) and kidney (P=0.0032) with accumulation of the PHCC within these three organs. It was apparent that 1 and 3 mg Br/L water administered to broilers or ingestion rates of 1.59 and 4.44 mg Br/L per bird per day, over a production period of 42 days did affect the production parameters of the birds sub-clinically. Another derivation to refine the water quality guidelines was that the administration of 0.7 mg I/L water or ingestion rates of 1.1 mg I/L per bird per day did alleviate the severe detrimental effect of the high PHCC. The 3 mg Br/L guideline is thus not too restrictive. Further research exposing the animals for a longer time period (exceeding 42 days) and exposing mature animals (producing nutritional products for human consumption) to these treatments would assist in quantifying these results.

Keywords: Broiler, Br, drinking water, I, PHCC

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LIST OF TABLES

LIST OF FIGURES

CONTENTS

1 CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Water quality investigations on communities sharing water sources with livestock were conducted by Casey and Meyer (2001), which revealed that high concentrations of bromine (Br) occur naturally within groundwater in South Africa, hence a potential health hazard. The water in the communities is used by livestock, poultry and humans located in the areas (Casey & Meyer, 2001). The high levels of detected Br pose both a direct adverse effect on the livestock and people, as well as a potential indirect adverse effect through human food products of livestock origin (Casey & Meyer, 2001). Analyses were done by Casey and Meyer (2001), on the following communities: the Barolong Resettlement located in the Northwest Province in the Potchefstroom District, Rietgat and Hartebeeslaagte in the Zeerust District, Jericho and Immerpan Districts.

The relevant safety guideline for Br recommended by Casey and Meyer (2001) is 0.01 mg/L. This guideline is considered conservative as another guideline specify 3 mg/L as a maximum permissible level and 6 mg/L as a crisis level, primarily due to the possibility of bromate formation (Casey & Meyer, 2001). The Safe Drinking Water Committee (1988) reported chronic exposure levels of Br to be 2.3 mg/L, this calculation was based on the Suggested-No-Adverse-Response-Level (SNARL) where a 70 kg human consuming 2 L of water per day was included. Russian researchers, El'piner *et al.* (1972), recommended a maximum bromide concentration of 0.2 mg/L in drinking water. The toxic end point in their calculation was based on presumed neurological effects in rats as measured by changes in response to a conditional stimulus.

In humans, blood bromide concentrations of less than 5 mg/L have a therapeutic effect, whereas 5 to 10 mg/L is toxic and 10 to 30 mg/L or more may possibly result in a coma and/or death (Ellenhorn *et al.*, 1997). The acceptable daily intake of bromide in dietary form was set at 1 mg/kg for humans by The Food and Agriculture Organisation/World Health Organization (NRC, 2005). Pond *et al.* (1995) recommend 200 mg/kg Br for cattle, sheep and pigs and 2500 mg/kg Br for poultry as maximum tolerable dietary levels. The Br measurements for Table 1.1, in particular the Immerpan March 1998 inscription where 30 to 132 mg Br/L were measured, indicate that livestock and humans could be exposed to highly toxic levels of Br in drinking water, far in excess of the guideline ranges. Additional samples confirming the final concentrations at point of use can be observed in Appendix A (Meyer, 2005a-e).

Table 1.1 Averages, minimum and maximum bromine (Br) levels (mg/L) for the sampled communities

Community	Season of sampling	Average	Standard Deviation	Median	Minimum	Maximum
Barolong Resettlement	February 1998	2.351	2.423	1.607	0.550	9.812
Barolong Resettlement	July 1999	0.211	0.175	0.196	0	0.558
Rietgat and Hartbeeslaagte	May 1997	0.0557	0.020	0.049	0.023	0.083
Jericho District	Summer	0.261	0.176	0.275	0	2.292
Jericho District	Winter	0.091	0.085	0.066	0.006	0.326
Immerpan	March 1998	65.097	34.676	52.426	30.768	132.678
Immerpan	November 1998	6.149	5.115	3.152	1.052	18.00
Immerpan	March 1999	0.389	0.372	0.244	0.085	1.196

(Casey & Meyer, 2001 and Casey *et al*., 2001)

The communities in which water quality investigations were done make use of boreholes as a water source and are dependent on subterranean water for household and livestock use. For example; the farmers in the Immerpan District depend greatly on subterranean water sources because of the distance between the Immerpan and other settlements, the absence of rivers in the area, and a low annual rainfall (Casey & Meyer, 2001).

1.2 Current water status in South Africa

South Africa is a semi-arid country receiving a mean annual rainfall of less than 500 mm a year, with 200 mm of rain a year falling in the western part, the driest part of the country (WRC, 2010). The eastern and western belts of the country receive the most rain (WRC, 2010). An average annual rainfall of more than 2500 mm falls in the wettest part of the country. The remainder of the country receives only about 27% of the total rainfall of South Africa (WRC, 2010). With only a few natural lakes in South Africa, rivers, dams and underground water are essential for water supply (WRC, 2010). It is estimated that the rivers of South Africa receive about 50 billion cubic metres of water a year, with another six billion cubic metres stored underground (WRC, 2010). In South Africa, water is mainly utilized within the following sectors: agriculture and irrigation (52%), forestry (4%), industrial (4%) and domestic (10%). About 19% of water is confined for the survival of the environment (WRC, 2010). Apart from the unpredictable rainfall and the low percentage of runoff in South Africa, which affects the reliability and variability of river flow, the

mean annual water evaporation is higher than the rainfall in all except for the few isolated areas where rainfall exceeds 1400 mm a year (WRC, 2010).

Even though South Africa has rich resources of gold, diamonds and platinum, the one resource that the country is not rich in is water. In fact, South Africa is one of the 30 driest countries in the world (Van Heerden *et al.*, 2008). This is the reason for the importance of water conservation, as emphasized by Van Heerden *et al* (2008); "The availability of water of acceptable quality is predicted to be the single greatest and most urgent development constraint facing South Africa. Virtually all the surface waters are already committed for use, and water is imported from neighbouring countries. Groundwater resources are rather limited; maintaining their quality and using them sustainably is a key issue."

1.3 Poultry production within South Africa

The poultry industry has become an important component of the South African agricultural sector and continues to dominate the agricultural sector within South Africa, as illustrated by Figure 1.1 (NAMC, 2007). The broiler industry is one of the largest consumers of yellow maize in South Africa, if not the largest; this agricultural subsector being responsible for an estimated turnover of R14 532 million at trade level, employed 54 000 people during 2005 (NAMC, 2007). At provincial level (listed from the leading to the smallest contributor) 73.5% of broiler production is concentrated in the Western Cape, North West, Gauteng and KwaZulu-Natal, as shown in Figure 1.2 (NAMC, 2007).

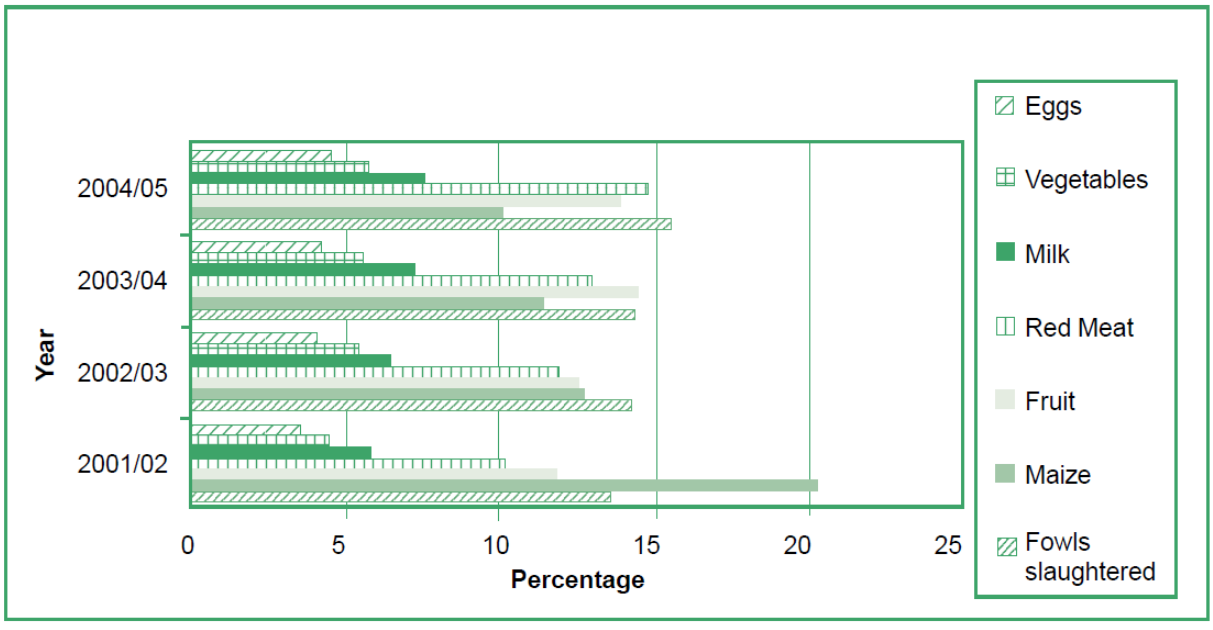


Figure 1.1 Gross value contribution of individual livestock products to Agriculture (NAMC, 2007)

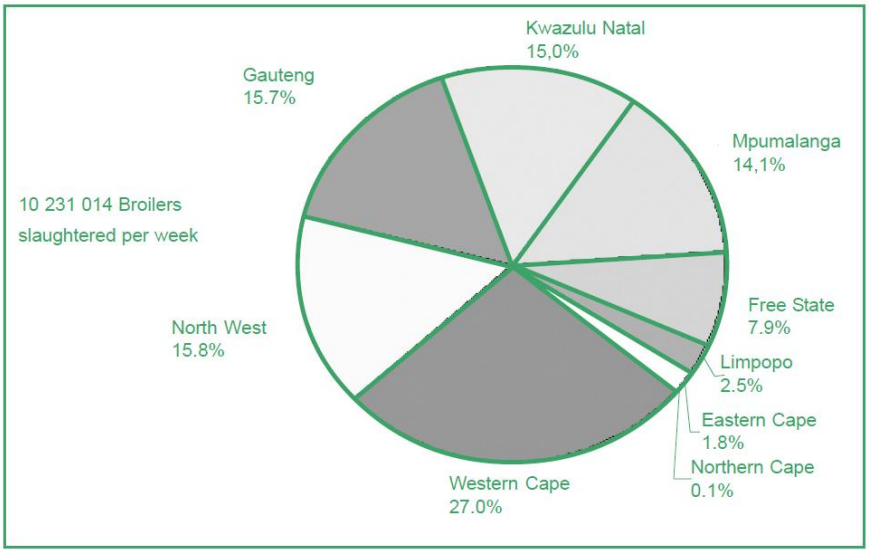


Figure 1.2 The geographical distribution of broiler producers in November 2005 (NAMC, 2007)

Chicken meat remains a highly affordable protein source relative to other meat protein sources (SAPA, 2008). The poultry industry is currently the main supplier of animal protein, since more poultry products are consumed per annum than all of the other animal protein sources combined (SAPA, 2008). In terms of consumption, beef is second and poultry has shown an upward trend in consumption over the past six years (Figure 1.3).

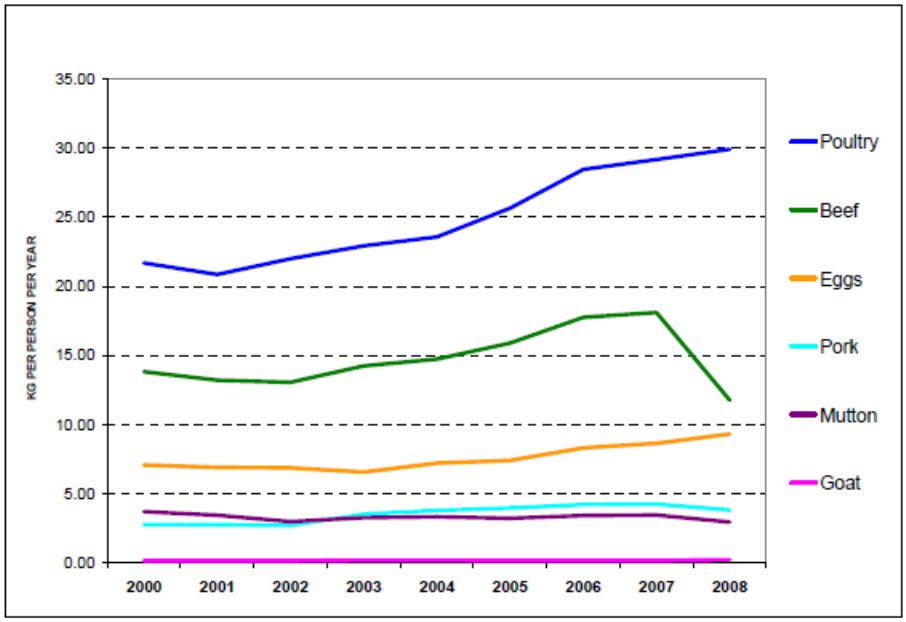


Figure 1.3 The per capita consumption of meat and eggs (SAPA, 2008)

The Southern African Poultry Association (SAPA, 2008) reported the total broiler production for 2008 to be 920.4 million. According to the Department of Agriculture, Forestry and Fisheries the producer level turnover for 2008 was R18.6 billion (SAPA, 2008). For the period of 2004 to 2008 the number of broilers slaughtered per week increased at an average rate of 6% per year, which amounted to a multifaceted growth of 30.6% over the last five years (SAPA, 2008), as seen in Figure 1.4.

Knowledge regarding water quality is important for poultry production as it provides the producer with managerial information to prevent the potential adverse consequences of specific concentrations of water constituents. These typically pertain to health and production parameters, the quality of the livestock product and the watering system of intensive poultry production systems. Health, feed conversion efficiency, feed costs or any unfavourable effects on poultry production can adversely affect the profitability of a poultry production system.

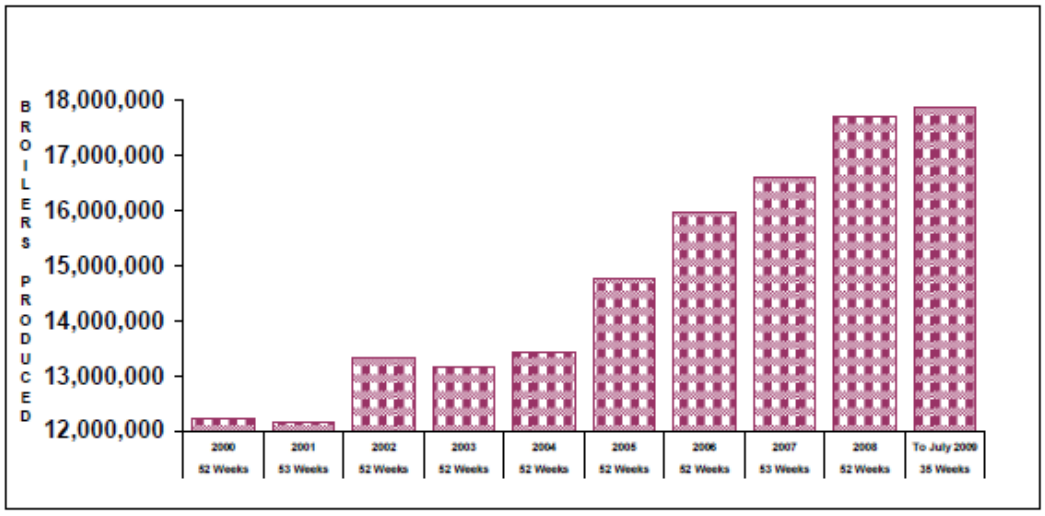


Figure 1.4 The average broiler production per week (potential slaughtering) (SAPA, 2008)

1.4 Motivation

Incorporation of water quality contribution towards drug interactions, feed and mineral requirements is essential due to poultry systems operating with large quantities of water. Broiler diets are formulated to provide the correct nutrient and mineral balance. Water, feed and drugs administered should complement each other for maximum growth and production in livestock. The relative scarcity of supplement provision in rural communal livestock production systems could lead to the occurrence of an abnormal appetite (pica) by livestock (Casey *et al*., 1998). Water containing high mineral concentrations should not be perceived as water with poor quality but seen as a valuable source of minerals (Casey *et al*., 1998).

Since commercially available components may be deficient in certain minerals, supplementation may be required to ensure adequate intake of trace minerals (Casey *et al*., 1998). However, combinations of elements in the same water and/or feed source can be synergistic or antagonistic and as a result end effects may mitigate or exacerbate toxicity. Dietary supplementation with trace elements should be undertaken with great care and consideration because of possible interactions (NRC, 2005; Casey *et al*., 1998). The difficult task of refining the current water quality guidelines is reflected by the question Casey and Meyer (2001) posed, which asks whether high concentrations recorded in the water source act as a valuable source of trace element supplement to the diets of both animals and humans, or if these concentrations contribute an additional burden to a diet already supplying amounts in excess of requirements.

Chickens will consume more water at high ambient temperatures. Water requirements for broilers increase by 6.5% when environmental temperature increases with 1º C over 21º C (Aviagen, 2002). Water consumption also varies with feed intake (Aviagen, 2002). In general, chickens consume approximately twofold the quantity of water as feed on a weight basis (NRC, 2005), for example 1.8 L of water will be consumed per 1 kg of feed consumed.

Table 1.2 Typical water consumption by broilers from Bell drinkers at 21º C in litres/1000 birds/day

Age (days)	Water intake
7	69
14	123
21	190
28	255
35	303
42	345
49	371
56	375

(Aviagen, 2002)

Water must be considered an essential nutrient (Casey & Meyer, 2001). Although it is not possible to state precise requirements, good quality is essential. The quantity required will depend on environmental temperature and relative humidity, the characteristic and consumption of the diet, especially salt content and the efficiency of kidney re-absorption of water in individual chickens (Casey & Meyer, 2001). Water is engaged in all the chemical reactions of poultry metabolism (Casey *et al*., 2001). It participates in temperature regulation of the body, digestion of food and waste elimination to name a few (Casey *et al*.,

2001). Thus, high Br concentrations might influence the water ingestion rates of the birds and consequently also their physiological parameters (Casey & Meyer, 2001). Many water sources used for poultry production purposes in South Africa contain mineral levels that exceed local and international guidelines by large margins (Casey & Meyer, 2001). On the basis of potential hazardous Br levels found in these water sources, it is recommended that research should be focused on identifying and testing outcome based manipulations, which allow for the continued use of these water sources (Casey & Meyer, 2001). This information is intended to refine current water quality guideline values.

1.5 Water intake and excretion

All life is strictly associated with water (Swenson & Reese, 1993). Water is the most vital nutrient to be knowingly supplied to poultry, yet it is taken for granted (Leeson & Summer, 2005). Swenson and Reese (1993) substantiate that water is the most essential nutrient to sustain life, as water accounts for 50 to 70 percent of body weight in adult terrestrial animals and as much as 80 percent for neonates. Both intracellular and extracellular spaces within body cells contain water (Swenson & Reese, 1993). A loss of 20 percent or more of body water is considered fatal (Swenson & Reese, 1993). Water requirements for animals depend on many physiological and environmental factors; consequently water intake is affected by body size, physiological state (gestation, growth and lactation) health and temperature to name a few (Casey & Meyer, 2001).

Table 1.3 Distribution of body fluids in White Leghorn female chickens, in percentage (%) of body weight

Age (weeks)	Weight (g)	Total body water	Intracellular water	Extracellular water		
				Interstitial	Plasma	Total
1	55.1	72.4	11.4	52.2	8.7	61
2	108.4	71.6	21	42.3	7.3	50.6
3	175.3	70.5	24.6	39.1	6.8	45.9
4	241.8	68.4	24.1	38.3	6	44.3
6	372.3			36.8	5.9	42.7
8	527.3	68.7	26.6	36.1	6.1	42.2
16	1137.3	64.8	34.8	24.8	5.2	30
32	1759.5	57.3	31.1	21.7	4.6	26.2

(North & Bell, 1990)

The metabolism, water intake and evaporation rate of smaller birds are accountable for the water turnover in a chicken and exists in an inverse relationship with body weight (Casey *et al.*, 2001). Fat contains less water than lean body tissue and the proportion of body-water content decreases with increasing fatness (Swenson & Reese, 1993). The water content of adult male bodies appears to be higher than that of females, which can lead to the conclusion that males have less body fat than females (Skadhauge, 1976). A higher percentage of body tissue consists of lean body tissue in young birds compared to adult birds; young birds furthermore have higher total body water than older birds (Swenson & Reese, 1993). Intracellular and extracellular body fluid distribution also varies with age. The highest water content is found to be in chicks at 1 week of age and the lowest in mature birds (Medway & Kare, 1959).

According to Swenson and Reese (1993) within the anterior hypothalamus the centre of thirst is found, which regulates the release of vasopressin or antidiuretic hormone. When osmolality in the blood increases (hyperosmolality) thirst is triggered but may also be triggered by a decrease in blood volume. The osmoreceptors in the hypothalamus are especially affected by changes in sodium chloride. Thirst will be triggered by a 1 to 2 percent increase in osmolality, which will be experienced as dryness of the mouth and throat. Extensive blood loss of greater than 10 percent results in thirst (Swenson & Reece, 1993).

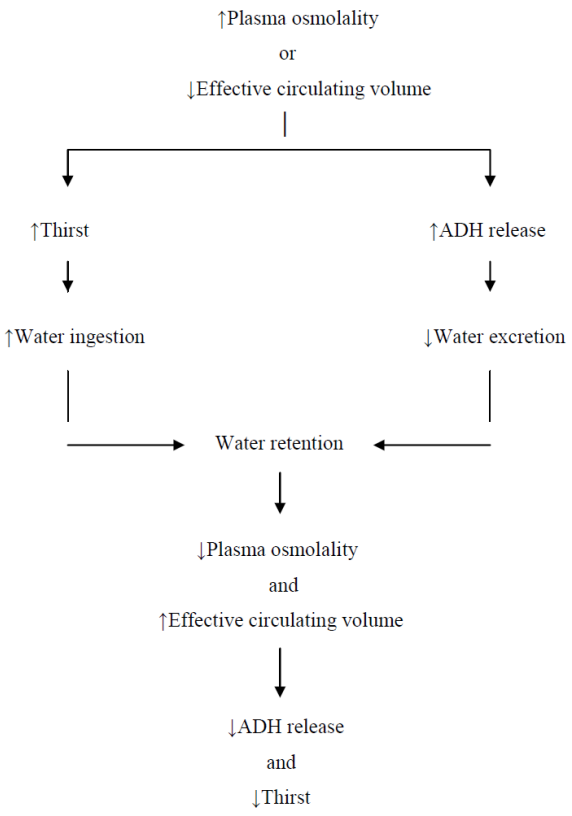


Figure 1.5 Cycle of events for the relief of hyperosmolality and hypovolemia (Swenson & Reece, 1993)

Sources of water for the body are water directly consumed by drinking or derived from feed as well as metabolic water from the oxidation of carbohydrates or fat in the body. Metabolic water is a less significant source of water, except in extreme circumstances (Casey *et al*., 2001). The consumption of water by birds increases with an increase in environmental temperature, live weight and the rate of egg production. Feed rations high in salt and protein also cause an increase in water consumption (Medway & Kare, 1959). Under normal conditions water intake and excretion are controlled in order to sustain homeostasis of the body (Swenson & Reese, 1993).

Drinking water must be available *ad lib* for broilers to prevent dehydration; a positive water balance in growing birds has shown to accommodate growth. A short term reduction in water intake leads to an adverse effect of reduction in feed intake (Leeson & Summers, 2005). Body water losses occur through urine and faeces excretion, expiration of air and perspiration. In birds pulmocutaneous and evaporative water loss are the predominant means of water loss (Casey *et al.*, 2001). An inverse relationship exists between evaporative water loss and body weight with the lack of temperature stress. The respiratory system and air sacs are mainly responsible for evaporative water loss, with a small contribution through the skin and plumage (Casey *et al.*, 2001). Birds store heat and constantly undergo an increase in body temperature within a hot environment. This is mainly a result from the inability of birds to produce adequate evaporative cooling by panting (Bartholomew & Cade, 1963). The function of birds to lose water by method of evaporative water loss decreases as birds mature. The amount of water loss by a day-old-chick, expressed as a ratio of body weight, is appreciably higher than that of an adult (Casey *et al.*, 2001). This occurrence can be explained by the result of marked respiratory loss due to rapid breathing during the first day. During the first day the nature of respiration of the chick changes from allantoic respiration to pulmonary respiration, which gives rise to relative anorexia (Casey *et al.*, 2001).

1.6 An approach to mineral importance and toxicosis

In broad-spectrum, minerals are defined as inorganic elements found in the outer layer of the earth (NRC, 2005) or the result of a geological course of action resulting in an element or chemical compound that is normally crystalline (Nickel, 1995). Essential minerals are vital for health and efficient body function; minerals therefore have definite and clear nutritional and biochemical roles in the maintenance, production and reproduction of animals (Upadhyay *et al*., 2006). Several unintentional minerals occur at trace levels in the nutrition of animals (NRC, 2005). These trace minerals are not typically suspected to play an important nutritional role and are thus considered as incidental contaminants (NRC, 2005). Nonetheless all minerals, essential or nonessential, can exert toxic effects when consumed in excess through water or diet, which emphasise the nutritional and managerial fundamentals of minerals (NRC, 2005).

An ample range of sources can expose animals to toxic levels of minerals. Animal feed, in particular plant derived feedstuffs, is a common source of potentially toxic levels of minerals (NRC, 2005). Molybdenum (Mb) and selenium (Se) are naturally found in soil, in certain areas Mb and Se might occur in adequate levels to cause specific plants to accumulate these levels which can be toxic to animals (NRC, 2005). Br occurs in soil and therefore accumulates in plant derived feedstuffs (detail is given in section 1.7). Mineral supplements are commonly added to animal diets in order to compensate for pasture, forage and other feedstuff deficiencies (NRC, 2005). Mineral supplements may contain potential toxic levels, which depends on the source of the supplement and the method of processing. Error in feed formulation and manufacturing or contamination during storage and transportation can lead to elevated levels of minerals in animal feed and cause acute toxicity and death, whereas other means of exposure will only lead to toxicity after chronic exposure (NRC, 2005). Naturally high levels of minerals occurring in soil can lead to surface water and domestic water supplies containing high mineral levels (Jolles, 1966; NRC, 2005). Not only natural occurring minerals can increase the mineral levels of water, minerals can also be introduced into water through industrial waste, pesticide contamination and other sources of pollution (Jolles, 1966; Pavelka, 2004; Lyday, 2007). Some minerals, including Br, can be introduced to water or feed sources as therapeutics or growth promoters (Bosshardt *et al.*, 1956; Huff *et al.*, 1956). Inaccurate methods have resulted in toxicosis (NRC, 2005). Water is an essential nutrient for living organisms and an exceptional solvent for compounds, including minerals (Swenson & Reece, 1993). Water can thus act as a medium for the consumption of excess minerals, undesirable minerals and other toxic substances (Swenson & Reece, 1993).

Water is only pure as a distillate. Generally it contains numerous diverse elements and compounds apart from its basic hydrogen and oxygen atoms (Stumm & Morgan, 1996). The characteristics of water, which include a high boiling point and heat of vaporization, a high surface tension, maximized density at 4º C and its ability to expand when frozen, demonstrate why water is an exceptional solvent and reactive with salt and polar molecules. The complex system of exchange between water and other elements involves atmospheric chemistry, water chemistry, sediment geochemistry, soil chemistry as well as kinetics (Stumm & Morgan, 1996). Thus, the elements found in water will exist in a variety of oxidation states, protons and non-protons, free ions, and complex ions (Stumm & Morgan, 1996). The mineral composition of groundwater is affected by the composition of rock through which water surges or in which it is stored, the solubility of the minerals contained within the rock, the soil type through which the water flows, the original mineral composition of the water source and evidently the pH of the water (NRC, 2005).

The reactivity and toxic level of mineral elements are related to the form in which the mineral exists (Forbes & Erdman, 1983). Therefore not only the concentration of the mineral content in water should be known to determine its toxicity (NRC, 2005). The term speciation refers to the form of a mineral or element in water (NRC, 2005). An element can exist in water as an uncomplicated hydrated ion, a molecule, a complex with other ions or molecules or many other supplementary forms (Stumm & Morgan, 1996). The principal forms of many minerals found in fresh water, are hydroxo and carbonate complexes (NRC, 2005). Minerals occur in a dissolved state in water and these particles often exist as very minute colloidal or suspensions (The Safe Drinking Water Committee, 1977). Many substances are not recognised as a particulate substance, but as a dissolved matter since they often exist as colloidal precipitates small enough to pass through filters (The Safe Drinking Water Committee, 1977). Most water analyses only provide information on the mineral quantity in the water and not any information on the identification of the mineral speciation (NRC, 2005).

The drinking water guidelines for humans generally offer a very conservative assessment of water quality compared to the livestock guidelines (Casey & Meyer, 2001). As a result the use of human enforceable and secondary water quality guidelines should generally provide safe guidelines for livestock and poultry (NRC, 2005). Due to the diverse physiology of humans and animals, research is needed to refine water quality guidelines for livestock and also to refine to what degree food producing animals concentrate potentially toxic minerals, consumed through water, in various tissues especially tissues frequently consumed by humans (NRC, 2005). NRC (2005) reported that embryonic development, growth and periods of stress such as infections or trauma, are periods when mineral toxicity susceptibility is high. Mineral tolerance often increases with age. Healthy, mature animals are usually more resistant to mineral toxicosis because they have completed important developmental phases, have well developed homeostatic mechanisms and have relative low rates of feed intake (NRC, 2005).

The concentration and the duration of exposure will determine the detrimental effects of minerals (Casey & Meyer, 2001). The effects range from slight homeostatic effects to growth and reproductive impairment, specific pathologies and death (NRC, 2005). In the process of diagnosing toxicities or when identifying the level at which a mineral becomes toxic it is constructive to understand the biochemical and physiological mechanisms by which the mineral exerts its adverse effect (NRC, 2005). Although the mechanism by which minerals cause their toxic effects can be diverse, several universal mechanisms have been identified (NRC, 2005).

The "maximum tolerable level" (MTL) of a mineral is defined as the dietary level, when fed for a defined period of time, that will not impair the performance or health of the animal (NRC, 2005). Incremental administrations of the mineral of concern in feed or water are experimentally used to distinguish between tolerable mineral levels and toxic levels by measuring the impact on performance and pathological signs of toxicosis (NRC, 2005). The duration time of exposure to the animal of the mineral of concern markedly influences the level that causes toxicosis (NRC, 2005). Three exposure durations can be considered as stated by NRC (2005): a single dose, acute dose and a chronic dose. A single dose is defined as an exposure due to the consumption of a single meal or gavages of the mineral. An acute dose is defined as an intake period of 10 days or less and a chronic exposure is defined as an exposure period of 10 days or more. NRC (2005) observed that, in practice the MTL is dependent on the form in which the mineral will be presented to the animal. The solubility in the digestive tract, valence state and the organic, inorganic or metallic form determine the bioavailability of the mineral.

Geohydrological characteristics of subterranean water sources are of importance in determining the chemical properties and mineral status of the drinking water of the specific sources (Moseki, 2001). Vast differences in water level and geochemical properties could occur in sources as close as 200 m of each other. Elsenbroek *et al.* (2003) revealed that the presence of potentially hazardous water constituents have been suspected of causing adverse health effects in livestock. Groundwater may impose a potentially hazardous threat to rural communities sharing the same water source as their livestock, when water contains a relative high concentration of a potentially hazardous chemical (Casey *et al*., 1998).

The application of animal manure as fertilizer might lead to diverse outcomes on human health, crop yields and the environment from a mineral content analysis (NRC, 2005). Excreta management involves environmental considerations, for example the location of manure disposal and climate factors can limit the levels of minerals in the soil for appropriate animal feeding (NRC, 2005). It is further stated by the NRC (2005) that environmental issues must be considered along with the mineral levels that are tolerated by animals in regulation of the mineral concentrations in the feed and water of animals.

1.7 Bromine

In 1825, Liebig isolated Br from mineral water but had considered Br to be a compound of iodine (I) and chlorine (Cl), which led to the discovery of Br in 1826 when Balard recognised Br as an element (Rauws, 1983). Jolles (1966) recorded the estimated amount of Br on earth to be 10^{16} tons, of which half is thought to be contained in living organisms. Br is present in a number of minerals including mica, kaolinite and Fe-bearing minerals and it can be emphasised that Br is a characteristic isomorphic impurity in sylvite.

The maximum Br content found in minerals is found in labradorite (Jolles, 1966). The main sources of Br are similar to Cl; they are plant material, weathering during exogenesis, sea water, volcanic activity and diagenetic–epigenetic solutions (Table 1.4).

Table 1.4 Clarke values of chlorine (Cl) and bromine (Br) (in decreasing order) for coals, rocks, soil, plants and water, measured in parts per million (ppm)

Object	Cl	Br
Multicellular algae	60 000	1 000
Deep- sea carbonates	21 000	70
Deep- sea clays	21 000	70
Brown algae	4 700	740
Angiospermae plants	2 000	15
Bituminous- anthracitic coals	1 100	
Coals	1 000	17
Mosses	670	
US coals	614	17
Lignitic- subbituminous coals	300	
Coaly chondrites	260	5
Acid rocks	240	1.7
Rocks	200	3
Granites	200	1.3
Shales	180	4
Lithosphere	170	2.1
Clays and Shales	160	6
Carbonates	150	6.2
Granodiorite	130	4
Magmic rocks	130	2.5
Intermediate rocks	100	4.5
Soils	100	5
Chomdrites	70	0.5
Basic rocks	60	3.6
Ultra basic rocks	50	0.5
Sandstones	10	
Sea water mg l^{-1}	19 000	65
Fresh water mg l^{-1}	7.8	0.021

(Vassilev *et al.*, 2000)

Water molecules, hydroxyl groups and exchangeable cations in various minerals play a leading role for the inorganic occurrence and distribution of Br (Vassilev *et al.*, 2000). Despite the similar chemical and geochemical properties of Cl and Br, some distinct differences are found in the association, behaviour and occurrence of Cl and Br in coal (Vassilev *et al.*, 2000).

Br, the only liquid non-metallic element, is ubiquitous and an abundant trace element, but it has not been conclusively shown to perform essential functions in plants, micro organisms or animals (Pavelka, 2004). On the other hand Huff *et al*. (1956) and Bosshardt *et al*. (1956) demonstrated a nutritional requirement of Br (8-15 mg Br/kg administered through the diet for 12-31 days) in rats and chicks when an 8-10% growth response was evident. Br may be an essential element, in 3 long term experiments with growing, pregnant and lactating goats, Br poor nutrition led to significantly reduced growth, haemoglobin and hematocrit quantities, conception, milk and fat yield, lower longevity of does and kids and increased abortion rates. Evidence for the essentiality of Br is lacking (Anke *et al.*, 1990). Naturally occurring Br is found bound to metals in the form of inorganic salts called bromide (Pavelka, 2004). Br deficiency is very difficult to achieve since many foodstuffs and water sources do contain levels of Br (Anke *et al.*, 1990). The contribution of Br to the composition of water is relatively insignificant and the absence of any confirmed adverse health-effect attributing to it this far, has resulted in Br receiving only scant attention (Stumm & Morgan, 1996).

Bromide occurs as a sodium salt in seawater and is considered toxic (NRC, 2005). The greatest pool of Br is considered to be seawater, containing levels of up to 65 mg/L Br. Saline deposits originating from evaporated lakes are also a rich source of Br. 1 to 20 mg/kg is the range of Br levels found in soil, although certain volcanic soils contain much higher levels of Br (NRC, 2005). Br-containing compounds are used widely in a range of agricultural, medicinal and industrial practises (Lyday, 2007). Bromide is excessively used in fumigation of soils prior to planting and after the harvesting of agricultural products (Greve, 1983). Medicinal uses of Br include the usage of bromide salts for epilepsy treatment in dogs and for the estimation of extracellular space in physiological research (March *et al.*, 2002). Salt-mining wastes, as well as water and food residue represent new roles of Br (Pavelka, 2004). High values of Br are a possible indication of groundwater pollution, probably due to prior use of Br containing agricultural products, but may also be related to naturally occurring geochemical abnormalities (Pavelka, 2004). Br concentrations exceeding 0.05 mg/L are suggestive of industrial contamination (Meyer, 2005a). Methyl bromide (CH_3Br) can diffuse through certain plastic and bromide treated soils have been observed to increase the inorganic bromide content of food (leafy vegetables accumulate more without phytotoxic symptoms) (Meyer, 2005a). Exposure to CH_3Br has been recorded from groundwater following leaching

of treated soils which were treated with concentrations of CH_3Br greater than 9mg/L (Meyer, 2005a). This occurs as bromide is water-soluble and can thus either be taken up by plants or leach into groundwater. Meyer (2005a) is of the opinion that the bromide ion is also routinely used as an aquifer tracer. Montreal protocol of 1991 defined CH_3Br as an earth ozone layer destructive chemical, which led to a reduction in its usage (NRC, 2005).

Br, classified as a trace mineral, is not typically supplemented to livestock diets (NRC, 2005). Dietary Br levels are a result of background levels in feed ingredients and contamination due to the use of fumigants or disinfectants (NRC, 2005). Crops naturally contain Br levels of 8 to 50 mg/kg on dry matter basis (Van Leeuwen & Sangster, 1987). Fishmeal, a common livestock dietary feedstuff, has the highest Br content (1206 mg/kg) of dietary feedstuffs (Greve, 1983). Another dietary source of Br includes salt prepared from brines containing high bromide levels. Decomposition of CH_3Br following fumigation of hay or feed ingredients result in inorganic bromide salts, which form the primary concern of Br toxicity in animal diets (NRC, 2005). 96 Percent of bromide can accumulate in the human body as well as their livestock (Vaiseman *et al.*, 1986). Accumulation can occur especially in the thyroid gland, liver and kidneys (Jolles, 1966) which can lead to an excessive intake of the mineral by humans if these animals were milked (Vreman *et al.*, 1985) or slaughtered (Vreman *et al.*, 1985) for human consumption purposes. Animals sensitive to potential hazardous constituents are young animals, livestock which are not adapted to their new water source, animals exposed to grazing with very low moisture percentages, animals exposed to high ambient temperatures and/or poor nutrition (Meyer, 2005a). Animals kept for breeding purposes may be more at risk than livestock destined for slaughter (Meyer, 2005a).

Safety guidelines and regulatory standards in terms of Br have been compiled by only a few organisations (Casey *et al*., 1998). These guidelines and standards often do not consider variations of available Br in food ingested, which is linked to the amount of available soil Br, which can vary according to season, land usage and region, the type of feed ingested and the resultant effect on metabolism of Br by the body (Casey *et al*., 1998). Physiological state (lactating, pregnant, growing), activity level, type and size of animal and health status are a few of the factors often neglected (Casey *et al*., 1998). Although the above mentioned factors need to be taken into consideration in establishing water quality guidelines, in researching the potential toxicity of a constituent the number of variables must be limited in order to avoid complex interactions. However, in order for the results to have a broad impact and be extrapolated to a number of different conditions, the fixed variables must be selected cautiously (Casey *et al*., 1998).

1.8 Bromine Absorption, Distribution and Excretion

Br, a halogen, is a brown-red, fuming, heavy and highly corrosive liquid with a specific gravity of 3.12 at 20º Celsius (Jolles, 1966). It is the only non-metallic element that is liquid at room temperature, at ambient pressure with a freezing point of -7.3 º Celsius and at 58.8 º Celsius Br will be boiling (Jolles, 1966). At a natural pH, Br exists as hypobromous acid (HOBr and OBr^-) of which HOBr is the predominant form (Kim *et al.*, 2000). When oxidized, bromide can result in the formation of organic and inorganic Br (Symons, 1999). Bromate (BrO_3) is the highest oxidation state of bromide (Symons, 1999); ozone (O_3) oxidizes bromide to form hypobromite (Glaze & Weinberg, 1993); hypobromite continues to be oxidized to form bromate or forms an unidentified species, possibly bromine dioxide (BrO_2), which regenerates bromide (Glaze & Weinberg, 1993).

The reaction:

$$O_3 + Br \rightarrow O_2 + OBr^-$$

$$O_3 + OBr^- \rightarrow O_2 + Br^-$$

$$O_3 + OBr^- \rightarrow 2O_2 + BrO_3^-$$

The presence of Br in a drinking water source, even at relatively low levels is a cause of concern from a drinking water regulatory perspective, because of the formation of bromate (Gillogly, 2001). The administration of ozone, a potent oxidizing agent, in drinking water reduces the formation of halogenated disinfection by-products, and is proficient in the treatment of chlorine resistant organisms (Bonacquisti, 2006). However, when bromide is present in the drinking water, ozone will convert Br to bromate (Gillogly, 2001; Symons, 1999; Singer, 1999; Glaze and Weinberg, 1993; Jacangelo, 1997; Faust & Aly, 1998), as explained in the above reaction. The current maximum contamination level (MCL) in the USA for bromate in water is 0.01 mg/L, therefore the ideal will be a 0.00 mg/L level of bromate, because of the possibility that bromate may function as a genotoxic carcinogen (Bonacquisti, 2006). The International Agency for Research on Cancer (IARC) classified potassium bromate as a group 2B carcinogen (possibly carcinogenic to humans) (IARC, 2006).

Animal tissues contain between 1 to 9 mg/L bromide, where bovine milk contains up to 2.5 mg/L bromide (Jolles, 1966). The mean bromide content of the human body is 1.7 to 30 mg/L and for blood bromide 5 to 15 mg/L has been recorded. These values depend on the diet of the animal as well as the time elapsed since intake (Jolles, 1966). Jolles (1966) further states that scant information is available on the metabolism of Br. Br metabolism in organisms occurs in the form of its ion i.e. bromide (Jolles,

1966). Injected or orally administered Br is generally well absorbed and is excreted by the kidneys through urine. The appearance of bromide in the blood after ingestion was observed significantly later in time than the other halogens, which was measured with radioisotopes (Jolles, 1966). Hellerstein *et al.* (1960) reported that species differences in tissue Br concentrations are small and that the element does not accumulate in a particular organ or tissue. Cole and Patrick (1958) and Jolles (1966) on the other hand did report relative proportions of bromide in various organs two hours after intraperitoneal administration of 50 micro curies (μc) ^{82}Br as potassium bromide (KBr), which are shown in Table 1.5.

Table 1.5 Relative proportions of bromide in various organs

Organ	Relative bromide proportion
Thyroid	1.000
Kidney	0.536
Adrenals	0.320
Liver	0.394
Brain	0.131
Blood	0.602

(Jolles, 1966)

Cole and Patrick (1958) reported that the brain appeared least active in ^{82}Br uptake; the liver and heart were significantly less active than any other organ except the brain. The other organs were ranked from least to most significantly active as follows: pancreas; adrenals; gonads; spleen; kidney and intestine.

After oral ingestion, bromide is rapidly and completely absorbed in the gastrointestinal tract and distributed almost exclusively to the extracellular fluid (Jolles, 1966). It does not pass through the cell membranes generously, except the erythrocyte membranes which have been recorded to contain relatively high bromide concentrations (Jolles, 1966). Bromide is minimally protein bound. Since the ionic radius of the bromide ion is of the same order as that of the chloride ion, Br replaces part of the extracellular Cl (Jolles, 1966). The similarity of bromide to chloride entails an important pharmacokinetic interaction; both ions compete for absorption by the kidney tubules (Rauws, 1983). Bromide is intermediate in abundance between Cl and F. The relative amounts of Cl^- : Br^- : I^- are the same in plant and animal tissue and are 1 000: 1: 0.01 respectively (Jolles, 1966). Bromide excretion is not affected by urinary volume (Palmer & Clarke, 1932). The biological half-life of bromide can be decreased by the administration of surplus I ions (Langley, 1958). On the contrary, the already long half-life of bromide, which is 14 to 94 hours for the thyroid gland, 88 to 235 hours for the liver and 22 to 197 hours for the whole body (Pavelka *et al.*, 1999), may be increased significantly by a salt-deficient diet (Rauws & Van Logten, 1975). Frances

et al. (2003) reported an elimination half-life for Br in blood of 10 days. Considering the chemical similarity of Br to I, goitrogenic effects of bromide intake may be assumed. An enhanced intake of bromide by the rat reduced iodide accumulation in the thyroid gland, as well as the skin (Velicky *et al.*, 2004).

Bromide is not bio-transformed by the liver and is eliminated unchanged, primarily by renal clearance (Pavelka, 2004). The excretion of bromide mainly occurs through the kidney, which reabsorbs bromide preferably to chloride (Jolles, 1966; Pavelka, 2004), hence the long Br half-life. Thus the chloride/ bromide ratio in urine is usually 2 to 10 times greater than that of blood serum. The chloride/bromide ratio in sweat was found to be lower than that in blood serum (Jolles, 1966; Pavelka, 2004). It is thus believed that in hot temperatures, bromide concentrations are regulated by the sweat glands. It has also been found that saliva eliminates excess bromide and counteracts the effect of bromide retention by the kidney (Jolles, 1966; Pavelka, 2004). The excretion of ^{82}Br in the faeces and urine of rats after subcutaneous injections of radioactive bromide has also been recorded. The excretion of bromide can also be effected through the stomach (Jolles, 1966). The acid secreting cells of mucosa form hydrobromic acid in the presence of extracellular fluid bromide (Jolles, 1966; Pavelka, 2004). The hydrobromic acid is neutralised in the alkaline juices of the duodenum and some of the bromide is reabsorbed (Jolles, 1966; Pavelka, 2004). Bromide excretion was demonstrated in dogs and horses (Jolles, 1966; Pavelka, 2004).

1.9 Iodine absorption, retention and excretion

I, a non-metallic element is of the halogen group and volatile at room temperature and pressure. In solid form I forms lustre blackish-blue plates (The Safe Drinking Water Committee, 1988), which sublimes into the gaseous form I_2 and release an intense violet vapour with a distinguishing odour (NRC, 2005). In water at 25 º C 34 g/L of I will dissolve (The Safe Drinking Water Committee, 1988). I is less active than other halogens (F, Cl and Br) and is derived from natural deposits of saltpetre ($NaNO_3$) (NRC, 2005). The Safe Drinking Water Committee (1988) illustrates that the iodide ion is oxidised by other halogens. The reaction involves nucleophilic displacement of Br by I with the intermediate formation of an interhalogen compound iodine monobromide (IBr).

The reaction:

$2I^- + Br_2 \rightarrow I_2 + 2Br^-$

I is the 64th most abundant element and exists in both organic as well as inorganic substances (NRC, 2005). The Safe Drinking Water Committee (1988) states that pharmaceuticals, antiseptics, photographic material, catalysts, analytical reagents and chemical purification are all applications of I. I is the only

essential trace element required for the synthesis of hormones (NRC, 2005). The I-containing hormones are involved in embryogenesis, differentiation, cognitive development, growth, metabolism and maintenance of body temperature (Underwood & Suttle, 1999). I is highly concentrated in the thyroid gland, which enlarges significantly with a deficiency in I (Underwood & Suttle, 1999). I is the most deficient trace element in the world with a recognised third of mankind functioning on a below optimal level due to its deficiency (Underwood & Suttle, 1999). The main source for I intake is by means of food, thus the proportion of total intake of I is negligible or very low (Underwood & Suttle, 1999). However, I levels in water often serve as an indicator of high or low I intake in an area and correlate inversely with high or low prevalence of goitre (Underwood & Suttle, 1999).

I is rapidly converted to the iodide ion and absorbed efficiently throughout the gastrointestinal tract. Small amounts may be absorbed through the skin (Perlman *et al.*, 1941). I vapour is converted to iodide and absorbed when it reaches the lungs. More than 70% of administered I in a study done by Perlman *et al.* (1941) was absorbed in one hour, 80% in 3 hours and 90% of the I absorption was completed in 12 hours. After 24 hours the small intestine contained only 1% of the administered I. After 3, 12 and 24 hours of administration 13%, 33% and 50% of I was excreted by urine. 5% or less of the I was excreted by faeces (Perlman *et al.*, 1941). The liver is responsible for rapid I uptake and loss. After 24 hours the administered I was completely eliminated from the hepatic tissue (Perlman *et al.*, 1941). The ability of the kidneys to increase and eliminate I concentration is very similar to the liver, as can be seen Figure 1.6. In this figure each animal received 0.5 mg of potassium iodide (KI) and each point indicates the average of four separate analyses on numerous animals (Perlman *et al.*, 1941).

The thyroid gland has 100 times the ability of the liver and the kidney to retain I (Perlman *et al.*, 1941). At the end of a 24 hour interval the thyroid gland still contained more than half of the administered I as shown by Figure 1.7. Each point on the graph (Figure 1.7) represents the average of four separate analyses on a number of animals. Only 20% of the total body I remained within the thyroid gland and release of I takes place at a remarkably slow rate (Perlman *et al*., 1941).

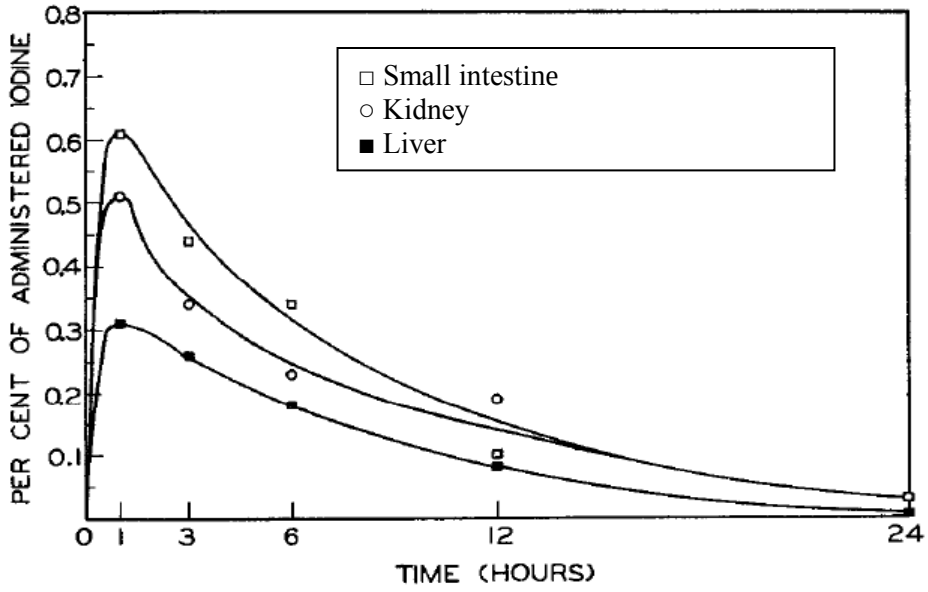


Figure 1.6 The uptake of iodine (I) in the small intestine, kidney and liver of the rat (Perlman *et al*., 1941)

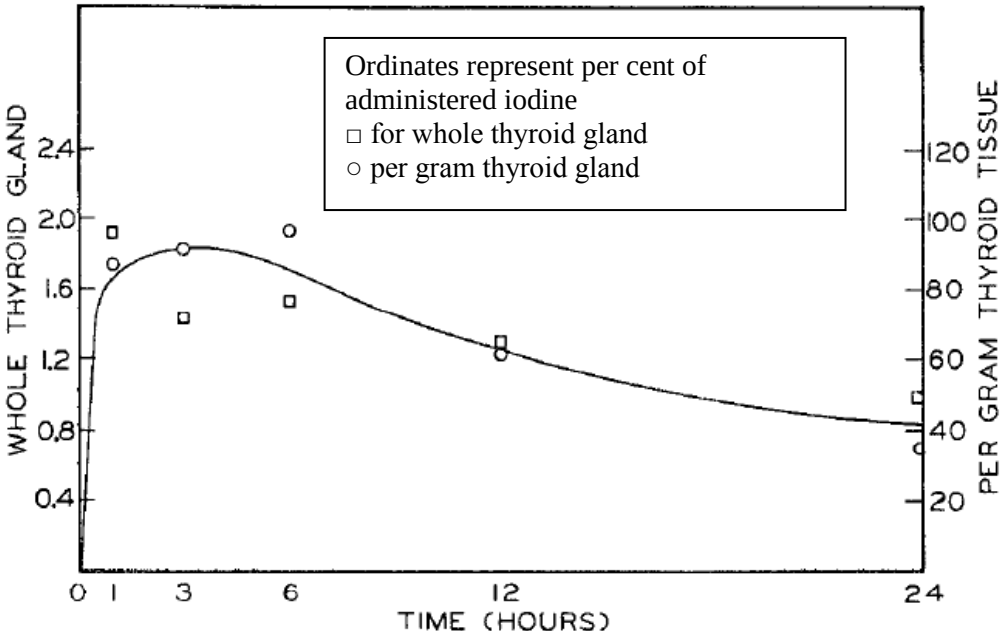


Figure 1.7 The uptake of iodine (I) in the thyroid gland of the rat (Perlman *et al*., 1941)

Because of the essentiality of I, Pechenkina (1964) recommended that levels of I in drinking water be no less than 0.002 mg/L. Dawson (1974) has recommended 10 mg/L of I as a safe level for drinking water, but this would result in a daily intake of at least 20 mg/person/day from water alone, which is excessively high.

1.10 Iodine deficiency disorders and the goitrogenic effect of bromide

Subclinical hypothyroidism- and hyperthyroidism can be recognized by investigating the biochemical profile, serum thyroid gland stimulating hormone (TSH) concentration and the free thyroid gland hormone levels of the animal (Demers & Spencer, 2003; Stone & Wallace, 2003). Therefore quality thyroid gland tests are essential for diagnosing and managing thyroid gland conditions. Current thyroid gland tests reviewed by The National Academy of Clinical Biochemistry on their clinical utility and technical performance are (Demers & Spencer, 2003; Stone & Wallace, 2003):

- Total thyroxine (T_4) and triiodothyronine (T_3) measurements;
- Free thyroxine (FT_4) and free triiodothyronine (FT_3) estimate measurements;
- Thyroid gland stimulating hormone (TSH) measurements;
- Thyroid gland autoantibody tests (TPOAb, TgAb and TRAb);
- Serum thyroglobulin (Tg) testing;
- Calcitonin (CT) and ret proto-oncogene measurements;
- Urinary I measurement;
- Thyroid gland fine needle aspiration (FNA) and cytology;
- Screening for congenital hypothyroid glandism (CH).

I is crucial for the health of the thyroid gland and is essential for the production of two key hormones, T_4 and T_3 (Barry *et al*., 1983). Both T_4 and T_3 play an important role in resting metabolic rate, heat production and the regulation of energy levels (Barry *et al*., 1983). Human thyroid gland hormones are synthesised from iodinated tyrosine (Linder *et al.*, 1994). Tyrosines are provided from a large glycoprotein scaffold called thyroglobulin, which is synthesized by thyroid gland epithelial cells and secreted into the lumen of the follicle (Linder *et al.*, 1994). A molecule of thyroglobulin contains 134 tyrosines, although only minute quantities are utilised to synthesize T_4 and T_3. Iodide is taken up from the blood by thyroid gland epithelial cells; these cells have a sodium-iodide symporter (an iodine-trap) on the outer plasma membrane (Linder *et al.*, 1994). Facilitated diffusion is the proposed transport mechanism for iodide, which involves oxidation of the halide anion by hydrogen peroxide, in order to pass the cell membrane (Linder *et al.*, 1994). Once inside the cell, iodide is transported into the lumen of the follicle along with thyroglobulin, as shown in Figure 1.8. The whole reaction is catalyzed by the thyroid gland peroxidase enzyme (Austgen *et al*., 2001).

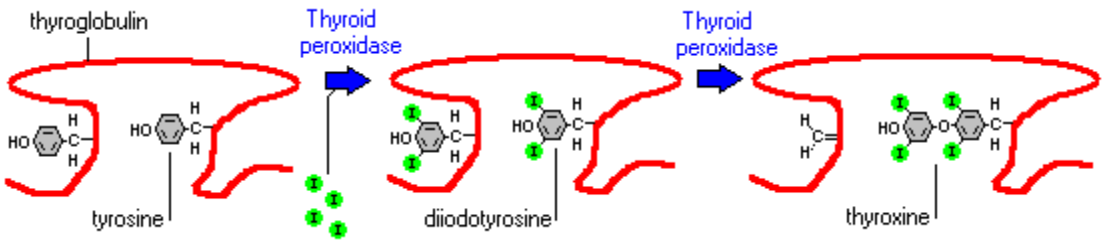


Figure 1.8 Fabrication of thyroid gland hormones (Austgen *et al.*, 2001)

Br is not oxidised by thyroid gland peroxidase and Br does not substitute I in thyroxine, but high levels of Br decrease I uptake by the thyroid gland (NRC, 2005). Br interferes through ring substitution and prevents I ions from entering the tyrosine ring thus suppressing the synthesis of T_4 and T_3 (Loeber *et al.*, 1983). This effect will lead to the disorder hypothyroidism and causes the metabolism to slow down and reduces the sensitivity of tissues. Dietary bromide, on the other hand, reduces I toxicity in chicks (Baker *et al*., 2003; NRC, 2005).

Austgen *et al.* (2001) explained that the thyroid gland is part of the hypothalamic-pituitary-thyroid gland axis and control of thyroid gland hormone secretion is exerted by the negative feedback mechanism, as shown in Figure 1.9. Thyroid gland-releasing hormone (TRH) secreted from the hypothalamus stimulates TSH release from the pituitary, which stimulates the release of thyroid gland hormones (Kelly, 2000). As blood concentrations of thyroid gland hormones increase, it inhibits both TRH and TSH release leading to a "shutdown" of thyroid gland epithelial cells (Kelly, 2000; Austgen *et al.*, 2001). When the thyroid gland hormone concentration in the blood is too low, the negative feedback mechanism comes to a stop, which initiates TSH and TRH release again (Kelly, 2000; Austgen *et al.*, 2001).

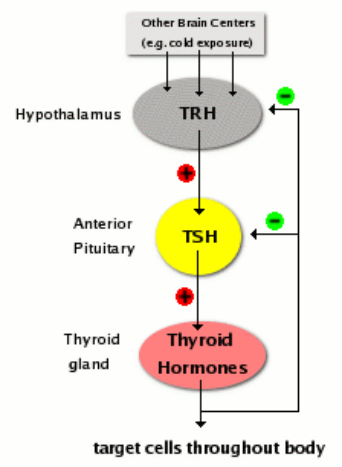


Figure 1.9 Control of thyroid gland synthesis and secretion (Austgen *et al.*, 2001)

It is estimated that there are about 1.6 billion people with an insufficient average I intake (Pavelka, 2004) and are at risk of health problems associated with I deficiency. Apart from the severity of I deficiencies, the symptoms and frequency of I deficiency disorders are also influenced by other goitrogenic factors and trace elements (Pavelka, 2004). In addition to the known goitrogens of plant origin, increasing importance is also given to goitrogenic agents of both organic and inorganic nature, especially in connection with the increasing contamination of the environment (Buchberger *et al.*, 1990). Above all Br, because of its chemical similarity to I, belongs among these goitrogens (Buchberger *et al.*, 1990). The goitrogenic effect of Br becomes particularly significant at moderate I deficiencies when Br can interfere with the production of thyroid gland hormones (Buchberger *et al.*, 1990).

There is a general assumption that the biological behaviour of Br is similar to Cl, so the administration of Br results in some displacement of body Cl (Hellerstein *et al*., 1960). Within the thyroid gland Br replaces I rather than Cl (Hellerstein *et al*., 1960). Many studies following the effects of enhanced supply of Br to an animal were carried out and indicated that Br toxicity is dependent on the state of I supply in the organism, thus the symptoms of hypothyroidism caused by Br intake were significantly enhanced under the conditions of simultaneous I deficiency (Pavelka, 2004). High levels of Br intake influence the I metabolism of the animal in two ways: by decreasing the iodide accumulation in the thyroid gland and skin (and in the mammary glands of lactating dams) and by increasing the iodide excretion by the kidneys (Pavelka *et al*., 2002). By accelerating the renal excretion of iodide, excessive bromide can also influence the amount of exchangeable iodide in the thyroid gland. High bromide intake in lactating rat dams dramatically decreased I and increased bromide transfer through the milk to the suckling young (Pavelka *et al*., 2002). The impact of greatly decreased I and increased bromide concentrations in the milk of lactating dams on the growth of their sucklings were found to be detrimental (Pavelka *et al*., 2002).

Pavelka *et al*. (2002) state prominently that apart from the above researched areas, the virtue of the toxic effects of bromide on the thyroid gland and mechanisms of its interference with the thyroid gland hormones have not been completely explained.

1.11 The effect of bromine on physiological parameters

Excessive Br intake induces a condition known as bromism (Horowitz, 1997; Golomb, 1999). This condition has neurological, psychiatric, dermatological as well as endocrine effects (Horowitz, 1997; Golomb, 1999). Neurological symptoms include headaches, tremors, slurred speech and blurred vision with distended and inadequate reaction of pupils, ataxia, increased or decreased tendon reflexes and brain or spinal cord abnormalities that affect nerve signalling to the muscles (Sticht & Käferstein, 1988;

Horowitz, 1997; Golomb, 1999). Psychiatric symptoms may include fatigue, lethargy, impairment of memory and concentration, irritability or emotional instability and depression. Dermatological symptoms: some patients will develop "bromoderma", an acne-like eruption of the face and hands; abnormal pigmentation of sun-exposed areas and a rash may also be seen (Golomb, 1999). Acute bromism does not occur often because bromide ions irritate the gastrointestinal tract, inducing vomit production before high bromide blood levels can be reached to cause bromism from short-term use. However, chronic bromism may develop because of slow excretion of bromide through the kidneys, giving bromide the opportunity to accumulate in the body when continuously ingested (Golomb, 1999). Masoud *et al.* (1973) estimated that the acute fatal dose of inorganic bromate is approximately 57 mg/kg. Ingestion produces a caustic reaction in contacted tissues accompanied by nausea, vomiting and epigastric pain. Death is due to acute renal failure, which is caused by a direct nephrotoxic effect of the bromate ion. Like chlorates, high doses of bromates also produce methaemoglobinemia (Masoud *et al.*, 1973).

The key constituent affecting Br toxicity is the Cl intake level. Cl attributes to increased renal excretion of both Br and Cl. This statement is illustrated by an experiment conducted by Rauws and Van Logten (1975) where the biological half-life of bromide in rats was recorded to be 3 days at normal dietary chloride levels, increasing to 25 days when sodium chloride was excluded from the diet. Br, as discussed previously, has an effect on growth, development and production in livestock. The biggest influence of Br toxicity is on the production of thyroid gland hormones (Rauws and Van Logten, 1975). Thyroid gland hormones are responsible for a sound metabolic rate and sensitivity of organs and tissues to epinephrine. Therefore, if thyroid gland hormone production is impaired the processes in the body will not proceed normally (Rauws and Van Logten, 1975).

It is known that both the growth and thyroid gland hormones acting together promote protein synthesis during the development stages of an animal (Shapiro *et al.*, 1978). The most sensitive bromide toxicosis indicators in rodents are behaviour and weight changes (NRC, 2005). Loeber *et al.* (1983) recorded a suppression of growth hormone release in male rats receiving sodium bromide (NaBr) enriched diets. A diet containing 19.2 g NaBr/kg showed evident growth retardation after 4 weeks and was more prominent after 12 weeks. A distinct increase in thyroid gland weight was noted in the rats receiving 12 g NaBr/kg for 4 weeks and rats receiving 19.2 g NaBr/kg showed thyroid gland weight increase after 4 and 12 weeks. This supports the goitrogenic effect of Br and is further supported by Velicky *et al.* (2004) who recorded a decrease in body weight and marked changes in the morphology of the thyroid gland in rats fed a semi synthetic purified diet containing a high concentration of Br. In contrary, Huff *et al*. (1956) and Bosshardt *et al*. (1956) demonstrated a nutritional requirement of Br (8-15 mg Br/kg administered

through the diet for 12 and 31 days) in rats and chicks when an 8-10% growth response was evident. A direct relationship between increased activity of the stomach and its uptake of Br has been reported; a similar observation has been made on the sciatic nerve of the cat (Jolles, 1966). An increase in the Br content of cerebrospinal fluid following nerve irritation has been reported (Jolles, 1966).

Doberenz *et al.* (1965) found the toxic dietary level of Br in chicks to be between 5000 and 10 000 mg/kg, based on body weight gain. 10 000 mg/kg Br including 1000 mg/kg F fed to chicks resulted in a decrease in body weight greater than when only F was fed. However 5000 mg/kg Br including 1000 mg/kg F fed to chicks did not cause a significant difference in body weight compared to when only F was fed to the chicks. A 100% mortality rate was achieved within 3 weeks when chicks were fed 10 000 mg/kg Br including 2000 mg/kg F. It was found that a 10% increase of dietary fat increased F retention with chicks (Bixler & Muhler, 1960), the same hypothesis was tested by Doberenz *et al*. (1965) for Br. A 10% fat inclusion in the above diets fed to 4 week old chicks had no significant effect on Br toxicity. The chicks had *ad lib* access to the treated feed. Table 1.6 is a summary of the effect Br exposure has on chickens recorded by Doberenz *et al.* (1965).

Table 1.6 indicates the toxic effect Br has on chickens. Broilers are fed for maximum growth and will therefore be slaughtered after a very short feeding period. This means that broilers have to put on a lot of muscle in a short period of time. Muscle consists of protein and adversely affected thyroid gland hormones cannot promote protein synthesis for growth hence negative production. Broilers are intended for the production of meat for human consumption. These recordings imply an adverse effect on healthy meat production, hence a potential health hazard to the consumer.

Table 1.6 Effect of bromine (Br) exposure on chickens

Age (days)	Quantity (mg/kg)	Source	Duration (days)	Route	Effect
1	2 500	NaBr	28	Diet	No adverse effects
1	5 000	NaBr	28	Diet	Reduced weight gain
1	10 000	NaBr	28	Diet	Reduced weight gain and increased mortality
1	20 000	NaBr	28	Diet	100% mortality

(Doberenz *et al*., 1965)

Renal and thyroid gland tumours were induced in rats exposed to potassium bromate (bromate is the substance formed when the bromide ion reacts with ozone in water) in drinking water. Bromate

treatments of 125 mg/L, 250 mg/L and 500 mg/L over a period of 2 years measured high incidences of renal tumours. The 500 mg/L treatment measured increased thyroid gland tumours (IARC, 1999). Potassium bromate was identified as a nephrotoxic compound by Geter *et al.* (2006). Williams *et al.* (2000) explained that halogen aliphatic chemicals, predominantly those with short alkyl chains and one or more Cl or Br atoms, constitute a group of chemicals that are acutely nephrotoxic and hepatotoxic in experimental animals. Nephrotoxicity induce renal vascular disease and a reduction in the glomerular filtration rate of the kidneys (Williams *et al*., 2000).

Disturbances in the digestive process due to possible changes in the digestive fluid might occur (Pavelka, 2004). Pavelka (2004) and Jolles (1966) explained that this is caused by bromide accumulation in the gastric mucosa which is secreted into the stomach lumen. The stomach produces hydrochloric acid (HCl) which digests proteins, activates certain gastric enzymes and has antibacterial properties. HCl can react with bromide to form hydrobromic acid (Jolles, 1966), a highly corrosive acid that can cause severe ulcers when it comes in contact with any body tissue (Pavelka, 2004).

An elevated dietary intake of bromide by rat dams in lactation was found to cause a very significant decrease in weight gain in the suckling offspring (Pavelka *et al.*, 2002). More trials revealed that only half of these sucklings survived and were in a very poor condition. This was complemented by stagnation in the extent of the consumption of diet, water and a drop in milk production rate during the nursing period (Pavelka, 2003). This suggests that bromide ingested by the dam was transported via the milk to the sucklings (Pavelka *et al.*, 2002). It is possible that without special dietary precautions animal tissues will contain Br as a result of maternal transfer to the offspring (Huff *et al.*, 1956). Winnek and Smith (1937) found egg albumin to contain a relative high content of Br (94 ppm). This indicates possibility of bromide being transferred to the eggs of chickens governed primarily by the Br intake of the hen. Egg quality and safety may be adversely influenced for consumption by humans and must be investigated.

Bromide ingested by cows as NaBr caused a reduction in milk fat and milk glucose concentration (Jolles, 1966). On a wet weight basis 1 to 12 mg/kg Br was recorded in milk of dairy cows fed 9.5 to 38 mg/kg dietary NaBr. Bromide in the milk was found to be proportional to the level of bromide in the blood, which was also proportional to the bromide level in the feed (NRC, 2005). Lipid turnover and increased blood cholesterol were recorded in dogs and rabbits. NaBr fed to rats resulted in a decrease in the calcium and phosphorus content of their bones with increased water content. Retarded growth, decreased thymus, enlarged thyroid gland and deformed spleen were also included in the recordings (Jolles, 1966). According to Anke *et al.* (1990) Br may be an essential element, in 3 long term experiments with

growing, pregnant and lactating goats, Br poor nutrition lead to significantly reduced growth, haemoglobin quantities, hematocrit quantities, conception, milk and fat yield, lower longevity of does and kids and increased abortion rates.

Bromide is also known to have an effect on reproduction. In trials where male rats received dosages of dibromoacetic acid, the serum testosterone levels were 17% less than compared to the control serum levels after 2 days. Abnormal head shape of spermatozoa, flagella degeneration (Linder *et al*., 1994), decreased spermatogenesis in testes, decreased prostate activity in males and a reduction in corpora lutea quantities found in the ovaries of females were also observed (Loeber *et al*., 1983). In humans, abortions have been recorded where fertilization occurred with spermatozoa of males accidentally exposed to Br vapour. A mild degree of spermatogenesis suppression and impaired reproductive performance follow paternal exposure to Br vapour (Potashnik *et al.*, 1992).

The residue levels in meat, milk and eggs of animals fed maximum tolerable dietary levels of Br are unknown (NRC, 2005). The MTL of Br for selected animals is thus very important to ensure good health, efficient growth, production and reproduction of livestock.

1.12 The treatment of bromine toxicity

Meyer (2005 a-e) is of the opinion that site specific recommendations, correct seasonal utilisation and management can decrease the toxic potential of potential hazardous chemical constituents (PHCC). A reduction in water intake from the source containing excessively high Br levels, to achieve acceptable ingestion levels mainly through dilution, is a practical solution when animals have access to alternative water sources. The allocation of poor quality water to less sensitive groups and antagonistic dietary application are possible mitigation options according to Meyer (2005 a-e).

Golomb (1999) explains that the well researched antagonism between bromide and chloride provides a management option for Br toxicity. Saline or sodium chloride loading has been performed to enhance kidney excretion of bromide. The chloride ions from sodium chloride compete with and replace the bromide throughout the body, which reduces the half-life of bromide significantly. Golomb (1999) further emphasises that this method must be performed with care as it may not necessarily reverse the hypothyroidism symptoms, as Cl has been identified as a natural thyroid gland suppressing chemical. Failing to react to the saline loading, mannitol or "loop" diuretics are used, which act on a specific part of the kidney by making use of mannitol and ethacrynic acid agents. Haemodialysis is also used to treat bromism (Golomb, 1999). Haemodialysis is a rapid promising method of mitigation of patients who fail

to respond to saline loading. For this reason, it may be appropriate to use haemodialysis to eliminate the bromide ions from the blood rather than relying on the kidneys (Golomb, 1999).

As explained earlier, bromide competes with I for a binding site during the formation of T_4 and T_3. Br is converted to bromide in the thyroid gland, proportional to its continuously increasing concentration, whilst the production of iodinated thyronines is decreased. Consequently Baker (2004) confirmed administration of iodide may be used as a possible treatment method for Br toxicity. Although I toxicity in animals and humans is rare, chronic over-consumption of I reduces organic binding of I by the thyroid gland and results in hypothyroidism or goitre (Baker, 2004). This should be stressed according to Baker (2004) to prevent an oversupply of I when Br toxicity is treated.

1.13 Aim

The aim of this trial is to establish the effect of Br and I in drinking water, on the physiological parameters of broilers.

2 CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

A great deal of research, as mentioned above, support the statement that Br is toxic to animal tissues and does affect the physiology and biochemistry of the body. For this trial the effect of Br on growth, development and production of broilers was of importance. Br retention and accumulation in the thyroid gland, liver and the whole body (Jolles, 1966; Golomb, 1999) further support the importance and essential information that can be gathered from this trial. Ishidao *et al.* (2002) states that inhaled Br vapour is rapidly decomposed and metabolised in the body. Therefore Br ion concentrations in the blood and urine decrease gradually. In general, the metabolism of inhaled toxic compounds increases after frequent exposure because metabolic enzymes are induced by the exposure (Ishidao *et al.*, 2002). However, when over-exposure to toxic materials occurs, the metabolic rates may decrease due to dysfunction of the metabolic system (Ishidao *et al.*, 2002).

Bioavailability studies have not been formally completed, but an orally administered bromide dose is 96% absorbed by humans (NRC, 2005). Nishikawa *et al.* (1985) noted a urinary excretion rate of bromide administered orally to be 5 to 9%, which thus provides a retention rate of 91 to 95%. The retention period for elevated bromide levels was studied in dogs (Jolles, 1966). An increase in the level of blood bromide was found to start within 2 to 4 hours after administration of NaBr and this level was maintained for 2 to 5 days before the NaBr started to subside. However, even after 19 hours, concentrations above normal were still recorded (Jolles, 1966).

Most of the previous work done on the effect of Br was done on rats and humans and administrated via the diet where high levels of Br were used. In this trial, treatments closer to the natural Br content of South African groundwater sources were used. This was achieved by administering different concentrations of Br by means of drinking water to broilers and observing the behavioural and physiological responses (feed and water intake, weight change and production). The main concerns for a commercial production system were the possibility of elevated tissue concentrations within the birds due to higher feed and water intakes and the concerns regarding high exposure rates to the local communities or resettlements with poor quality and quantity of nutritional diets, when these birds were consumed.

As indicated earlier, Casey and Meyer (2001) noted the target guideline range for Br in drinking water for livestock to be 0.01 mg/L. It was concluded that this guideline value may be too restrictive since it does

not take the production system, exposure time, species tolerance and ingestion rate of the water quality variable into account.

2.2 Broiler trial

540 mixed (male and female) Ross 708 Broiler day-old chicks were hatched and vaccinated by a reputable organisation to standard practices of the poultry industry and were employed as experimental animals. Chicks received New Castle disease and bronchitis vaccines at the hatchery and received vaccinations for Gumburo disease on day 12. Vaccines were administered in the form of eye drops and purchased from a reputable animal and veterinary pharmaceutical supplier. Water was administered to each pen from a bell drinker connected to a calibrated 15 L perspex cylinder (Figure 2.1). The bell drinkers were suspended from the roof of each cage and kept at the correct drinking height for the birds. Bell drinkers were cleaned on a daily basis and fresh water was dispensed into the 15 L cylinders daily. The cylinders had removable lids for easy access for treatment administration and an outlet at the bottom to simplify cleaning and refilling. Water was accessible *ad lib* (Figure 2.1).

Figure 2.1 Calibrated 15 L Perspex cylinder

Chickens were kept in a mechanically ventilated broiler house with sawdust as bedding material. The house was divided into 3 blocks by making use of a complete randomised block design, with 6 pens per

block. Each pen (3m x 2m) housed 30 chickens. Ventilation shafts were opened and electronic fans functioned for the duration of the trial to prevent ammonia accumulation and heat stress. Chickens did not receive a specific lighting programme but were subjected to a 24 hour light period. The house temperature was measured twice a day.

Each of the treatment groups received the same standard commercial broiler starter (ME = 11.2 MJ/kg, Protein = 22 %, Day 1-18), finisher (ME = 11.4 MJ/kg, Protein = 18 %, Day 19-42) and post-finisher diet (ME = 11.6 MJ/kg, Protein = 17 %, Day 19-42). The feed were supplied by a reputable commercial feed supplier and formulated for a 42-day commercial broiler program. A 42-day production period is representative of a small scale production system (NAMC, 2007). This production system was chosen for the reason of elevated financial implications within a commercial production system. The feed composition is shown in Table 2.1.

Table 2.1 Standard commercial broiler starter, finisher and post-finisher diets

Ingredient %	Starter	Finisher	Post-finisher
Protein	22	18	17
Lysine	1.05	0.95	0.85
Fat	2.50	2.5	2.5
Calcium	1.05	0.95	0.9
Phosphorus	0.45	0.4	0.38
Sodium	0.20	0.2	0.2
Potassium	0.65	0.65	0.65
Chlorine	0.12	0.12	0.12
Chlorine mg/kg	450	450	0
ME/kg DM	11.2	11.4	11.6

One pan feeder per pen was used during the first week. After 7 days, one feeder was suspended from the roof of each cage. The brim of the feeder was kept at the correct adjusted height as the chickens grew. Feed was available and accessible *ad lib*.

Water intake, feed intake, body weight and mortalities were recorded over a 42-day period from Day 1 post-hatching. At the end of weeks 4 and 6, five chickens of each replicate were removed and blood were sampled before the birds were slaughtered, in the conventional way by experienced personnel at the abattoir on the Hatfield Research Farm for thyroid gland, kidney and liver sampling. The trial design

consisted of 6 combinations (1 control and 5 treatments) of these constituents with three repetitions and 30 birds per replicate (6 x 3 x 30).

2.2.1 Treatments

Br and I were administered to the birds via the drinking water at the inclusion levels given in Table 2.2.

Table 2.2 Treatment inclusion levels of bromine (Br) and iodine (I) in water administered to broilers over a 42-day period from Day 1 post-hatching.

Treatment	Bromine (mg/L)	Iodine (mg/L)
Control	0	0
Treatment 1	1	0
Treatment 2	0	0.7
Treatment 3	1	0.7
Treatment 4	3	0.7
Treatment 5	3	0

The water containing chemicals delivered a final concentration of 1 mg Br/L and 3 mg Br/L as sodium bromide (NaBr), as well as 0.7 mg I/L as potassium iodide (KI).

1 and 3 mg Br/L were selected because 1 mg/L is the recommended level for Br. Kempster *et al.* (1980) initially found 3 mg/L to be the maximum permissible level (maximum limit for insignificant risk) and 6 mg/L as crisis limit (maximum limit for low risk), as a general guideline for human consumption. Due to the fact that the presence of 3 mg Br/L can lead to bromate formation the recommended limit (maximum limit for no risk) was then set to 1 mg Br/L (Kempster *et al.*, 1980). These quality criteria were set for human drinking water and not for livestock, but as stated by McKee and Wolf (1963) water safe for human consumption may assumable be used safely by livestock. But the physiology, metabolism and tolerance of animal species and humans differ, which indicates the importance of this trial. Since no experimental data on Br administered via drinking water is currently available, except for studies done on fish (McKee & Wolf, 1963) or rats. By the administration of 1 mg Br/L, the recommended level of Br can be verified or it can indicate by revealing no sub-clinical signs of toxicity, that this recommended limit is too strict. Throughout the Water Research Commission Reports (Casey *et al.*, 1998; Casey *et al.*, 2001; Casey & Meyer, 2001) the average Br level recorded is slightly higher than 1 mg/L. The target water quality range for Br is 0 to 1 mg/L, with a relevant safety guideline of 0.01 mg/L. 6mg/L is recorded as the crisis level for Br. These results can also indicate the potential hazardous effect people and livestock

are exposed to in certain areas in South Africa. For example 30 mg Br/L in drinking water is the minimum recorded level of Br occurring naturally in the drinking water in the rural communities of the Immerpan district, with a maximum of 132 mg /L (Casey & Meyer, 2001).

The inclusion of I is necessary to indicate a possible alleviator effect for these high Br water levels, to determine whether I can possibly alleviate the occurrence of this particular water quality constituent (WQC) in the tissue of the animal.
In order to implement I as a possible financially viable alleviator treatment, certain inquisitions should first be confirmed (Casey & Meyer, 2001):

1. To what extent does the presence of the other WQC mitigate or exacerbate the adverse effect due to a single WQC?
2. To what extent does high WQC concentration contribute significantly to alleviating existing trace mineral deficiencies in the diet of the communities involved?
3. To what extent does WQC present in animal products such as milk, eggs and in this case meat and organs add to dose intake already experienced by humans from the WQC present in water?
4. To what extent can alleviator treatment be effective in livestock used to mitigate adverse effects in humans?
5. What about the financial viability of building the necessary structures enabling a single water source to receive multiple treatments, each specific to a different water user?

0.7mg I/L was selected because this level falls within the Target Water Quality Range for I which is 0 to 1 mg/L. AR- grade chemicals were used to produce the water treatments at the NutriLab premises at the Department of Animal and Wildlife Sciences, University of Pretoria. Final concentrations were confirmed by testing samples taken at the specific water sources of use at the Agricultural Research Council-Institute for Soil, Climate and Water (ARC-ISCW). NaBr and KI are ideal for testing the effect of Br exposure since both are highly soluble in water (for NaBr 790g/L will dissolve at 20 ºC and KI 1270g/L will dissolve at 20ºC) (Merck, 2009).

2.2.2 Water sample analyses

Water from the Pretoria Municipal Source was used. Chemical analyses of water samples collected throughout the trial were conducted once a week to determine the Br and I levels in the drinking water as well as for control purposes. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) techniques were performed by the ARC-ISCW in Pretoria as well as by making use of full quantitative

and semi-quantitative procedures (Looke *et al*., 1998) the water samples were analysed. ARC-ISCW prepared the sampling bottles including a protocol for sampling procedures as described in: "Water analyses methods as described by Looke, Philpott and van Vliet" (Looke *et al.,* 1998). Separate sample bottles were used for macro- and trace elements during these procedures.

2.2.3 Tissue sample analyses

The tissue samples of two chickens from each replicate were analysed for the presence of elements by ARC-ISCW. The samples were stored in a freezer and later delivered to the ARC-ISCW for analyses. The ARC-ISCW conducted the laboratory analyses of all the samples by making use of standard inductively coupled mass spectrometry (ICP-MS) techniques to determine trace element concentrations. The samples were digested by microwave assisted acid digestion of siliceous and organically- based matices; herewith the procedure:

A representative tissue sample of up to 0.5 g was digested in 9 ml of concentrated nitric acid and 3 ml hydrofluoric acid for 15 minutes, using microwave heating with a suitable laboratory microwave system. The vessel was sealed and heated in the microwave system. The temperature profile was specified to permit specific reactions, reaching 180 ± 5ºC in less than 5.5 minutes. It remained at 180 ± 5ºC for approximately 9.5 minutes for the completion of the specific reaction. After cooling, the vessel contents were filtered, centrifuged or allowed to settle and then decanted, diluted to volume and analysed by the appropriate ICP-MS method.

ICP-MS measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originated in a liquid are nebulised and the resulting aerosol is transported by argon gas and introduced in the plasma, and sorted according to their mass-to-charge ratios and quantified with channel electron multiplier interferences (ARC-ISCW, 2009). The quantities of Br and I measured within each organ were quantified on a dry matter basis.

2.2.4 Histopathological analyses

Thyroid gland, liver and kidney samples collected from 2 chickens of each replicate were placed in buffered formalin and delivered to INDEX Laboratories, Onderstepoort for histopathology analyses. Only the control, Treatment 4 (3 mg/L Br and 0.7 mg\L I) and Treatment 5 (3 mg/L Br) samples were analysed, for cost effective reasons.

The tissues were block selected and processed in an automated histological tissue processor before specific wax blocks were produced. Sections of 5µm were cut on a microtome and the tissue slides were stained with Haematoxylin and Eosin staining in an automated histological stainer.

2.2.5 Free T_4 and free T_3 hormone analyses

Blood was sampled from 2 chickens of each replicate and the quantitative determination of Free T_3 (T_3) and Free T_4 (T_4), concentration in chicken serum was done by LANCET Laboratories. The method used for determination was Chemiluminescence Enzyme Immunoassay (CLIA).

In the T_3 CLIA, as explained by Rongen *et al*. (1994), a certain amount of anti- T_3 antibody was coated on microtitre wells. A measured amount of chicken serum and a constant amount of T_3 conjugated with horseradish peroxidase were added to the microtitre wells. During incubation, T_3 in the samples and conjugated T_3 competed for the limited binding sites on the anti-T_3 antibody of the wells. After 60 minutes of incubation at room temperature, the wells were washed 5 times by wash solution to remove unbound T_3 conjugate. A solution of chemiluminescent substrate was then added and relative light units in a Luminometer were read. The intensity of the emitting light was proportional to the amount of enzyme present and was inversely related to the amount of unlabeled T_3 in the sample. By reference to a series of T_3 standards assayed in the same way, the concentration of T_3 in the unknown sample was quantified (Rongen *et al.*, 1994).

The precise method of T_3 was repeated for T_4 quantity determination.

2.2.6 Statistical analyses

Broiler chickens and treatments were randomly allocated in the house. The SAS (Statistical Analyses System®) software system was used to determine the significance of differences between treatments for WQC analyses. General linear estimate and hypothesis tests were used for regression analyses. The significance of the differences between treatments were determined by means of the Fisher's test at a $P< 0.05$ significance level.

3 CHAPTER 3: RESULTS

3.1 Bromine and iodine ingestion rates

The water samples collected throughout the trial were submitted to chemical analyses, as explained in section 2.2.2. The results indicated that the Pretoria municipal water source contained negligible and relative constant quantities of Br and I (Appendix B) throughout the duration of the 42-day trial. The Br and I ingestion rates of the broilers were calculated using the following formulas:

- Average water intake over the total trial period x Br concentration in the water for the specific treatment
- Average water intake over the total trial period x I concentration in the water of the specific treatment

The calculated average intakes for Br and I are indicated in Table 3.1.

Table 3.1 Average bromine (Br) and iodine (I) intakes (mg/bird/day) of broilers for the production period

Treatment	Total Br intake	Total I intake
0 mg Br/L + 0 mg I/L	0.096	0.032
1 mg Br/L	1.591	0.030
0.7 mg I/L	0.093	1.114
1 mg Br/L + 0.7 mg I/L	1.567	1.064
3 mg Br/L + 0.7 mg I/L	4.618	1.087
3 mg Br/L	4.442	0.029

3.2 Water intake

The different levels of I, irrespective of Br, had no significant effect (P=0.8053) on water intake. The overall interaction of Br and I also had no significant effect (P=0.0928) on water intake. The different treatments of Br, irrespective of I, had a significant effect (P=0.0232) on water intake. Table 3.2 shows that the control group had significantly higher water intakes than Treatment 1 (1mg Br/L) and Treatment 5 (3mg Br/L), Br clearly played a role.

The average weekly water intakes are shown in Table 3.3 and cumulative intakes in Table 3.4.

Table 3.2 Average water intake (ml/bird/day) of broilers (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

	Levels of I	
Treatments of Br	0 mg/L	0.7 mg/L
0 mg/L	267.75 (15.93)[1a]	254.99 (8.10)[1a]
1 mg/L	250.23 (2.74)[2a]	250.60 (7.23)[1a]
3 mg/L	241.94 (2.05)[2a]	251.51 (5.44)[1a]

[ab] *Row means for different I levels within Br treatments differ significantly (P<0.05) according to the Fischer's Test*
[12] *Column means for different Br treatments within I levels differ significantly (P<0.05) according to the Fischer's Test*

Table 3.3 Weekly average water intake (ml/bird/day) of broilers (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P-value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	56.75 [a] (3.78)	49.70 [a] (9.16)	52.08 [a] (8.30)	53.81 [a] (4.64)	54.37 [a] (8.22)	54.34 [a] (3.37)	53.51 (5.42)	0.2447
2	132.97 [a] (13.80)	123.23 [a] (7.67)	125.24 [a] (0.87)	126.89 [a] (2.14)	127.79 [a] (7.09)	127.10 [a] (7.19)	127.20 (7.88)	0.7582
3	252.55 [ab] (10.37)	240.26 [b] (8.64)	257.15 [a] (9.18)	241.45 [abc] (5.59)	245.06 [ab] (8.03)	227.96 [bc] (8.98)	244.07 (9.12)	0.0684
4	320.48 [a] (25.23)	309.01 [ab] (9.63)	318.43 [ab] (8.37)	307.94 [ab] (13.85)	316.03 [ab] (8.22)	292.85 [b] (5.99)	310.79 (14.28)	0.3910
5	349.02 [a] (29.23)	313.40 [a] (14.04)	333.19 [a] (27.82)	325.74 [a] (25.05)	319.46 [a] (15.44)	313.10 [a] (7.70)	325.65 (22.20)	0.4879
6	494.75 [a] (26.30)	465.76 [ab] (28.93)	443.86 [b] (18.51)	447.78 [b] (8.08)	446.36 [b] (29.13)	436.29 [b] (7.96)	455.80 (19.26)	0.0391

[abc] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

Within Table 3.3 week 3 had no overall effect (P = 0.0684) on water intake, but Br irrespective of any other element or factor had a significant influence on water intake (P = 0.0147). Week 6 had a significant influence (P = 0.0391) on water intake. Within week 6, the control group had a significantly higher water intake than Treatment 5 (3mg Br/L). The overall trend throughout all 6 weeks is that the control group had the highest water intake and that Treatment 5 (3mg Br/L) had consumed the lowest. Treatment 4 (3mg Br/L and 0.7mg I/L) reported higher water consumption than Treatment 5 (3mg Br/L). Treatment 4 did not differ significantly from the control group. Also Treatment 3 (1mg Br/L and 0.7mg I/L) had higher water intake than Treatment 1(1 mg Br/L) but this was not significant. Figure 3.3 and Figure 3.4 evidently indicate how water intake gradually increased at each week and at week 6 minimum consumption of water occurred within Treatment 5 (3mg Br/L) and maximum consumption within the control group.

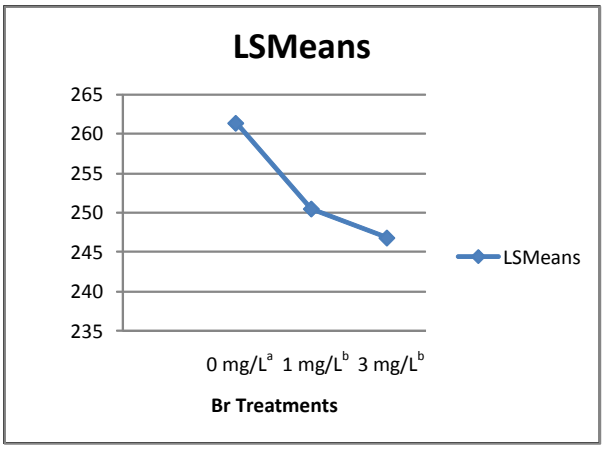
Table 3.4 Average cumulative water intake (ml/bird/day) of broilers (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	507.78 (34.17)^{a}	428.48 (69.68)^{a}	464.58 (71.97)^{a}	467.00 (17.17)^{a}	474.33 (72.34)^{a}	480.05 (35.06)^{a}	470.37 (45.39)	0.2034
2	1542.89 (123.03)^{ab}	1409.36 (104.25)^{b}	1559.03 (54.83)^{a}	1535.63 (112.07)^{ab}	1485.69 (106.35)^{ab}	1464.20 (33.03)^{ab}	1499.47 (81.45)	0.1634
3	3408.02 (200.37)^{ab}	3205.89 (165.31)^{ac}	3477.92 (23.31)^{b}	3255.38 (55.27)^{abc}	3291.25 (110.17)^{abc}	3140.43 (30.28)^{c}	3296.48 (123.18)	0.1009
4	5706.49 (389.05)^{a}	5432.50 (208.37)^{ab}	5761.92 (90.86)^{a}	5462.75 (179.50)^{ab}	5537.79 (175.04)^{ab}	5244.76 (82.69)^{b}	5524.37 (228.04)	0.2540
5	8196.13 (606.31)^{ab}	7667.69 (75.16)^{ac}	8264.24 (139.99)^{b}	7754.21 (314.90)^{abc}	7824.73 (238.89)^{abc}	7465.87 (64.36)^{c}	7861.65 (291.26)	0.0635
6	11245.66 (669.21)^{a}	10509.56 (114.92)^{b}	10827.36 (342.33)^{ab}	10347.21 (316.60)^{b}	10563.45 (228.38)^{bc}	10161.50 (86.05)^{c}	10609.12 (331.26)	0.0410

^{abc} *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

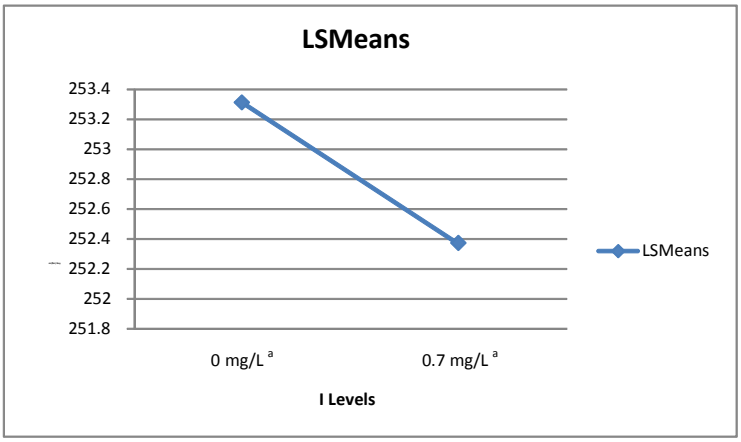
In weeks 3 (P = 0.1009) and 5 (P = 0.0635) there were no overall effect on the cumulative water intake but again Br on its own had an effect (week 3 P = 0.0163, week 5 P = 0.0112). Overall only week 6 had a significant influence on cumulative water intake (P = 0.0410), where Br again on its own had a significant effect (P = 0.0100) and were probably the cause of this trend, Figure 3.1 supports this observation.

Again the overall trend throughout the 6 weeks was that the control group had the highest water intake and that Treatment 5 (3mg Br/L) had the lowest. Treatment 4 (3mg Br/L and 0.7mg I/L) had higher water consumption than Treatment 5 (3mg Br/L). Treatment 4 (3mg Br/L and 0.7mg I/L) did not differ significantly from the control. Also Treatment 3(1mg Br/L and 0.7mg I/L) had higher water intake than Treatment 1 (1mg Br/L) which was not significant, supported by Figure 3.2. Figure 3.2 indicates that I inclusion levels of up to 0.7 mg/L didn't decrease water intake.



[ab] *Means with different superscripts differ significantly (P<0.05), according to the Fischer's Test*

Figure 3.1 The Least Square Means of the different bromine (Br) treatments for the average water intake (ml/bird/day) over the production period (P= 0.0232)



[ab] *Means with different superscripts differ significantly (P<0.05), according to the Fischer's Test*

Figure 3.2 The Least Square Means of the different iodine (I) levels for the average water intake (ml/bird/day) over the production period (P= 0.8053)

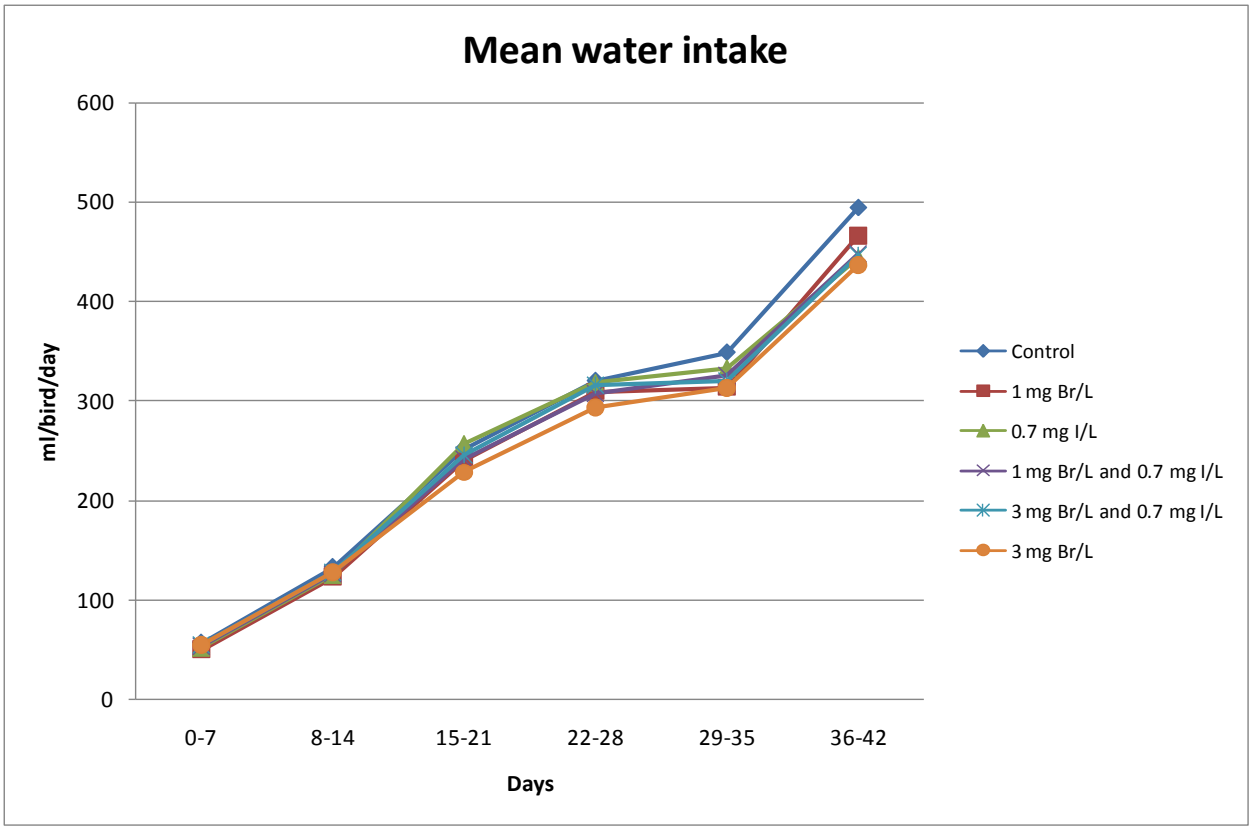


Figure 3.3 Average water intake

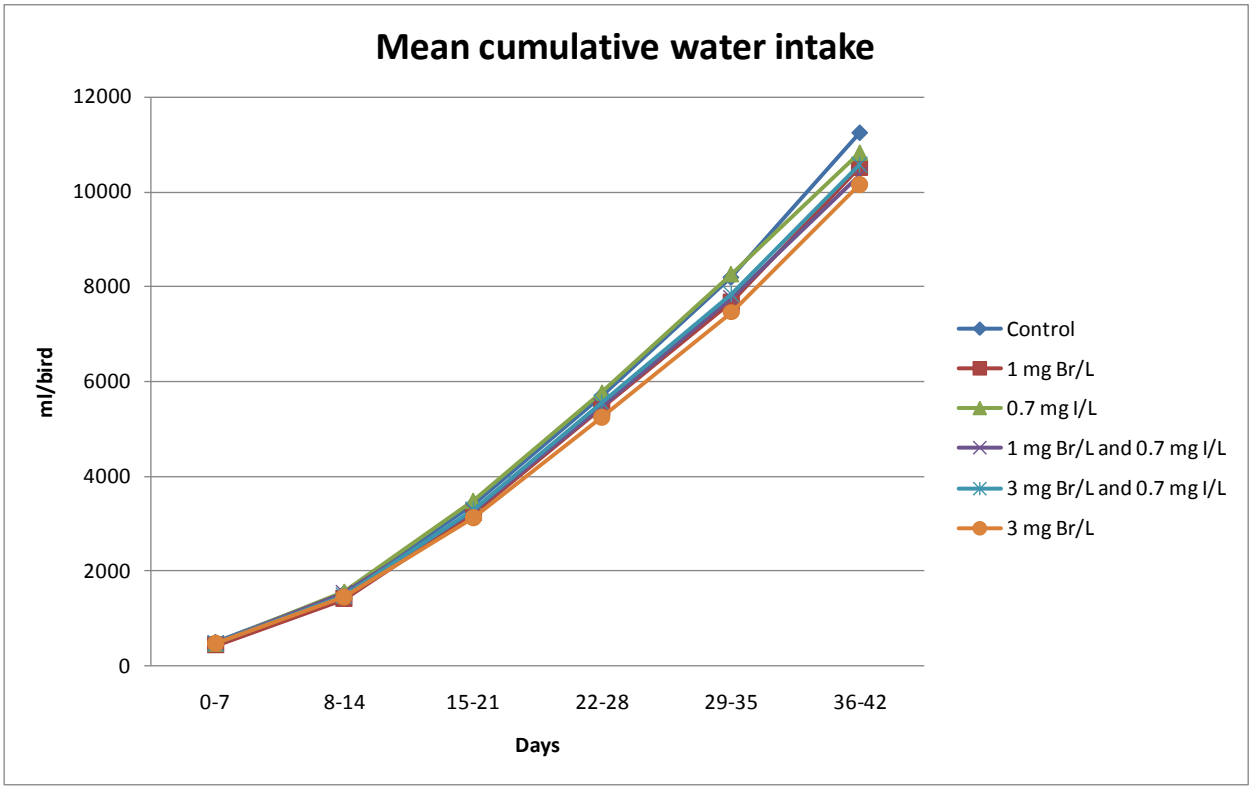


Figure 3.4 Average cumulative water intake

3.3 Feed intake

Table 3.5 Average feed intake (mg/bird/day) of broilers (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

	I levels	
Br treatments	0 mg/L	0.7 mg/L
0 mg/L	120.30[1a] (2.73)	117.18[1b] (0.93)
1 mg/L	116.23[2a] (0.58)	113.59[2b] (2.29)
3 mg/L	117.80[2a] (0.46)	114.81[12b] (1.07)

[ab] *Row means for different I levels within Br levels differ significantly (P<0.05)*
[12] *Column means for different Br levels within I levels differs significantly (P<0.05)*

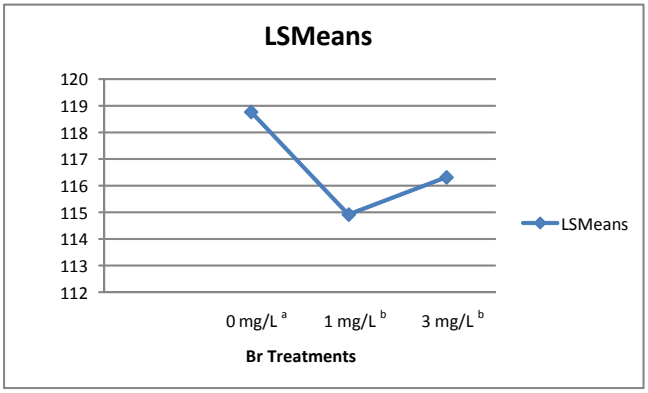
The mean weekly feed intakes are shown in Table 3.6 and cumulative intakes in Table 3.7. The 5 Treatments had an overall effect on feed intake (P = 0.0054). The different levels of I (P = 0.0018) and Br (P = 0.0035) separate and on their own had a significant influence on feed intake. Figure 3.5 indicates that the control had significantly higher feed intakes than the 1 or 3 mg Br/L treatments. Figure 3.6 on the other hand shows the effect of the 0.7mg I/L treatments, where there was significantly less feed consumed. The overall effect of the interaction between Br and I did not have an effect (P = 0.9593), but in Table 3.5 it is reported that the control group differs significantly from Treatment 1(1mg Br/L) and Treatment 5 (3mg Br/L). Also all of the treatments where 0 mg/L I were administered, higher feed intakes occurred than in the treatments where 0.7mg I/L were administered together with the Br.

Table 3.6 Average feed intake (g/bird/day) of broilers (means and SD) over the 6 week production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	24.27 [a] (0.37)	25.34 [a] (2.85)	24.54 [a] (1.17)	27.63 [a] (3.63)	24.84 [a] (0.55)	26.18 [a] (1.48)	25.47 (2.04)	0.4211
2	48.30 [a] (0.50)	43.89 [b] (3.20)	47.87 [ab] (0.53)	44.01 [b] (0.41)	46.27 [ab] (1.37)	45.75 [b] (0.50)	46.02 (1.44)	0.0254
3	95.76 [ab] (1.94)	94.01 [b] (3.09)	98.89 [a] (1.99)	93.56 [b] (1.65)	95.79 [ab] (1.60)	95.90 [ab] (1.92)	95.65 (1.83)	0.0529
4	145.66 [a] (2.21)	143.01 [ab] (2.05)	142.23 [ab] (1.82)	141.29 [b] (4.30)	142.91 [ab] (2.94)	144.00 [ab] (0.58)	143.18 (2.38)	0.2888
5	173.90 [a] (10.81)	164.05 [ab] (2.08)	164.55 [ab] (2.13)	164.31 [ab] (7.07)	162.17 [b] (5.11)	171.19 [ab] (2.02)	166.70 (5.71)	0.1880
6	233.93 [a] (6.15)	227.11 [ac] (3.64)	225.01 [acd] (5.54)	210.73 [bd] (1.14)	216.85 [d] (9.70)	223.77 [cd] (3.32)	222.90 (5.16)	0.0064

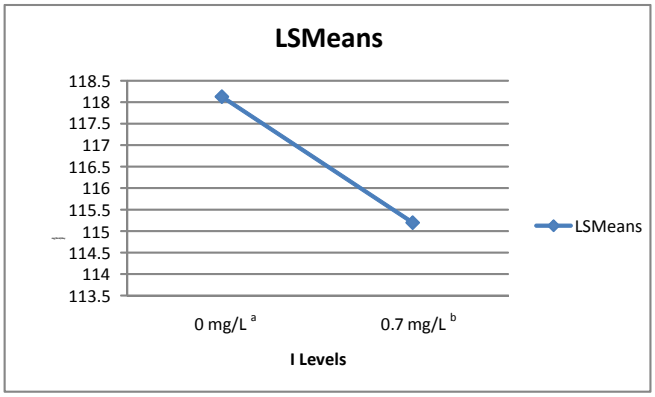
[abc] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

In Table 3.6 there was overall a significant difference (P = 0.0254) in feed intake at week 2, where Br irrespective of I had a significant influence (P = 0.0020). At week 2, Treatment 1 (1mg Br/L) and 5 (3mg Br/L) had significantly (P = 0.0038) lower feed intakes than the control group. Treatment 4 (3mg Br/L and 0.7mg I/L) did not differ significantly from the control treatment. In week 3 there was no overall influence (P = 0.0529) on feed intake but Br did have a significant influence (P = 0.0225). Overall in week 6 there was a significant difference (P = 0.0064) in feed intake. Br (P = 0.0106) and I (P = 0.0013) had individual significant influences, where Treatment 5 (3mg Br/L) significantly consumed less feed than the control group.



[ab] Means with different superscripts differ significantly (P<0.05), according to the Fischer's Test

Figure 3.5 The Least Square Means of different treatments of bromine (Br) of the average feed intake (mg/bird/day) over the production period (P= 0.0035)



[ab] Means with different superscripts differ significantly (P<0.05), according to the Fischer's Test

Figure 3.6 The Least Square Means of different levels of iodine (I) of the average feed intake (mg/bird/day) over the production period (P= 0.0018)

Table 3.7 Average cumulative feed intake (g/bird) of broilers (means and SD) over the 6 week production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	205.45 [a] (5.21)	209.50 [a] (19.53)	207.95 [a] (8.97)	224.89 [a] (25.57)	206.89 [a] (7.00)	215.95 [a] (11.45)	211.77 (14.48)	0.5213
2	583.67 [ab] (3.53)	561.22 [b] (13.09)	586.44 [a] (11.78)	579.00 [ab] (26.17)	576.77 [ab] (14.03)	583.39 [ab] (11.71)	578.42 (13.08)	0.1819
3	1304.86 [ab] (8.62)	1270.43 [c] (18.40)	1328.04 [b] (11.88)	1281.02 [ac] (33.42)	1292.35 [ac] (6.36)	1303.90 [ab] (8.20)	1296.77 (18.11)	0.0679
4	2367.18 [a] (10.15)	2310.87 [b] (26.60)	2365.26 [a] (9.44)	2311.35 [bc] (40.51)	2336.43 [abc] (27.97)	2357.05 [ac] (12.87)	2341.36 (25.22)	0.1026
5	3626.23 [a] (82.19)	3494.02 [b] (12.28)	3553.60 [ab] (12.39)	3482.26 [b] (104.73)	3494.85 [b] (56.10)	3568.07 [ab] (18.78)	3536.50 (60.62)	0.1402
6	5052.80[b] (114.71)	4881.83[a] (24.63)	4921.65[ac] (38.85)	4770.67[c] (96.08)	4821.86[a] (44.97)	4947.51[bc] (19.23)	4899.39 (61.51)	0.0054

[abc] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

For week 3 there was no overall effect on cumulative feed intake (P = 0.0679), but Br had a significant effect (P = 0.0098).Week 4 also had no overall effect (P = 0.1026) but Br again had a significant effect (P = 0.0108). Again in week 5 the same observation was made; no overall influence (P =0.1402), but Br had an effect (P = 0.0459). Week 6 had an overall effect on cumulative feed intake (P = 0.0054), where Br (P = 0.0035) and I (P = 0.0017) irrespectively had a significant effect.

Figure 3.7 and Figure 3.8 clearly indicate how feed intake gradually increased at each week and it also shows at week 6 there was a minimum feed intake by Treatment 3 (1mg Br/L and 0.7mg I/L) and a maximum at the control group.

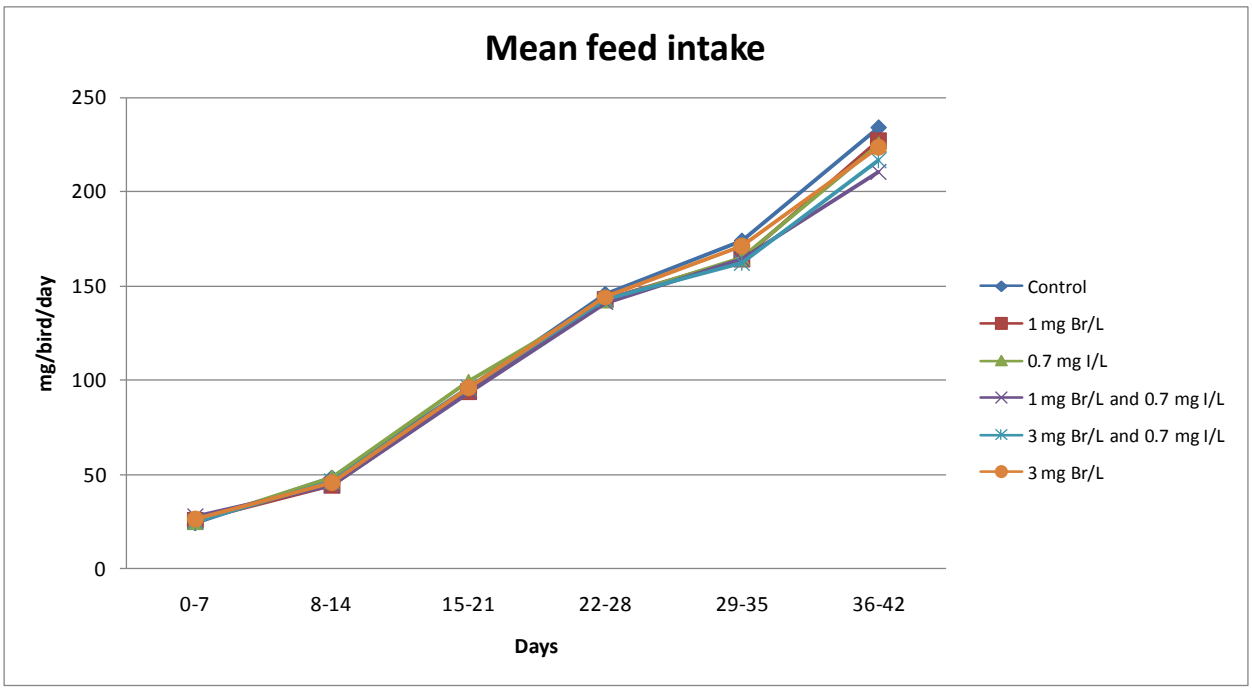


Figure 3.7 Average feed intake

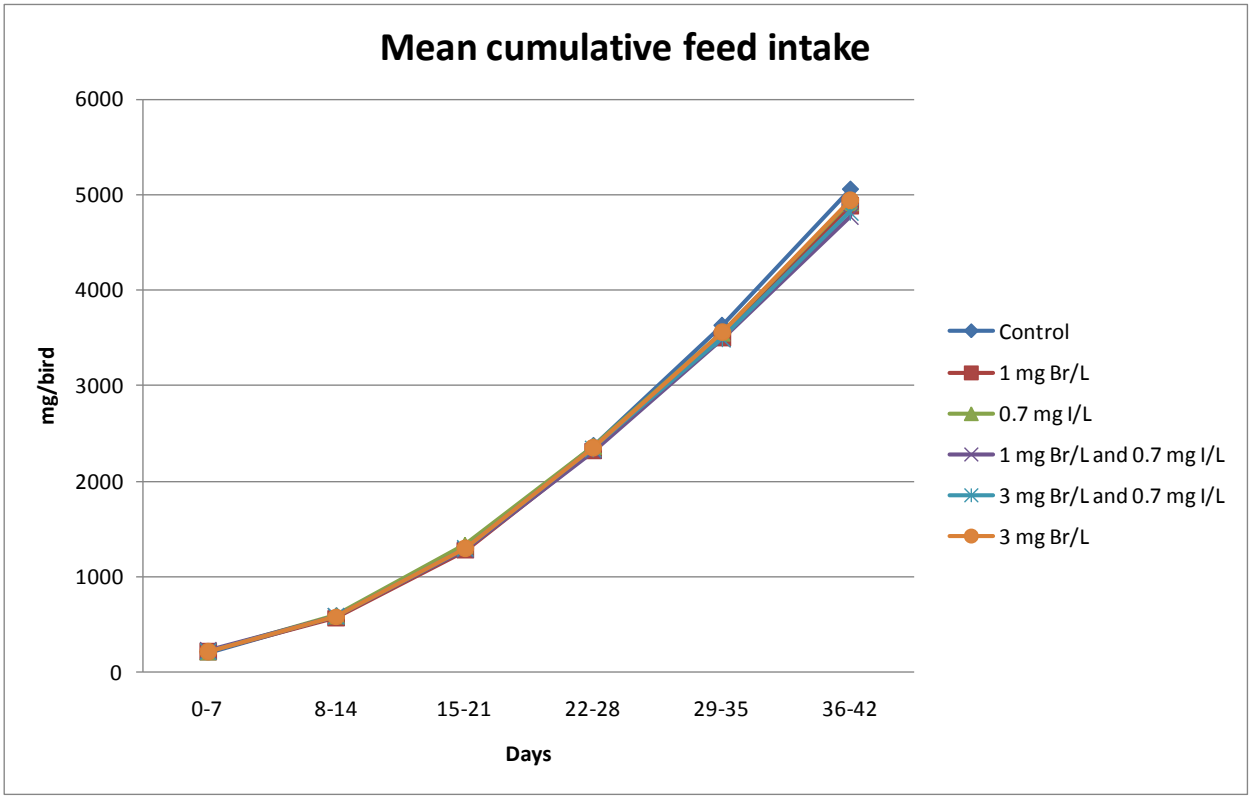


Figure 3.8 Average cumulative feed intake

3.4 Live Weight

Table 3.8 Average daily gain (g/bird/day) of broilers (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

	I levels	
Br treatments	0 mg/L	0.7 mg/L
0 mg/L	72.08[1a] (2.17)	68.87[1a] (3.10)
1 mg/L	72.52[1a] (2.61)	72.74[1a] (6.50)
3 mg/L	70.10[1a] (1.04)	69.41[1a] (1.91)

[ab] *Row means for different I levels within Br levels differ significantly (P<0.05)*
[12] *Column means for different Br levels within I levels differ significantly (P<0.05)*

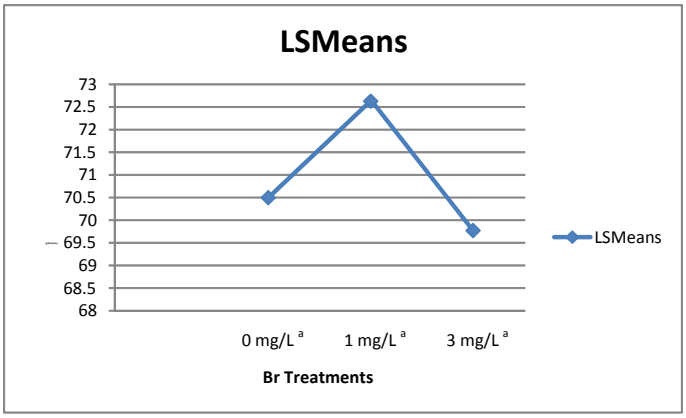


Figure 3.9 The Least Square Means of different bromine (Br) treatments on the average daily gain (g/bird/day) over the production period (P= 0.3055)

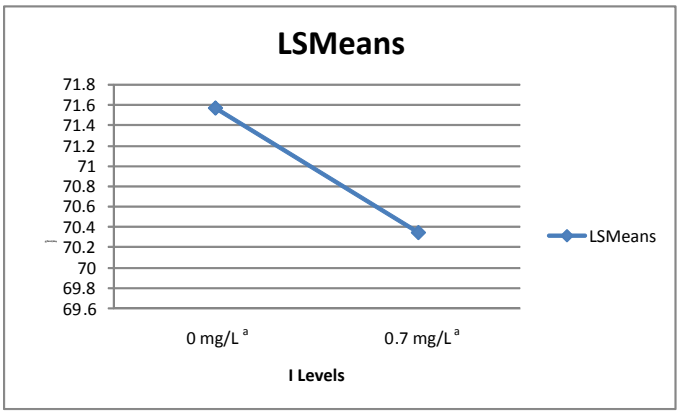


Figure 3.10 The Least Square Means of different iodine (I) levels on the average daily gain (g/bird/day) over the production (P=0.4313)

The mean daily gain and SD are shown in Table 3.9 and the cumulative live weights in Table 3.10. The 5 Treatments did not have an overall effect on average daily weight gain (P = 0.4157). The different levels of I (P = 0.4313) and Br (P = 0.3055) irrespective of any other element or factor also had no significant influence on average daily gain. The interaction between Br and I (P = 0.6382) also had no influence on average daily gain, as shown in Table 3.8.

Figure 3.9 confirms the above because there is no significant difference in the higher average daily gain in the 1 mg Br/L group compared to the 0 and 3 mg Br/L groups. The same can be said for I, where no significance were reported for the 1.3g/bird/day difference between 0 and 0.7 mg I/L treatments (Figure 3.10).

Table 3.9 Average daily gain (g/bird/week) of broilers (means and SD) over the 6 week production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	21.24 [a] (0.57)	20.25 [b] (0.12)	21.26 [a] (0.41)	21.00 [a] (0.36)	21.01 [a] (0.53)	18.93 [c] (0.34)	20.62 (0.38)	0.0003
2	42.25 [a] (0.58)	41.69 [a] (1.20)	42.77 [a] (0.56)	42.25 [a] (1.71)	42.04 [a] (0.91)	42.54 [a] (1.45)	42.26 (1.06)	0.5797
3	70.40 [ab] (3.23)	70.85 [ab] (2.91)	71.85 [a] (1.44)	67.66 [b] (1.61)	69.83 [ab] (1.93)	68.59 [ab] (1.03)	69.86 (2.11)	0.2675
4	96.56 [a] (2.19)	95.70 [a] (4.96)	91.88 [a] (3.71)	93.05 [a] (1.02)	92.35 [a] (2.59)	92.61 [a] (3.55)	93.69 (3.49)	0.6507
5	91.02 [a] (12.01)	87.37 [ab] (3.38)	81.94 [ab] (7.51)	78.04 [b] (4.19)	86.40 [ab] (1.60)	84.83 [ab] (6.26)	84.93 (6.73)	0.3621
6	111.04 [ab] (4.40)	120.58 [ab] (10.26)	103.63 [a] (21.47)	135.20 [b] (9.29)	104.84 [ab] (14.96)	111.04 [ab] (1.39)	114.39 (16.70)	0.2390

[abc] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

Only for week 1 there was a significant overall effect on average daily gain (P = 0.0003), where Br (P = 0.0006) and I (P = 0.0003) individually had significant influences as well as the interaction between Br and I (P = 0.0026), but not for any of the other weeks. After the first 7 days of the trial Treatment 3 (1mg Br/L and 0.7 mg I/L) had the highest average daily gain and Treatment 5 (3mg Br /L) the lowest, but there was no significant difference between Treatment 3 (1mg Br/L and 0.7 mg I/L) and the control group and Treatment 4 (3mg Br/L and 0.7 mg I/L). Treatment 1 (1mg Br/L) had a significantly lower average daily gain than the control group but higher than Treatment 5 (3mg Br /L).

Table 3.10 Average live mass (g/bird) of broilers as weighed once a week (means and SD) over the production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	194.44 [a] (3.98)	187.56 [b] (0.84)	194.64 [a] (2.85)	192.83 [a] (2.52)	192.89 [a] (3.72)	178.31 [c] (2.42)	190.11 (2.65)	0.0003
2	490.22 [a] (4.99)	472.34 [b] (14.46)	494.00 [a] (1.44)	483.27 [ab] (12.58)	487.19 [ab] (4.50)	490.60 [a] (10.46)	486.27 (9.49)	0.2280
3	946.33 [a] (52.88)	968.27 [ab] (23.75)	996.97 [b] (10.40)	956.90 [ab] (20.36)	975.99 [ab] (9.60)	970.74 [ab] (14.40)	969.20 (27.44)	0.4778
4	1658.94 [a] (5.79)	1635.98 [a] (56.69)	1640.16 [a] (34.93)	1608.25 [a] (20.67)	1622.47 [a] (27.70)	1619.03 [a] (14.95)	1630.81 (32.15)	0.5591
5	2296.11 [a] (84.20)	2247.60 [ab] (71.82)	2213.75 [ab] (34.36)	2154.53 [b] (46.51)	2227.29 [ab] (27.72)	2212.82 [ab] (46.12)	2225.35 (60.13)	0.3355
6	3073.37 [a] (91.09)	3091.65 [a] (109.61)	2939.14 [a] (130.13)	3100.91 [a] (273.23)	2961.15 [a] (80.07)	2990.12 [a] (43.59)	3026.06 (132.86)	0.4164

[abc] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

There was an overall significant difference for week 1 (P = 0.0003), where Br (P = 0.0006), I (P = 0.0003) and the interaction of Br and I also had an influence (P = 0.0027), but not for any of the other weeks. When weights were compared in week 6 there were no significant differences in the weights of the different treatments.

Figure 3.11 clearly illustrates the average daily gain difference between Treatment 2 (0.7 mg I/L) and Treatment 3 (1mg Br/L and 0.7 mg I/L). It also shows how at week 5 the same treatment had less average daily gain than the previous weeks. It is clearly visible how the average daily gain of the Treatments were increasing every week but after week 4 there was a negative growth in average daily gain, which increased again after week 5. This was not repeated in Figure 3.12.

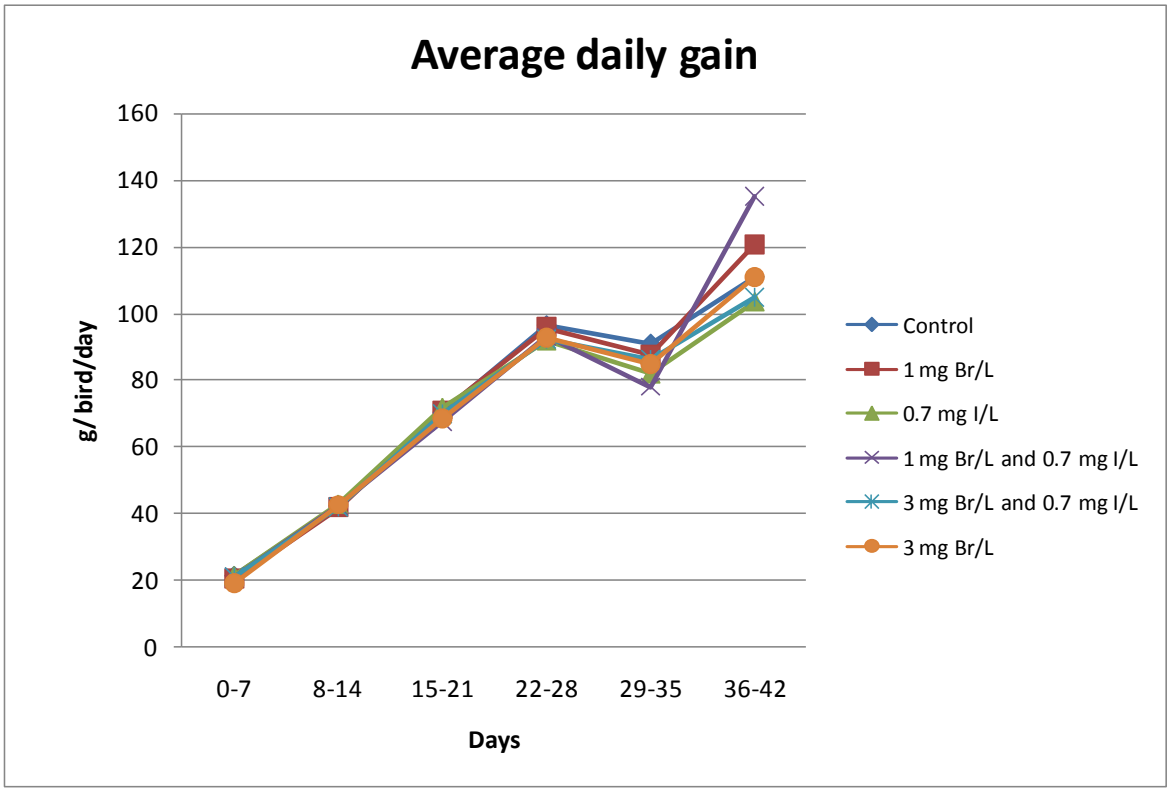


Figure 3.11 Average daily gain

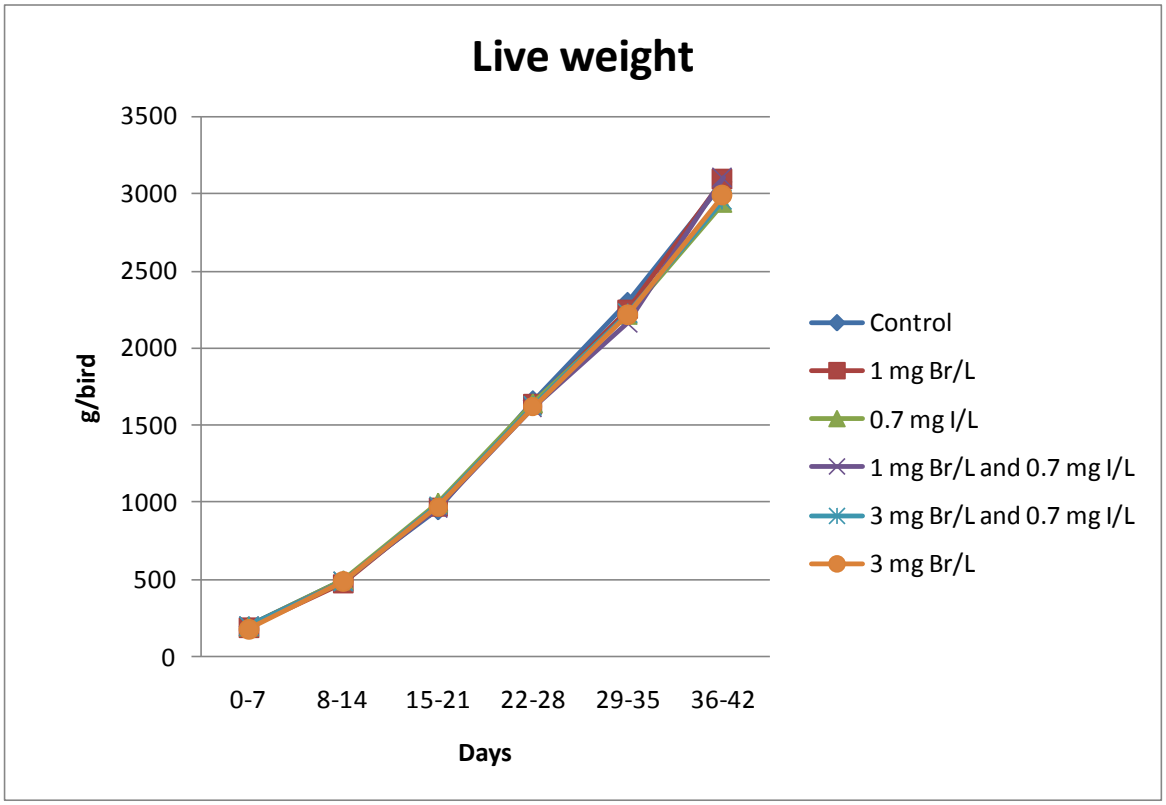


Figure 3.12 Average live weight

3.5 Feed Conversion Ratio (FCR)

Table 3.11 Average FCR (means and SD) of broilers over the production period with the different bromine (Br) and iodine (I) inclusion levels

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
1	1.06 [a]	1.12 [a]	1.07 [a]	1.17 [a]	1.07 [a]	1.21 [a]	1.12	0.3035
	(0.02)	(0.10)	(0.06)	(0.15)	(0.05)	(0.08)	(0.08)	
2	1.19 [a]	1.19 [a]	1.19 [a]	1.20 [a]	1.18 [a]	1.19 [a]	1.19	0.4977
	(0.01)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	
3	1.38 [a]	1.31 [b]	1.33 [ab]	1.34 [ab]	1.32 [ab]	1.34 [ab]	1.34	0.3911
	(0.09)	(0.02)	(0.02)	(0.01)	(0.01)	(0.02)	(0.04)	
4	1.43 [ab]	1.41 [a]	1.44 [ab]	1.44 [ab]	1.44 [ab]	1.45 [b]	1.44	0.4023
	(0.01)	(0.03)	(0.03)	(0.02)	(0.01)	(0.01)	(0.02)	
5	1.58 [ab]	1.55 [a]	1.60 [ab]	1.62 [b]	1.61 [ab]	1.57 [b]	1.59	0.2688
	(0.03)	(0.05)	(0.02)	(0.02)	(0.03)	(0.01)	(0.03)	
6	1.64 [ab]	1.58 [ab]	1.68 [a]	1.54 [b]	1.63 [ab]	1.65 [a]	1.62	0.1727
	(0.03)	(0.05)	(0.09)	(0.11)	(0.05)	(0.02)	(0.06)	

[ab] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

There is no significant difference in the weeks for FCR, but Br (P = 0.0407) irrespective of I and any other factors did have a significant influence on FCR within week 6.

From Figure 3.13 it is apparent that after week 1 the control and Treatment 2 (0.7mg I /L) groups had the most efficient FCR and that Treatment 5 (3mg Br/L) had the most uneconomical FCR, but they did not differ significantly from each other or any of the other treatments. Then at week 6 the most efficient FCR was recognized as Treatment 3 (1mg Br/L and 0.7mg I/L) with the most significant uneconomical treatments identified as Treatment 2 (0.7mg I/L) and Treatment 5 (3mg Br/L).

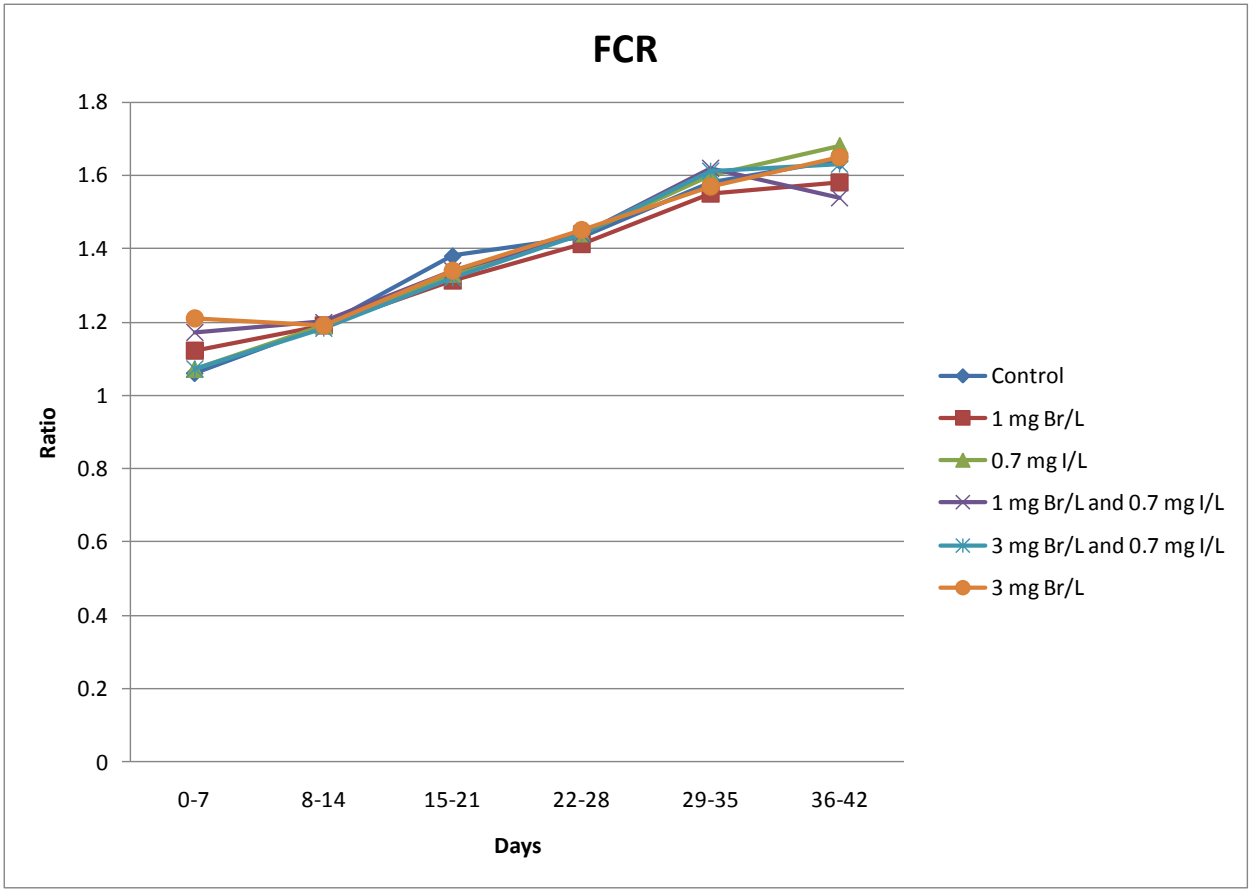


Figure 3.13 Feed Conversion Ratios

3.6 T_3 and T_4 concentration

Table 3.12 Average T_3 hormone levels (pmol/L) (means and SD) of broilers with the different bromine (Br) and iodine (I) levels compared within weeks

Week	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
4	3.33 [a] (0.94)	3.55 [a] (0.74)	3.27 [a] (0.32)	4.10 [a] (0.30)	3.37 [a] (1.46)	4.60 [a] (1.53)	3.70 (0.89)	0.2503
6	2.20 [b] (0.30)	2.78 [a] (0.50)	2.07 [b] (0.35)	2.55 [a] (0.28)	2.53 [a] (0.33)	2.17 [b] (0.16)	2.38 (0.18)	0.0010

[ab] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

Overall when the interaction between Br and I as well as the effect of weeks are taken into consideration there are no significant effects on T_3 for week 4 (P = 0.2503) but there was significance for week 6 (P = 0.0010). Where I (P = 0.0273) irrespective of Br and the interaction between I and Br (P = 0.0019) had an

effect, control and Treatments 2 (0.7 mg I/L) and 5 (3 mg Br/L) had significantly lower T_3 hormone levels than Treatments groups 1 (1 mg Br/L), 3 (1 mg Br/L and 0.7 mg I/L) and 4 (3 mg Br/L and 0.7 mg I/L).

Table 3.13 Average T_4 hormone levels (pmol/L) (means and SD) of broilers with the different bromine (Br), iodine (I) levels compared within weeks

Week	Treatments						Means	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
4	6.27 [a] (0.89)	6.38 [a] (1.54)	5.52 [a] (0.40)	5.77 [a] (0.73)	6.50 [a] (0.60)	5.42 [a] (0.28)	5.98 (0.91)	0.7412
6	6.00 [a] (1.09)	5.59 [a] (0.62)	5.89 [a] (0.75)	5.80 [a] (0.24)	5.10 [a] (0.00)	5.29 [a] (0.33)	5.61 (0.63)	0.5360

[ab] *Row means with different superscripts within weeks differ significantly (P< 0.05) according to the Fischer's Test*

When all of the interactions between Br, I and the effect of week 4 are taken into consideration there were no overall effect on T_4 (P = 0.7412). Again there was no significant effect on T_4 levels when the interaction between Br and I and the effect of week 6 were taken into account (P = 0.5360). The treatments with 3 mg Br/L inclusion levels had the lowest T_4 levels but did not differ significantly from the other treatments.

Table 3.14 Average T_3 hormone levels (pmol/L) (means and SD) of broilers compared between weeks

Week	Treatments						Means
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L	
4	3.33 [a] (0.94)	3.55 [a] (0.74)	3.27 [a] (0.32)	4.10 [a] (0.30)	3.37 [a] (1.46)	4.60 [a] (1.53)	3.70 (0.89)
6	2.20 [b] (0.30)	2.78 [a] (0.50)	2.07 [b] (0.35)	2.55 [b] (0.28)	2.53 [a] (0.33)	2.17 [b] (0.16)	2.38 (0.18)

[ab] *Column means with different superscripts between weeks differ significantly (P< 0.05) according to the Fischer's Test*

Overall when all of the different elements and factors are taken into account there was an overall effect on T_3 hormone levels (P = 0.0005). Figure 3.14 illustrates that Treatment 5 had the most prevalent decrease in T_3 hormone level from week 4 (4.60 pmol/L) to week 6 (2.17 pmol/L) with Treatment 3 (1 mg Br/L and 0.7 mg I/L) following, at week 4 T_3 measured 4.10 pmol/L and 2.55 mpol L in week 6. Treatment 1 (1 mg Br/L) also incurred a decrease in T_3 but not significantly. Treatment 4 (3 mg Br/L and 0.7 mg I/L) and the control group had less T_3 reported at week 6 than week 4, where the control group had a significant decrease. The control groups' levels were adjacent to the levels of Treatment 2 (0.7 mg I/L), which also experienced a significant decrease in T_3 levels from week 4 (3.33 and 3.27 pmol/L) to week 6 (2.20 and 2.78 pmol/L).

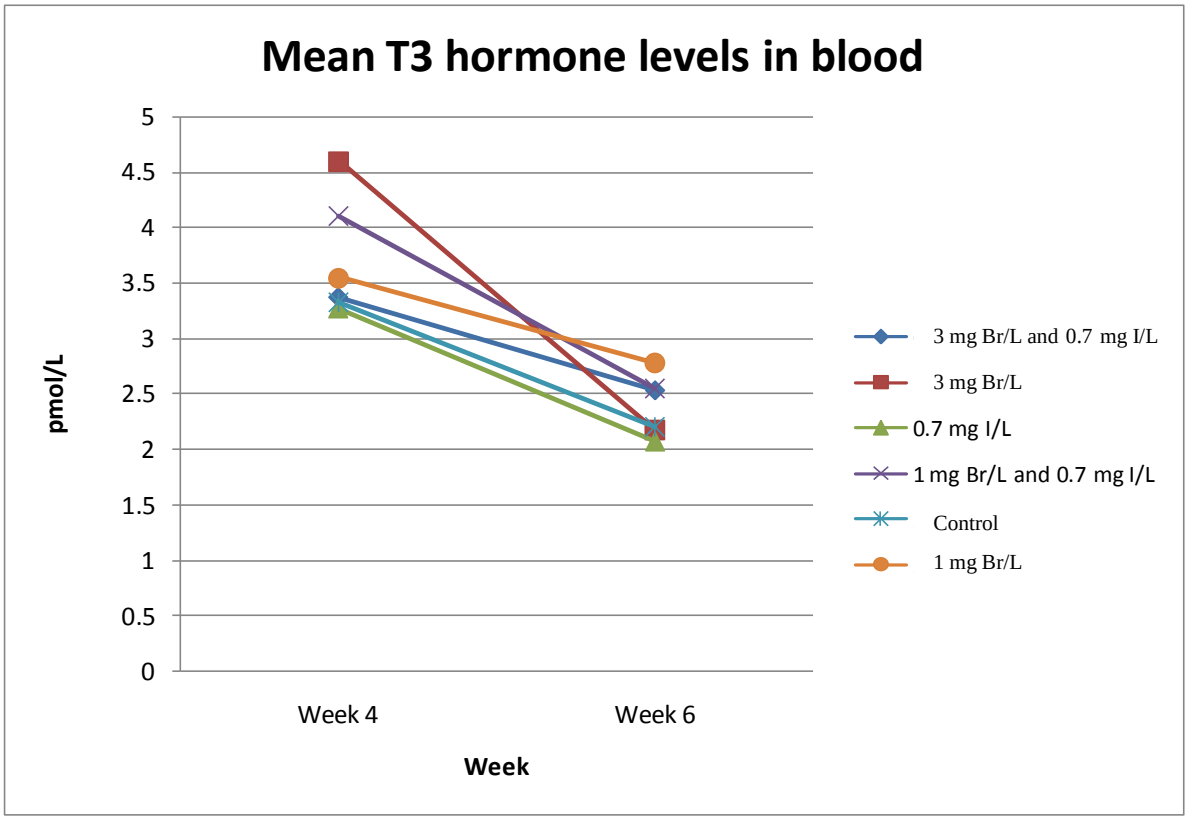
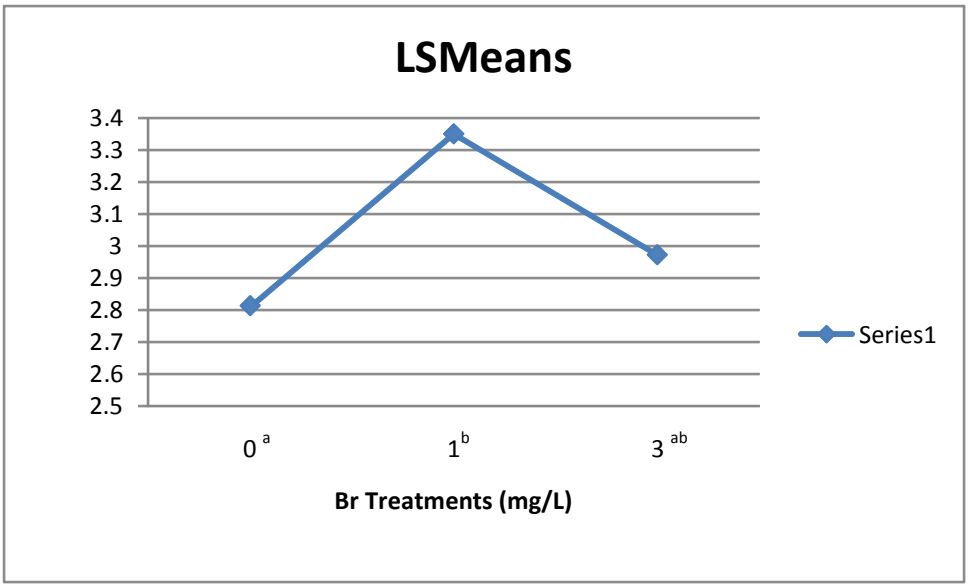


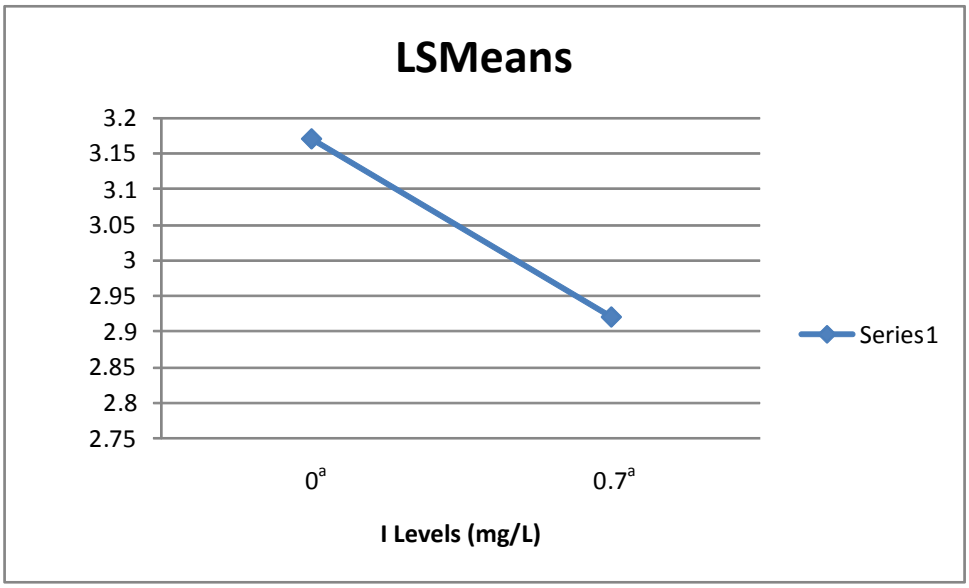
Figure 3.14 Mean T_3 hormone levels in the blood

Further statistical analyses were done on the hormone levels to locate what might have been the cause of the above mentioned effects. Figure 3.15 reveals that 1 mg Br /L treatment had significantly higher T_3 levels in the blood than 0 mg Br/L, but the difference between the 1 mg Br/L was not significantly higher than the 3 mg Br/L treatment, indicating that Br may have an advanced effect, with an elongated Br exposure period. Figure 3.16 confirms than I had no effect on T_3 production. Figure 3.17 confirms that the time exposure period had an influence on T_3 production. For all of the Br treatments irrespective, of I, week 4 had significantly higher T_3 hormone production than for week 6 (Figure 3.19). The same trend was observed when the effect of weeks and I levels, irrespective of Br, was tested; week 6 had lower T_3 levels than week 4 (Figure 3.20). From these figures the observation can be made that Br had the biggest influence on T_3 hormone levels.



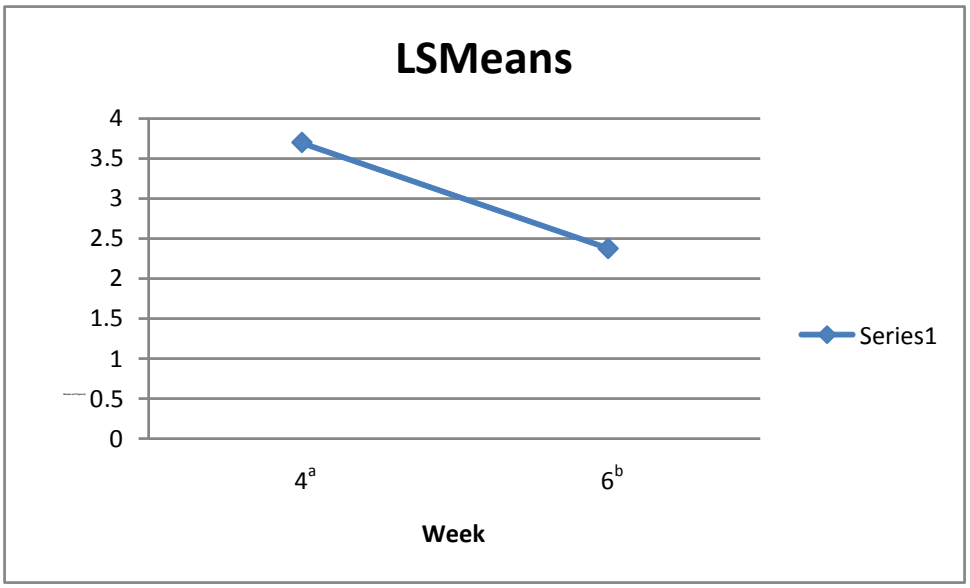
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.15 Least Square Means of T_3 hormone levels (pmol/L) of broilers when only bromine (Br) in the drinking water over the production cycle, was taken into account



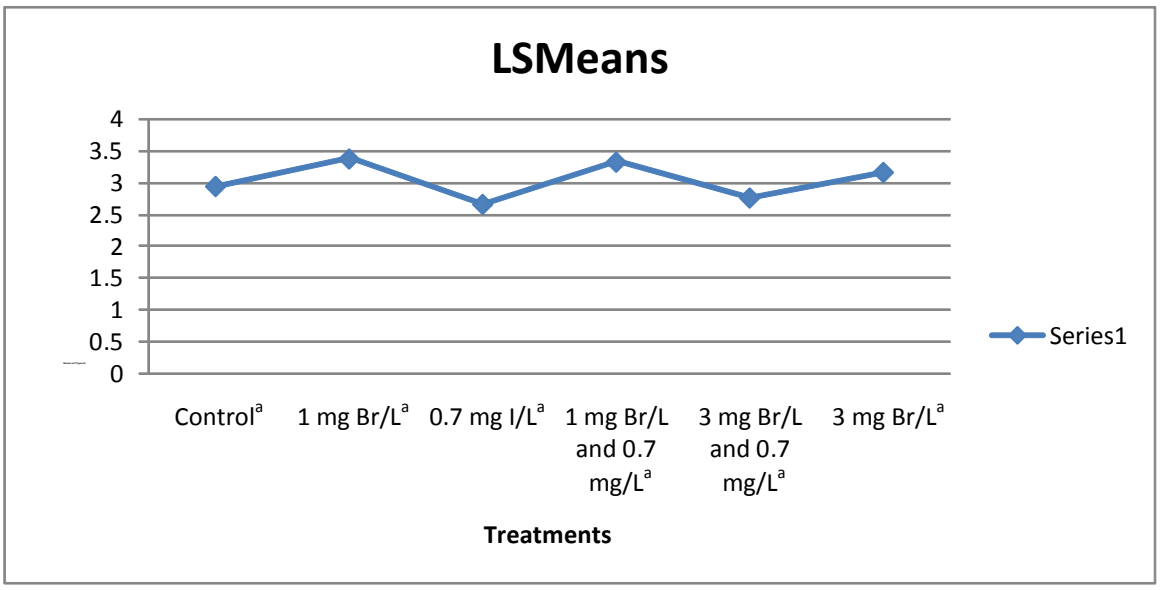
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.16 Least Square Means of T_3 hormone levels (pmol/L) of broilers when only iodine (I) in the drinking water over the production cycle, was taken into account



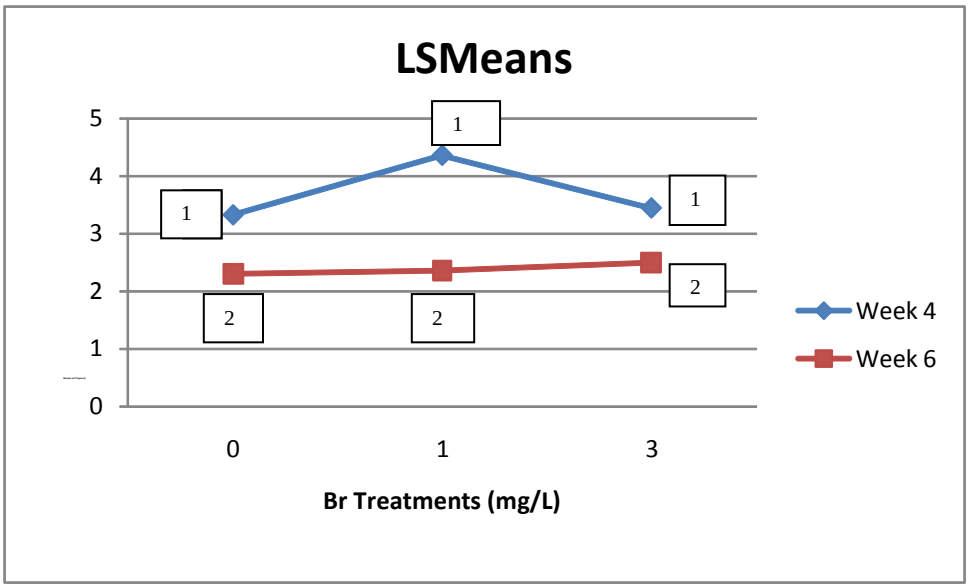
[ab] Weeks with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.17 Least Square Means of T_3 hormone levels (pmol/L) of broilers when only the week effect, was taken into account



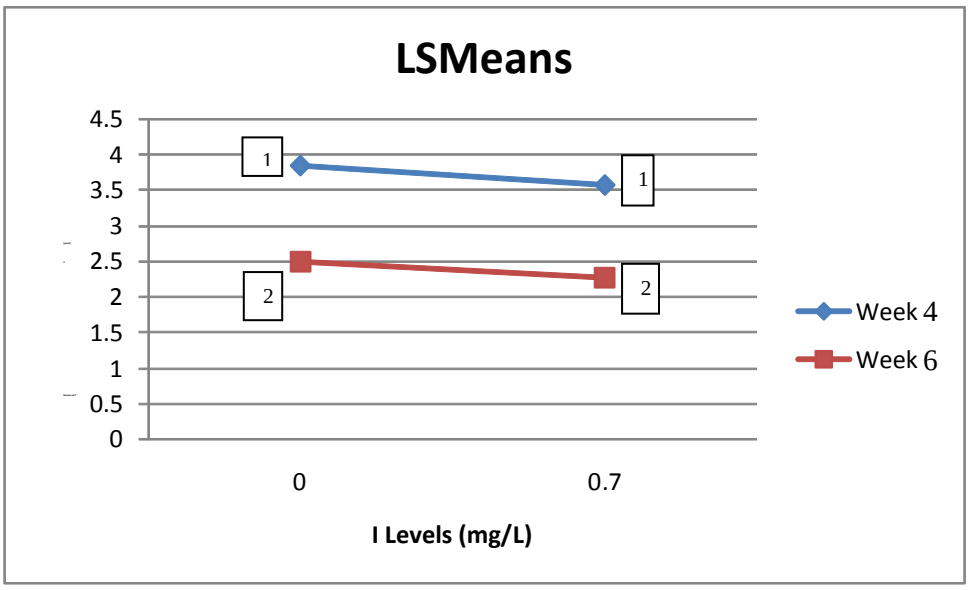
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.18 Least Square Means of T_3 hormone levels (pmol/L) of broilers when the interaction between bromine (Br) and iodine (I) over the production cycle, was taken into account



[12] Means with different superscripts between weeks differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.19 Least Square Means of T_3 hormone levels (pmol/L) of broilers when the interaction between bromine (Br) and weeks over the production cycle, was taken into account



[12] Means with different superscripts between weeks differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.20 Least Square Means of T_3 hormone levels (pmol/L) of broilers when the interaction between iodine (I) and weeks over the production cycle, was taken into account

Table 3.15 Average T_4 hormone levels (pmol/L) (means and SD) of broilers compared between weeks

Week	Treatments						Mean
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L	
4	6.27 [a] (0.89)	6.38 [a] (1.54)	5.52 [a] (0.40)	5.77 [a] (0.73)	6.50 [a] (0.60)	5.42 [a] (0.28)	5.98 (0.91)
6	6.00 [a] (1.09)	5.59 [a] (0.62)	5.89 [a] (0.75)	5.80 [a] (0.24)	5.10 [b] (0.00)	5.29 [a] (0.33)	5.61 (0.63)

[ab] *Column means with different superscripts between weeks differ significantly (P< 0.05) according to the Fischer's Test*

From Figure 3.21 it is clear that Treatment 4 (3 mg Br/L and 1 mg I/L) had a significant decrease in T_4 levels from week 4 to week 6. In addition the control group and Treatment 1 (1 mg Br/L) experienced a decrease in T_4 levels, which was not significant. The T_4 levels of Treatments 2 (0.7 mg I/L) and 3 (1 mg Br/L and 0.7 mg I/L) in fact increased from week 4 (5.52 and 5.77 pmol/L) to week 6 (5.89 and 5.80 pmol/L), but these increases were insignificant. Treatment 5 (3 mg Br/L) virtually remained constant.

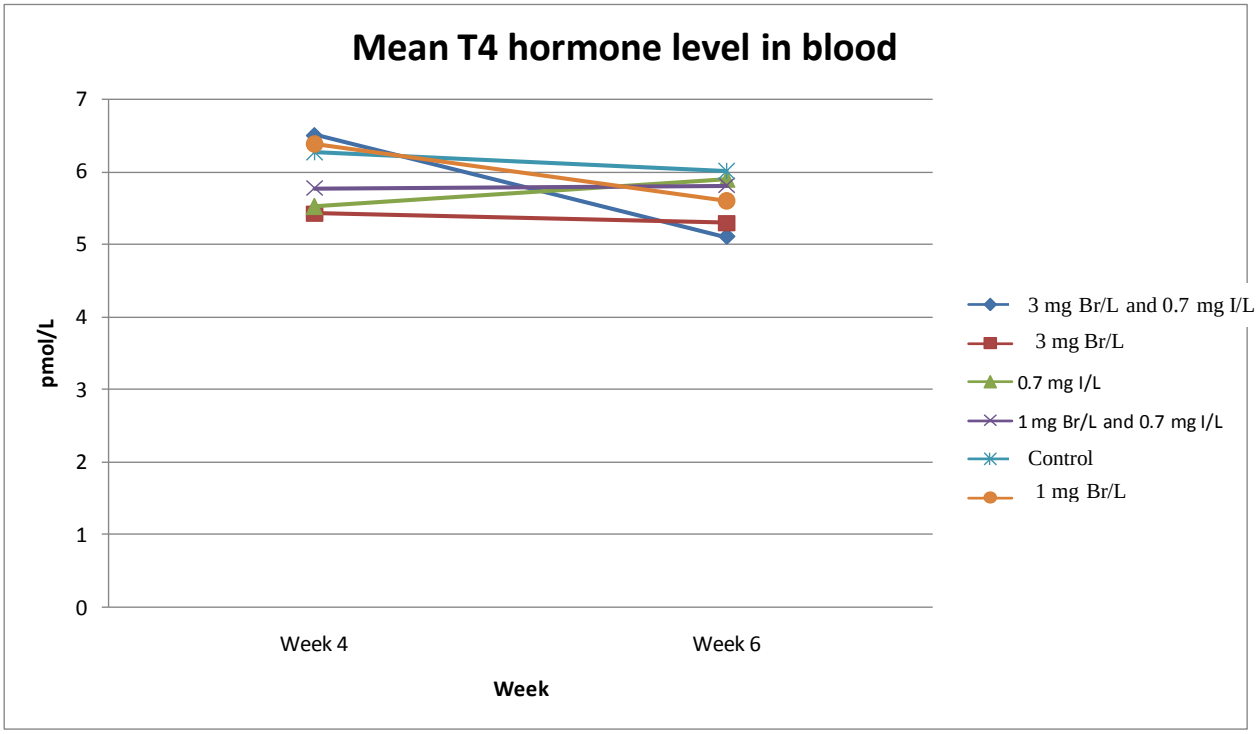
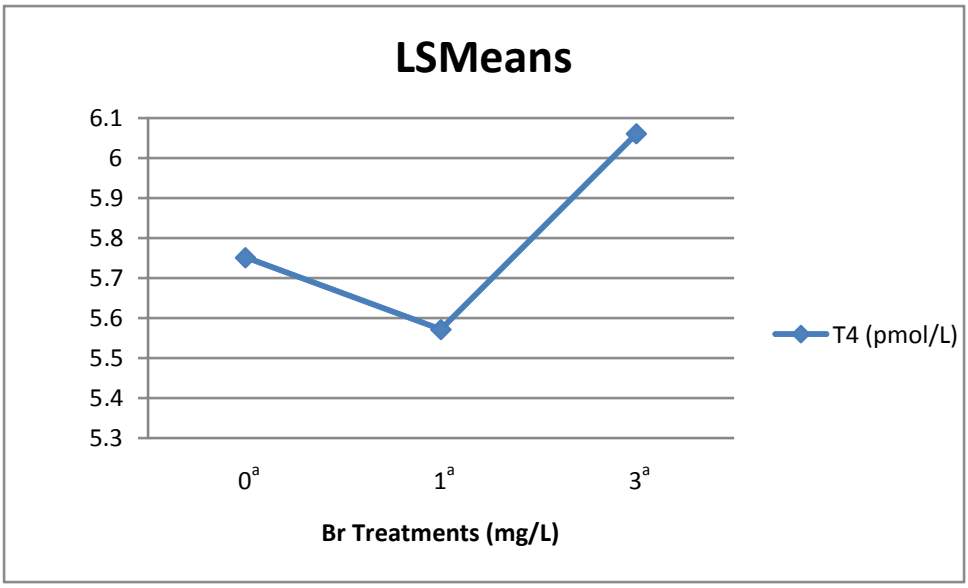


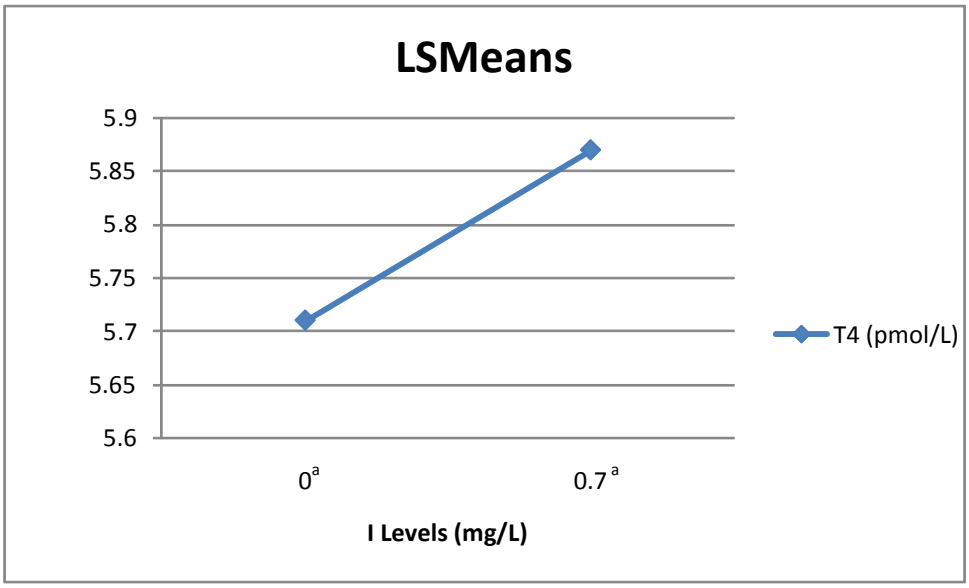
Figure 3.21 Mean T_4 hormone levels in the blood

No significance occurred with more comprehensive analyses of T_4.hrmone levels, within week 4 and 6; but Figure 3.27 illustrates the significant difference between week 4 and week 6. In this figure the 0.00 mg I/L inclusion level had a significantly more T_4 production at week 4 than at week 6. But the T_4 hormone levels measured at a 0.7 mg I/L level was the same for week 4 and week 6. This figure illustrates the essentiality of I for T_4 hormone production.



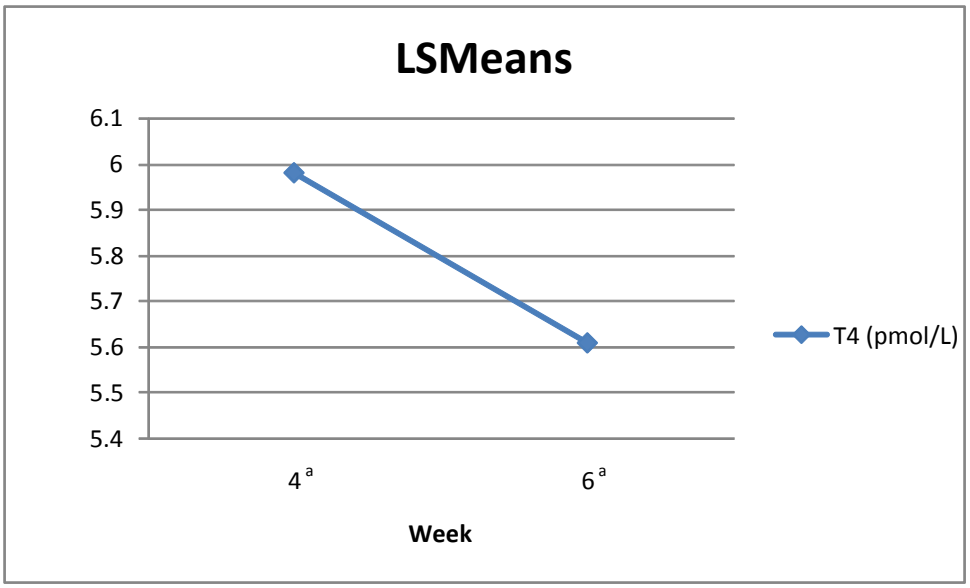
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.22 Least Square Means of T_4 hormone levels (pmol/L) of broilers when only bromine (Br) in the drinking water over the production cycle, was taken into account



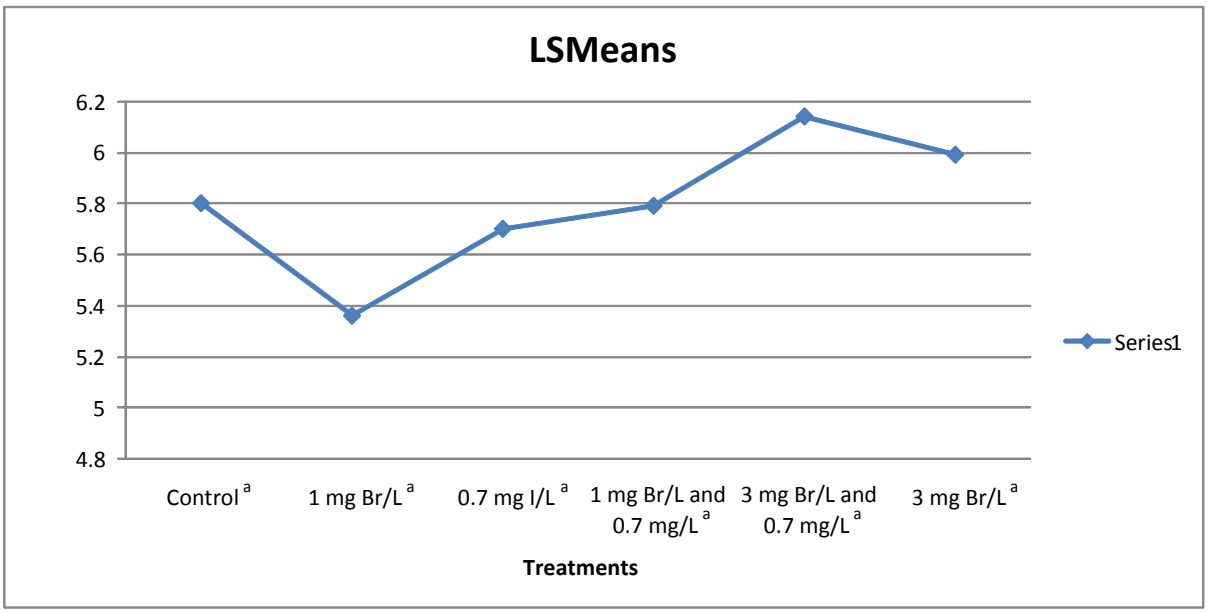
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.23 Least Square Means of T_4 hormone levels (pmol/L) of broilers when only iodine (I) in the drinking water over the production cycle, was taken into account



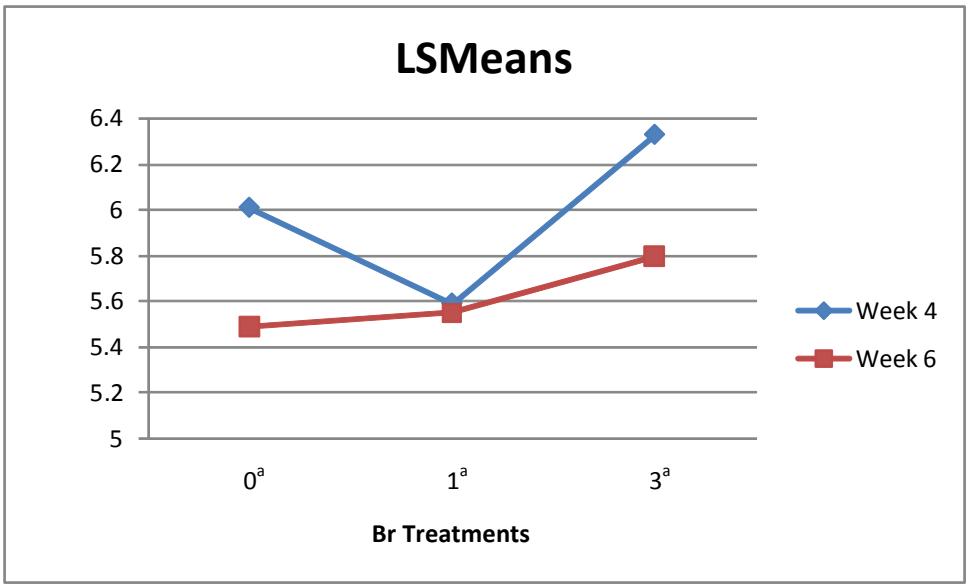
[ab] Weeks with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.24 Least Square Means of T_4 hormone levels (pmol/L) of broilers when only the week effect, was taken into account



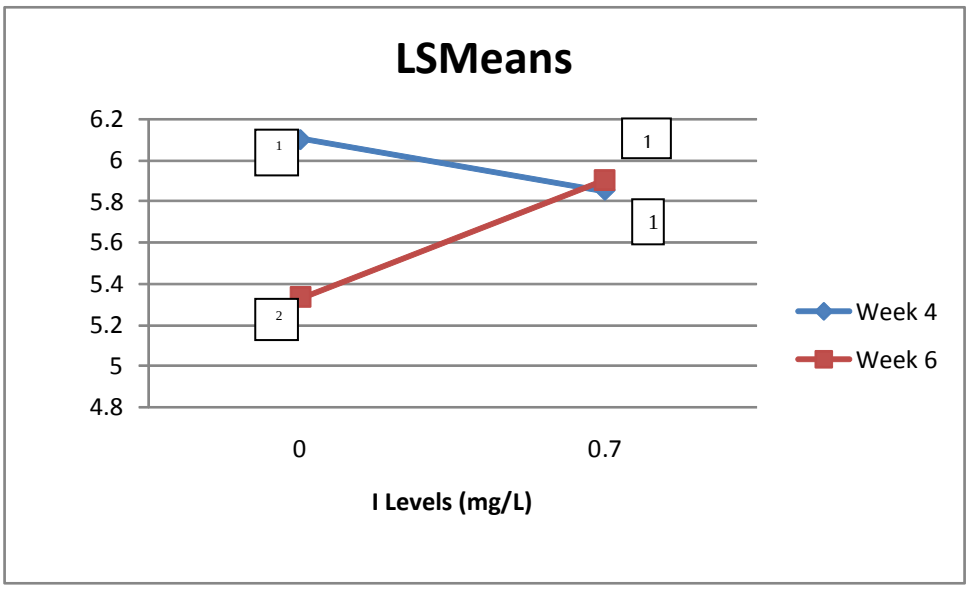
[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.25 Least Square Means of T_4 hormone levels (pmol/L) of broilers when the interaction between bromine (Br) and iodine (I) over the production cycle, was taken into account



[ab] Treatments between weeks with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.26 Least Square Means of T_4 hormone levels (pmol/L) of broilers when the interaction between bromine (Br) and weeks over the production cycle, was taken into account



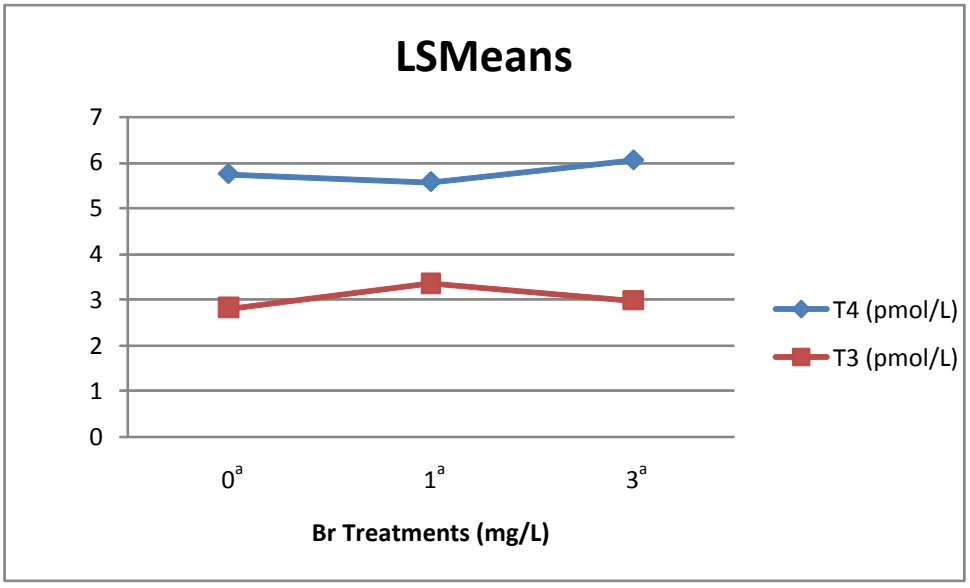
[12] Means with different superscripts between weeks differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.27 Least Square Means of T_4 hormone levels (pmol/L) of broilers when the interaction between iodine (I) and weeks over the production cycle, was taken into account

When comparing T_3 and T_4 with each other Figure 3.28 shows how T_4 and T_3 had the opposite effect on Br treatments, where T_4 levels first decreased and then increased as Br treatments increased, T_3 hormone levels first increased and then decreased. The same behaviour was observed in Figure 3.29, as I

treatments increased T_4 increased as well, and then as I increased T_3 levels decreased. In Figure 3.30 the effect of weeks was the same for T_3 and T_4 hormone levels both decreased in week 2. Figure 3.31 again illustrates the opposite reactions of the two hormones, when the interaction of Br and I is taken into account.

In Figure 3.32, where the interaction of Br and weeks was tested; it is exceptionally clear that the difference between the amounts of T_3 produced in week 6 was considerably less than in Week 4; the same trend was observed for the T_4 production but at a significantly lower level. Figure 3.33 represents the interaction between I and weeks and a reduction in T_3 production was seen but T_4 levels decreased in week 4 but increased at week 6 as I levels increased.



[ab] Treatments with different superscripts' means differ significantly (P< 0.05) according to the Fischer's Test

Figure 3.28 Comparing T_4 and T_3 hormone levels at different bromine (Br) treatments

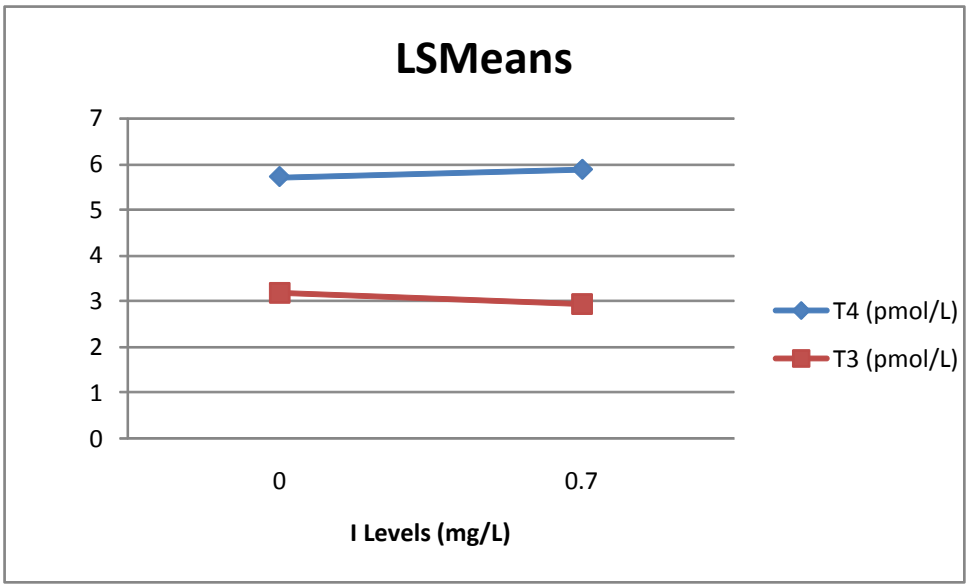


Figure 3.29 Comparing T_4 and T_3 hormone levels (pmol/L) at different iodine (I) levels

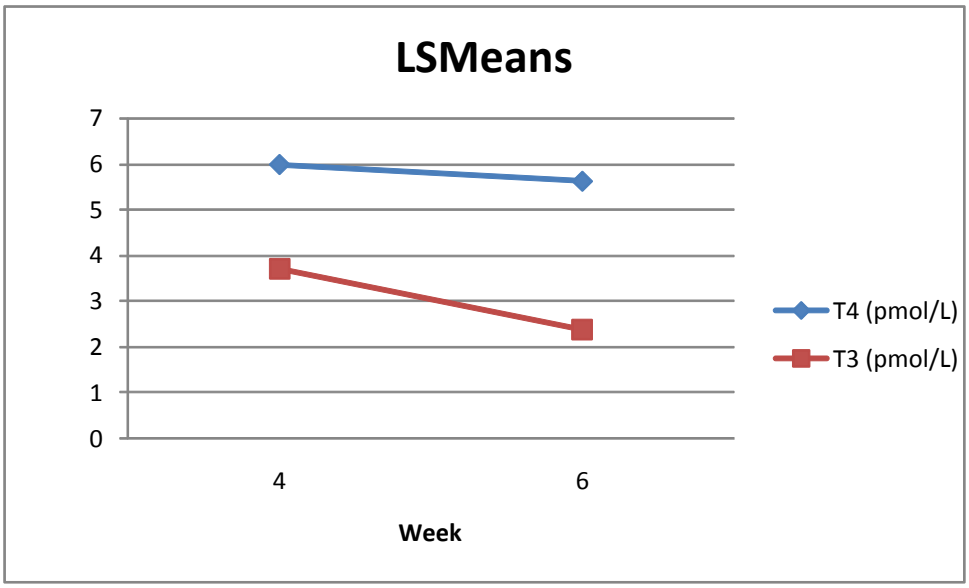


Figure 3.30 Comparing T_4 and T_3 hormone levels (pmol/L) at weeks

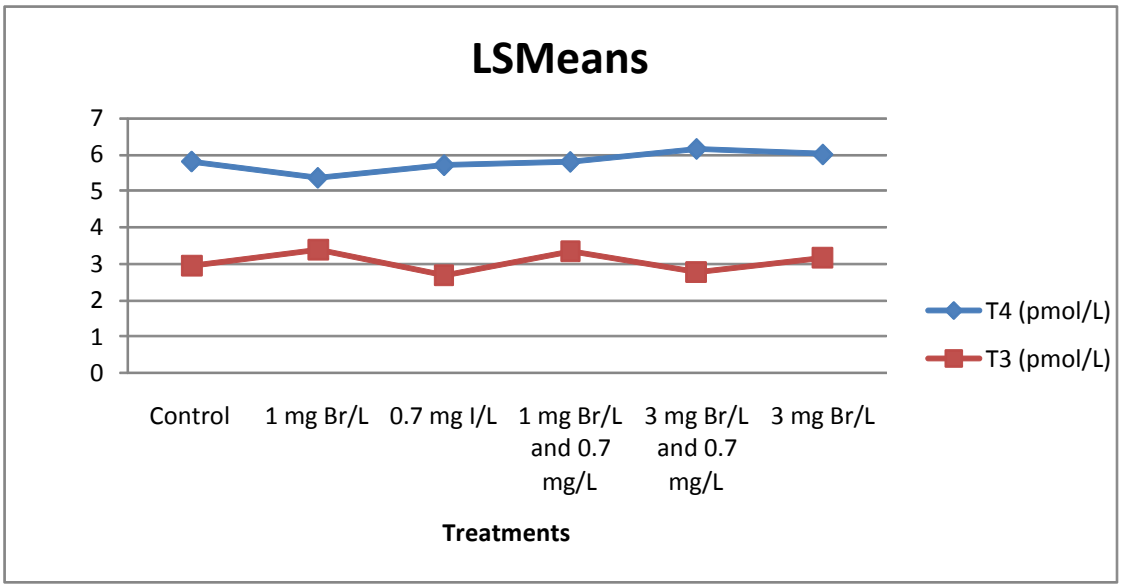


Figure 3.31 Comparing T_4 and T_3 hormone levels (pmol/L) at different treatments of bromine (Br) and iodine (I) interactions

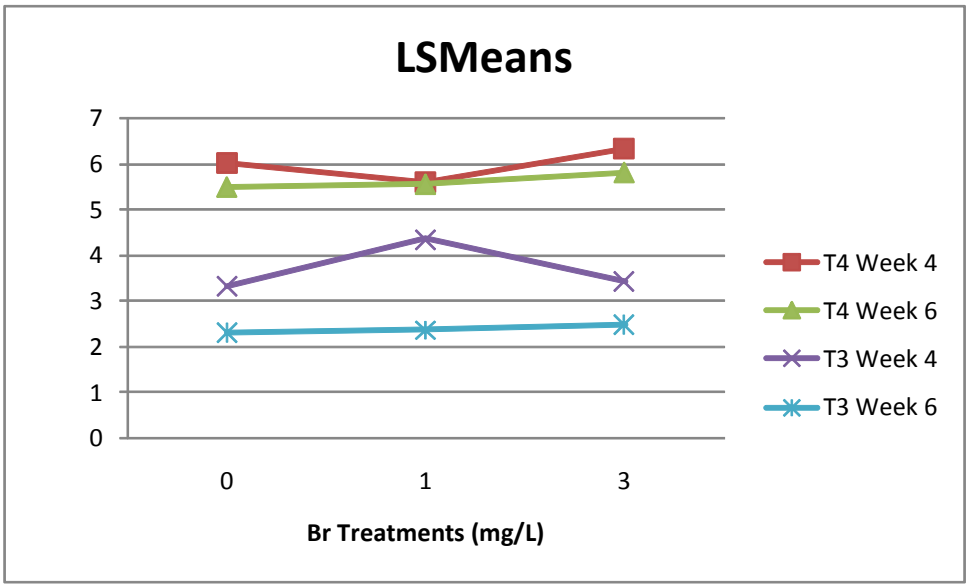


Figure 3.32 Comparing T_3 and T_4 (pmol/L) when bromine (Br) and week effects were taken into account

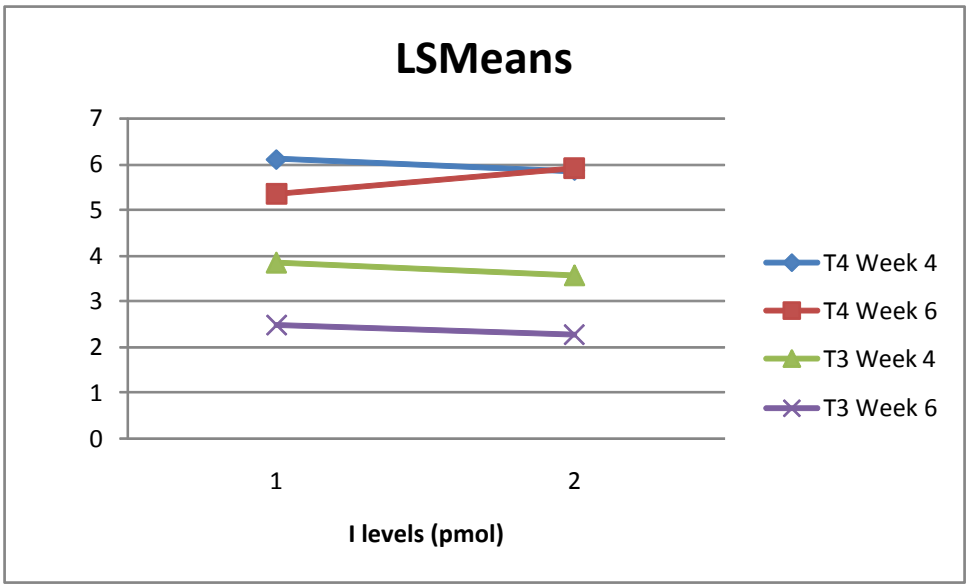


Figure 3.33 Comparing T_3 and T_4 (pmol/L) when iodine (I) and week effects were taken into account

3.7 Thyroid gland

Table 3.16 Average bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of chicken thyroid gland after 6 weeks of different Bromine (Br) treatments

Br treatments (mg/L)	Br content	I content
0	10.49 [a] (9.36)	1.67 [a] (4.50)
1	26.77 [ab] (25.99)	0.22 [a] (0.11)
3	46.74 [b] (66.50)	0.20 [a] (0.13)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

The overall effect of Br is shown in Table 3.16 and for I in Table 3.17, and the interaction between Br and I in Table 3.18. Br had an overall effect (P = 0.0457) on the thyroid glands' Br and I content. It can be seen from Table 3.16 that as Br administration increased the Br content within the thyroid gland increased. Treatment 5 (3 mg Br /L) had significantly accumulated more Br in the thyroid gland than the control group. Treatment 1 (1 mg Br/L) does not differ significantly from the control group, due to high a high standard deviation (SD). Although the control treatment had 8 times the I content than that of the Br treated groups the difference was not significant (Table 3.16 and Figure 3.34).

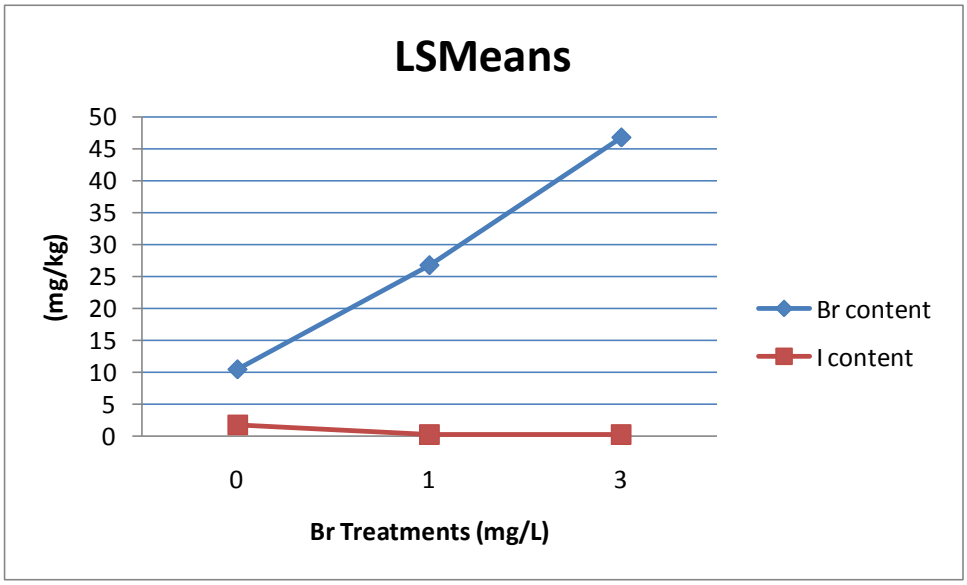


Figure 3.34 Bromine (Br) and iodine (I) (mg/kg) content of chicken thyroid gland after 6 weeks of different bromine (Br) treatments

Table 3.17 Average bromine (Br) and iodine (I) (mg/kg) content (means and SD) of chicken thyroid gland after 6 weeks of different iodine (I) treatments

I levels (mg/L)	Br content	I content
0	31.85 [a] (55.53)	1.02 [a] (3.67)
0.7	24.15 [a] (26.57)	0.37 [a] (0.69)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

As I levels increased within the drinking water, the Br concentrations quantified within the thyroid gland decreased and the I concentrations increased, but the differences were insignificant (P = 0.3950). As can be seen in Table 3.17 the Br content in the thyroid gland was less for the 0.7mg I/L than when the 0 mg I/L was administered, which indicates that an increase of I intake diminished the accumulation effect of Br. However, as Table 3.17 indicates there were no significant differences between the different levels of I because the SD's were high. The I content of the thyroid gland was not altered by the different I treatments (Figure 3.35).

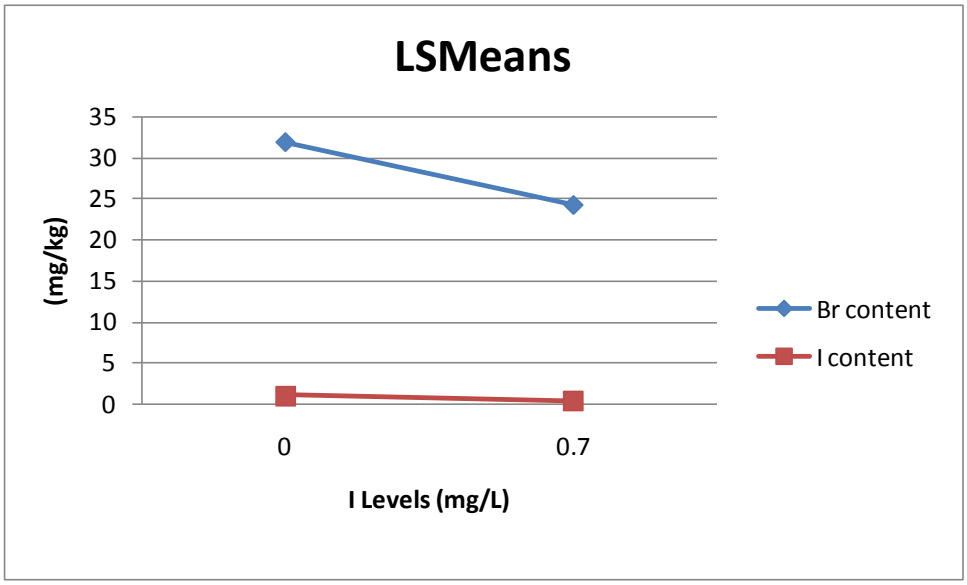


Figure 3.35 Bromine (Br) and iodine (I) (mg/kg) content of chicken Thyroid gland after 6 weeks of different iodine (I) treatments

Table 3.18 Average thyroid gland bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of broilers exposed to the interaction of bromine (Br) and iodine (I) over the production cycle

Element	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
Br	12.52 [ab] (11.25)	28.87 [ab] (33.48)	8.46 [a] (7.50)	24.67 [ab] (18.85)	39.32 [ab] (37.65)	54.16 [b] (90.43)	27.80 (38.04)	0.0457
I	2.70 [a] (6.37)	0.18 [a] (0.10)	0.63 [a] (1.20)	0.26 [a] (0.13)	0.24 [a] (0.14)	0.17 [a] (0.12)	0.70 (2.60)	0.3950

[ab] *Row means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

Within the thyroid gland, the interaction between Br and I had a significant effect on the Br content of the thyroid gland (P = 0.0457) but not on the I content (P = 0.3950). Within the thyroid gland Treatment 2 (0.7mg I/L) had the lowest content of Br and Treatment 5 (3mg Br/L) had the highest Br content. The difference between these two treatments was significant (P = 0.0467). The Br content measured in Treatment 4 (3mg Br/L and 0.7mg I/L) was less than Treatment 5 (3mg Br/L) but the difference was insignificant. Treatment 4 (3mg Br/L and 0.7mg I/L) did not have significantly higher Br content than the control group. Although Treatment 5 (3 mg Br/L) and Treatment 1 (1 mg Br/L), the Br treated groups, had the lowest I content the differences weren't significant (P = 0.3950) from any other treatment.

3.8 Liver

Table 3.19 Average bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of chicken liver after 6 weeks of different bromine (Br) treatments

Br treatments (mg/L)	Br content (mg/kg)	I content (mg/kg)
0	9.11 [a] (9.33)	0.26 [a] (0.15)
1	24.93 [a] (24.41)	0.34 [a] (0.16)
3	86.59 [b] (111.88)	0.24 [a] (0.13)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

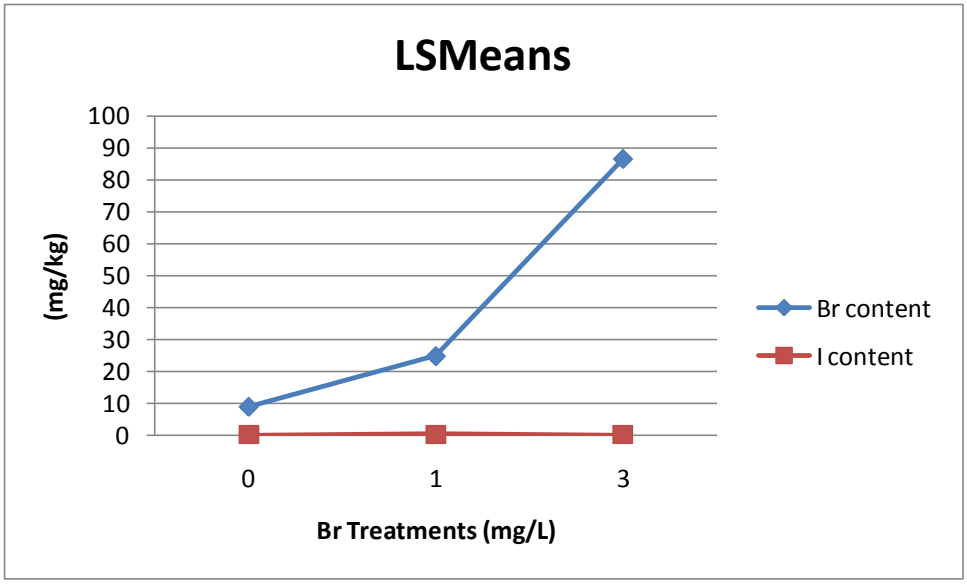


Figure 3.36 Bromine (Br) and iodine (I) (mg/kg) content of chicken liver after 6 weeks of different bromine (Br) treatments

The overall effect of Br is shown in Table 3.19 and for I in Table 3.20. The interaction between Br and I is shown in Table 3.21. Overall Br, irrespective of I, had a significant influence on the liver (P = 0.0025) where the different treatments of Br had a significant influence (P = 0.0056) on the Br and I content of the liver. Again as was seen with the thyroid gland, as the Br treatments increased the quantity of Br was significantly higher within the liver. A significantly higher amount of Br accumulated within the liver when 3 mg Br/L was administered than with the control group or in the 1mg Br /L treatment. However there was no significant difference between the control group and the 1 mg Br/L group. Treatment 5 (3 mg Br/L) measured the lowest I concentrations within the thyroid, but these levels weren't significantly less than the other treatment groups (Figure 3.36).

Table 3.20 Average bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of chicken liver after 6 weeks of different iodine (I) treatments

I levels (mg/L)	Br content (mg/kg)	I content (mg/kg)
0	46.58 [a] (80.36)	0.30 [a] (0.15)
0.7	33.83 [a] (66.06)	0.27 [a] (0.14)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's*

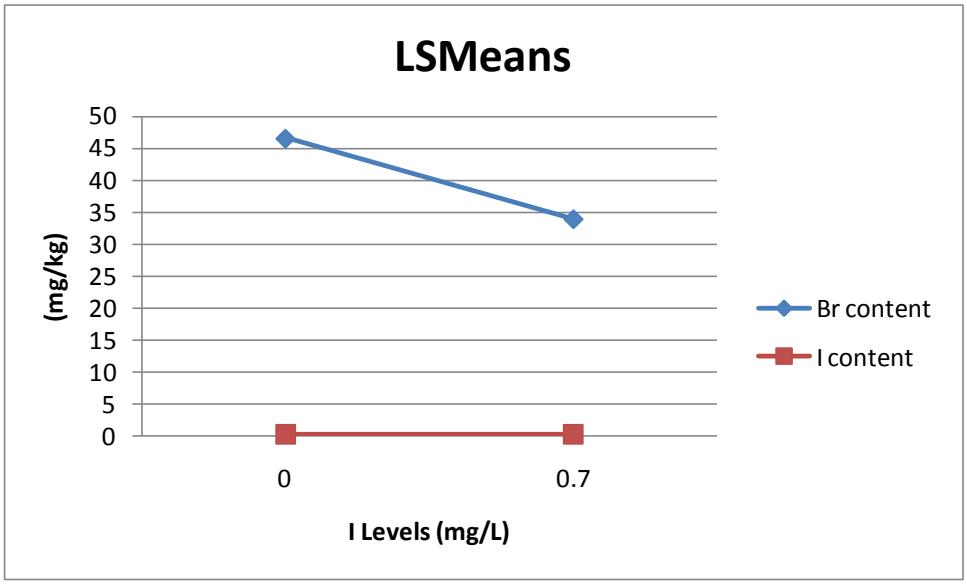


Figure 3.37 Bromine (B) and iodine (I) (mg/kg) content of chicken liver after 6 weeks of different iodine (I) treatments

As reported in Table 3.20, the measured Br content in the thyroid gland was less, where 0.7 mg I/L was administered, which indicates that an increase of I intake diminished the accumulation effect of Br, but there was no significant difference between the control group and Treatment 2 (0.7 mg I/L) because the SD was very high. No difference in the I content was observed (Figure 3.37).

Table 3.21 Average liver bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of broilers exposed to the interaction of bromine (Br) and iodine (I) over the production cycle

Element	Treatments						Mean	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
Br	12.04 [ab] (11.92)	32.11 [abc] (27.56)	6.18 [b] (5.36)	17.75 [ab] (20.68)	77.58 [ac] (104.19)	95.59 [c] (128.41)	40.21 (56.57)	0.0025
I	0.27 [a] (0.15)	0.35 [a] (0.18)	0.26 [a] (0.16)	0.33 [a] (0.14)	0.21 [a] (0.12)	0.28 [a] (0.14)	0.28 (0.13)	0.0235

[abc] *Row means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

The interaction of Br and I had a significant influence on the accumulation of Br (P = 0.0025) and I (P = 0.0235) within the liver. Again it was observed that Treatment 2 (0.7 mg I/L) had the lowest content of Br accumulated and Treatment 5 (3 mg Br/L) had the highest concentration of Br. The difference between these two treatments was significant and this was also seen in the thyroid gland. There was also significantly less Br accumulation in the liver within the control group compared to Treatment 5 (3 mg Br/L). Even Treatment 4 (3 mg Br/L and 0.7 mg I/L) had significantly higher Br levels in the kidney than Treatment 3 (1 mg Br/L and 0.7 mg I/L), but Treatment 4 (3 mg Br/L and 0.7 mg I/L) did not differ significantly from the control group.

3.9 Kidney

Table 3.22 Average bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of chicken kidney after 6 weeks of different bromine (Br) treatments

Br treatments (mg/L)	Br content (mg/kg)	I content (mg/kg)
0	27.27 [a] (31.70)	0.29 [a] (0.15)
1	87.36 [ab] (87.09)	0.43 [b] (0.16)
3	185.54 [b] (254.86)	0.27 [a] (0.15)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

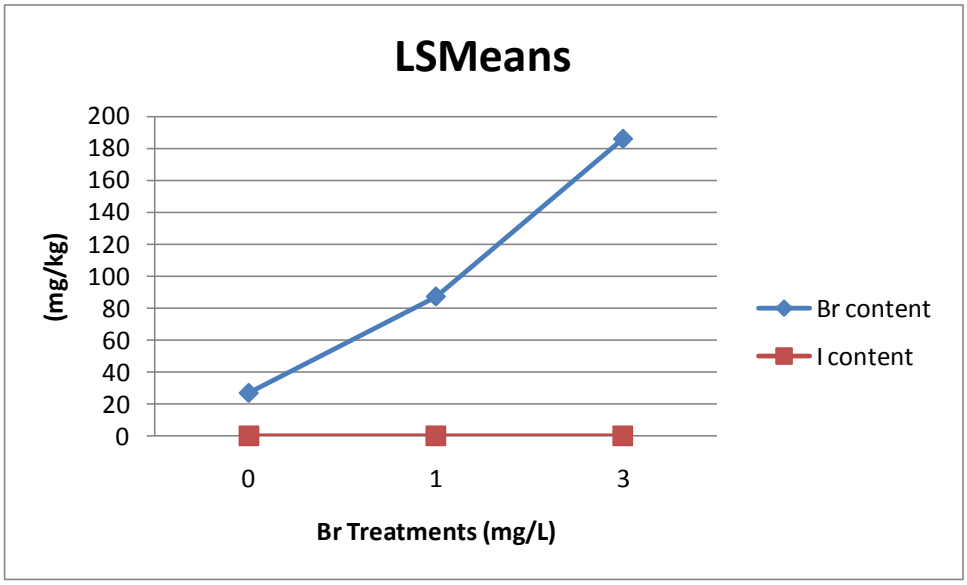


Figure 3.38 Bromine (Br) and iodine (I) (mg/kg) content of chicken kidney after 6 weeks of different bromine (Br) treatments

The overall effect of Br is shown in Table 3.22 and for I in Table 3.23 and the interaction between Br and I in Table 3.24. Overall Br, irrespective of I, had a significant influence on the kidney (P = 0.0032) where

the different treatments of Br had a significant influence (P = 0.0198) on the Br and I content of the liver. Again it was observed that as the Br treatments increased, the amount of Br accumulated within the kidney was significantly higher. This significant observation was also made in the thyroid gland and the liver. The 1 mg Br/L treatment does not differ significantly from the 3 mg Br/L treatment due to very high SD.

The kidney had an additional observation compared to the other two organs. Br had a significant influence on the levels of I deposits within the kidney. At the 1 mg Br/L treatment a significantly higher level of I in the kidney was measured than in the control and 3mg Br/L treatments (Figure 3.38).

Table 3.23 Average bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of chicken kidney after 6 weeks of different iodine (I) treatments

I levels (mg/L)	Br content (mg/kg)	I content (mg/kg)
0	115.00 [a] (199.68)	0.32 [a] (0.19)
0.7	85.11 [a] (127.50)	0.34 [a] (0.15)

[ab] *Column means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

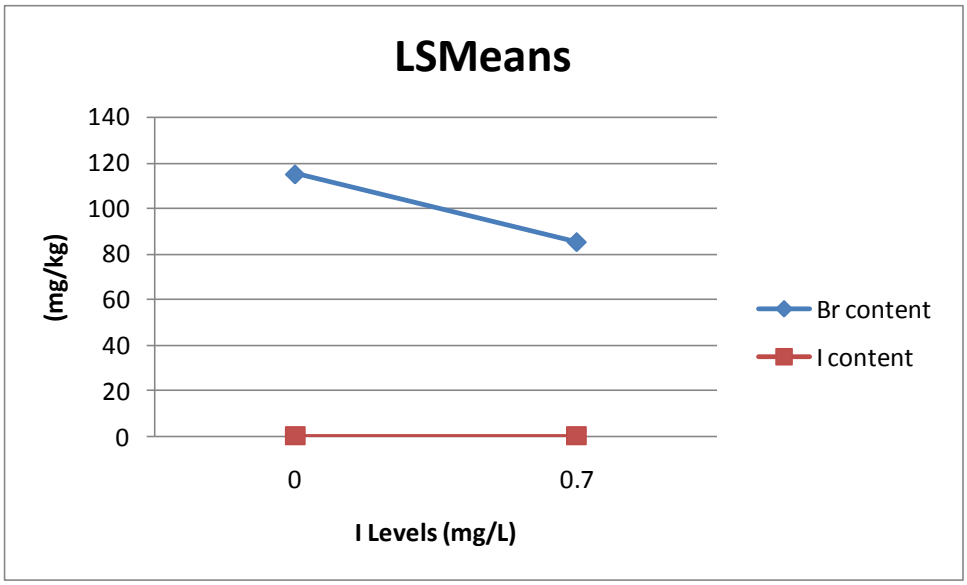


Figure 3.39 Bromine (Br) and iodine (I) (mg/kg) content of chicken kidney after 6 weeks of different iodine (I) treatments (P=0.0141)

There was an overall effect on the kidney due to the I levels (P = 0.0141). This can be ascribed to the effect of Br (P = 0.0167). As can be seen in Table 3.23 the Br content in the kidney was less where the 0.7mg I/L level was administered, which indicated that an increased I intake diminished the accumulation effect of Br, but was not significantly different, because the SD was very high. As Br decreased with

higher I administration, the measured I levels increased, but these too were not significant mainly due to large SD (Figure 3.39).

Table 3.24 Average kidney bromine (Br) and iodine (I) (mg/kg) quantities (means and SD) of broilers exposed to the interaction of bromine (Br) and iodine (I) in the drinking water over the production cycle

Element	Treatments						Means	P- value
	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L		
Br	32.08 [a] (40.69)	102.86 [ab] (108.87)	22.46 [a] (22.37)	71.85 [ab] (65.25)	161.02 [ab] (196.65)	210.05 [b] (320.61)	100.05 (130.12)	0.0032
I	0.30 [ab] (0.19)	0.42 [ab] (0.22)	0.28 [b] (0.13)	0.45 [a] (0.10)	0.29 [ab] (0.16)	0.26 [b] (0.16)	0.33 (0.14)	0.0141

[ab] *Row means with different superscripts differ significantly (P< 0.05) according to the Fischer's Test*

The interaction of Br and I had an overall significant effect on the accumulation of Br in the kidney (P = 0.0032) as well as on the I content (P = 0.0141). From Table 3.24 it is seen that Treatment 2 (0.7 mg I/L) had the lowest content of Br and Treatment 5 (3mg Br/L) had the highest quantity of Br; the difference between these two treatments was significant (the same trend was seen in the thyroid gland and in the liver). Also there was significantly less Br accumulation within the liver of the control group than when Treatment 5 (3mg Br/L) was administered. I too had an overall influence on the accumulation of the above measured elements in the kidney (P = 0.0141). Treatment 2 (0.7 mg I/L) had the lowest I accumulation and Treatment 4 (3 mg Br/L and 0.7 mg I/L) the highest. Treatments 2 (0.7 mg I/L) and 5 (3mg Br/L) differed significantly from Treatment 3 (1mg Br/L and 0.7mg I/L).

3.10 Histopathology of livers and kidneys and thyroid glands

A remarkable difference was observed between the liver morphology in the control birds, compared to the livers from both Treatment 4 (3 mg Br/L and 0.7 mg I/L) and Treatment 5 (3mg Br/L). The hepatocellular hypertrophy appeared to be most severe in Treatment 5 (3mg Br/L) and less prominent among Treatment 4 (3 mg Br/L and 0.7 mg I/L).

The vacuolar degeneration was due to swelling of the intracytoplasmic endoplasmic reticulum and may have followed on different forms of insult to the hepatocytes. It tended to show the same pattern as found in hepatocellular hypertrophy recorded in the liver sections. Fatty change, characterized by round fat droplets within the cytoplasm, did not show any specific pattern among the treatment groups or the control birds. The renal and thyroid gland appeared similar without any specific pathological changes among the treatment groups or in the control birds (Appendix B).

Table 3.25 Histopathological lesions in liver sections from broilers sampled on 4 weeks of age

Hepatic lesion	0 mg Br/L + 0 mg I/L			3 mg Br/L + 0.7 mg I/L			3 mg Br/L		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Hepatocellular hypertrophy	-	-	-	1+	-	1+	2+	3+	1+
Vacuolar degeneration	-	-	-	1+	1+	1+	3+	3+	2+
Fatty change intracytoplasmic	-	-	-	-	-	1+	-	-	-

[1+] Mild organ damage; [2+] Moderate organ damage; [3+] Severe organ damage

Table 3.26 Histopathological lesions in liver sections from broilers sampled on 6 weeks of age

Hepatic lesion	0 mg Br/L + 0 mg I/L			3 mg Br/L + 0.7 mg I/L			3 mg Br/L		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Hepatocellular hypertrophy	-	-	-	-	1+	-	2+	3+	2+
Vacuolar degeneration	-	-	-	-	1+	-	2+	3+ [c]	2+
Fatty change intracytoplasmic	-	-	-	1+	1+	1+	3+	1+ [a]	1+

[1+] Mild organ damage; [2+] Moderate organ damage; [3+] Severe organ damage

When Table 3.25 is compared to Table 3.26 it is noted that the damage to Treatment 4 (3 mg Br/L and 0.7 mg I/L) is similar at both week 4 and week 6. At treatment 5 (3mg Br/L) the fatty change intracytoplasmic reported no damage at week 4, but at week 6 severe to mild damage was recorded.

3.11 Mortalities

Table 3.27 Mortalities (%)

Treatment	Mortalities (%)
0 mg Br/L + 0 mg I/L	7.78
1 mg Br/L	14.44
0.7 mg I/L	11.11
1 mg Br/L + 0.7 mg I/L	10.00
3 mg Br/L + 0.7 mg I/L	10.00
3 mg Br/L	4.44

No abnormal mortalities were reported for the duration of the broiler trial.

4 CHAPTER 4: DISCUSSION

Certain farms and rural areas in South Africa contain natural high levels of Br in the groundwater. The investigation into rural communal water quality, by Casey and Meyer (2001) indicated that the close association between animals and humans serves as a limiting factor in the successful application of the solution to problematic water sources. Shared utilisation of water sources by humans and animals prohibits the application of certain alleviator treatments, on account of their potential adverse effects on humans (Casey & Meyer, 2001).

Broiler production is one of the most successful protein production systems in South Africa. Chicken meat remains a highly affordable protein source relative to other meat protein sources. SAPA (2008) reported that the per capita consumption of chicken meat has increased significantly from 29.6 kg per individual per annum in 2007 to 30 kg in 2008. The rise in chicken meat consumption to some extent results from a consumer driven preference behaviour of food service operators where value-added, brand names and convenient products and not only commoditised volume packs are sought after (SAPA, 2008).

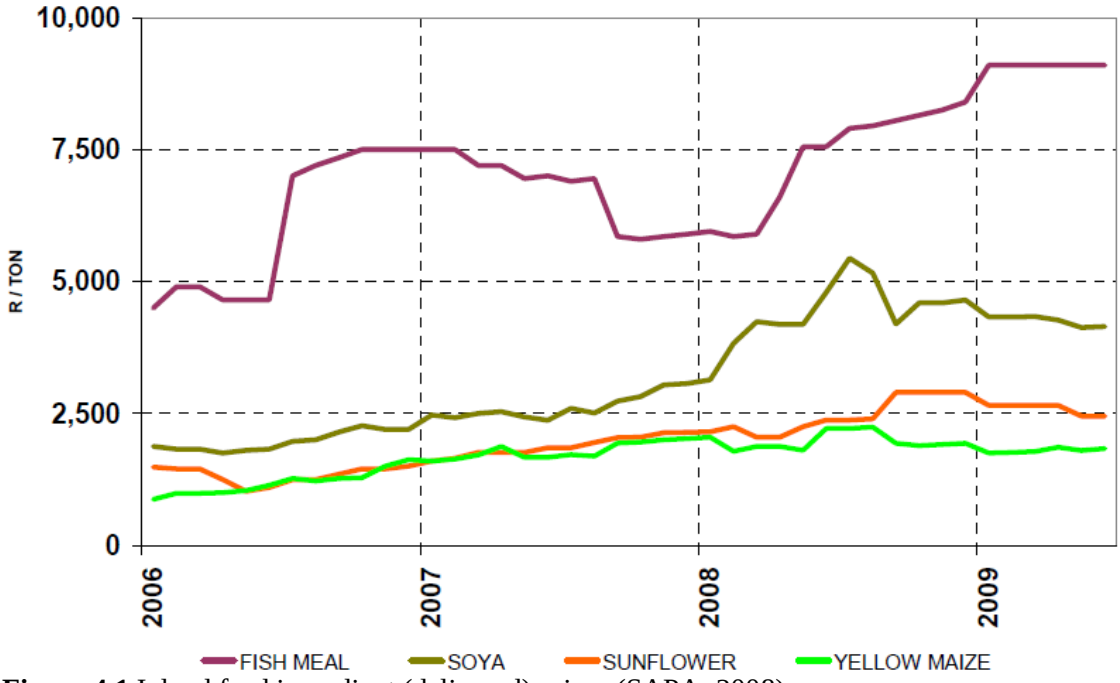


Figure 4.1 Inland feed ingredient (delivered) prices (SAPA; 2008)

Broiler production systems operate on intensive management and precise feeding in order to produce the end product within just a few weeks. Feed costs continuously have a significant influence on the profit margin in the poultry industry, especially within the past year. Dramatic feed price increases, as can be seen in Figure 4.1, are the result of the rising prices of maize and soya, the main raw materials in broiler

feed (SAPA, 2008). Profit margins are declining as feed costs continue to increase. It is therefore risky to involve any adverse amendments in the environment, feed or breeding programmes of the chick and young bird, as changes can lead to major economic losses. The poultry industry faces complex challenges, including the rise in feed costs (as explained above), environmental issues and bird health (SAPA, 2008). Water quality plays a major role in all of the above mentioned challenges.

The economic viability of a broiler production system to be operating in areas with high levels of Br in the groundwater and the effect of Br on the growth and production parameters of broilers is questioned as being detrimental to economic returns. Additional questions arise regarding the possibility of I to alleviate the effect of Br to relieve or even remove the unfavourable economic results. Another consideration is whether broiler production in these areas is safe for human consumption to ensure consumer confidence in broiler meat. Food safety is a key component in the maintenance of consumer confidence in chicken meat.

I deficiency still exists in some areas of South Africa such as the Limpopo Province, Mpumalanga Province and the North West Province as reported by the Department of Health South Africa (2002). The question now arising is what the effect of high Br naturally occurring in the groundwater of South Africa will have on the I status of the South African population. A comparison between the performance of the birds reared on brominated water compared to alleviated water treated with I and a reference broiler production system without brominated water is therefore essential. The brominating trial was done under standard housing, managerial and nutritional conditions, the only variables being the Br and in certain treatments the I content of the water and a low stocking density.

For the above mentioned comparisons, data from the Ross 708 Broiler Performance Objectives (Aviagen, 2007), Ross Broiler Management Manual (Aviagen, 2002), the National Agricultural Marketing Council Subsector study: Chicken meat (NAMC, 2007) and the control group and Treatment 1 group (As 0.1 mg/L; Br 1 mg/L; Pb 0.1 mg/L) of a study done by Mamabolo (Mamabolo, 2008) are used as reference broiler data, due to the lack of trial data where Br was administered in the drinking water of chickens. These data are representative of the commercial Ross broiler population and any differences and deviations that occur are therefore significant.

The reviewed economic traits were:

- Feed Conversion Ratio
- Production efficiency factor

Feed conversion is the unit of feed consumed per unit of live mass produced. Feed intake and live mass produced from mortalities are not included in the calculation. The factor of production efficiency is calculated by multiplying mean live weight in kg by the percentage of survivors and then dividing this by feed conversion multiplied by age (Morley & Thomson, 1984).

Since Br is likely to alter the metabolism of the birds, the above economic traits were selected because they include the broiler production indices most likely to be influenced by metabolism namely:

- Feed intake
- Live mass (growth)
- Mortality

4.1 Feed Conversion Ratio (FCR)

Table 4.1 Feed conversion ratio

Week	Reference birds			Treatments					
	Aviagen (2007)	NAMC (2007)[1]	NAMC (2007)[2]	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
1	0.87			1.06	1.12	1.07	1.17	1.07	1.21
2	1.13			1.19	1.19	1.19	1.20	1.18	1.19
3	1.29			1.38	1.31	1.33	1.34	1.32	1.34
4	1.44			1.43	1.41	1.44	1.43	1.44	1.45
5	1.58			1.58	1.55	1.60	1.62	1.57	1.61
6	1.73	2.43	1.77	1.65	1.58	1.68	1.54	1.63	1.66

[1]*The small farmer slaughters at 42 days of age* [2]*The commercial farmer slaughters at 38 days of age.*

Br, irrespective of I or the interaction of Br and I or any other factor, had a significant influence on FCR only at week 6 (Table 3.11), indicating that a longer exposure period is required for observing trends and more significant influences on FCR. A longer production period, higher stocking density or rural systems may provide different FCR results.

In week 4 and 5 the reference and treatment chickens had an equal performance in terms of FCR but in week 6 the treatment broilers outperformed the reference broilers (Table 4.1). There was more meat produced for less feed consumed, which is economically more viable. This observation is probably due to the short production period, low stocking density and controlled environment and high quality feed and daily clean feed and water provision.

4.2 Mortalities

Mortalities were the highest for Treatment 1 (1 mg Br/L) and lowest for Treatment 5 (3 mg Br/L) (Table 4.2), indicating no direct relationship between mortalities and Br administration. The mortalities of the reference birds for comparison are 7.85% for the small farmer and 5.04% for the commercial farmer, where the small farmer slaughters at 42 days of age and the commercial farmer slaughters at 38 days of age. This provides liveability values of 92.15% and 94.96% respectively (NAMC, 2007). The reference broilers (small farmer 7.85%) showed higher mortalities than the control (7.78%) and high Br treatment groups (3 mg Br/L) (4.44%) of this trial. The high moralities of the trial (14.44%, 11.11% and 10.00%) can overall be explained by high feed intakes (Table 3.6), which occurred in these treatment groups and led to more Sudden Death Syndrome (SDS) "flip over" occurrences. SDS is caused by high carbohydrate intake, very high rate of feed intake and methods which ensure increased feed intake, for example lighting programmes must be adjusted to prevent further occurrences (Leeson & Summers; 2005). When liveability is compared, there is no definite difference between the treatment groups and the reference birds.

Table 4.2 Mortalities and liveabilities

Treatments	Total mortalities	Mortalities (%)	Liveability (%)
0 mg Br/L + 0 mg I/L	7	7.78	92.22
1 mg Br/L	13	14.44	85.56
0.7 mg I/L	10	11.11	88.89
1 mg Br/L + 0.7 mg I/L	9	10.00	90.00
3 mg Br/L + 0.7 mg I/L	9	10.00	90.00
3 mg Br/L	4	4.44	95.56

4.3 Production Efficiency Factor (PEF)

$$\text{The production efficiency factor} = \frac{\text{Mean live weight x \% of survivors}}{\text{Feed conversion x age (days)}} \times \frac{100}{1}$$

Table 4.3 Production efficiency factor

Treatments	PEF
0 mg Br/L + 0 mg I/L	411
1 mg Br/L	398
0.7 mg I/L	373
1 mg Br/L + 0.7 mg I/L	431
3 mg Br/L + 0.7 mg I/L	389
3 mg Br/L	410

The higher the PEF the better the technical performance of the broilers. This factor is used extensively to compare flocks but PEF values cannot be used to compare flock performances between countries. The South African PEF improved from 150 to 263, while the international PEF are constantly represented between 270 and 300 (NAMC, 2007). The mean production efficiency of the Br and I trial is 402 (Table 4.3). Thus although there were relatively high mortalities throughout all the treatments, the PEF is still very high which implies that the increased live weights, low stocking density and more efficient feed conversions compensated for the mortalities overall. The data in Table 4.3 indicates that Treatment 5 (3mg Br/L) returned high production efficiency as well as the reference groups and the rest of the treatments. It is not unexpected for this trial as 3 mg Br/L was not expected to cause mortality in such a short exposure period, but rather explicit subclinical damage and Br accumulation within organs.

4.4 Water intake

Significantly less water was consumed by the Br treated (1 and 3 mg Br/L) broiler groups compared to the control group, over the 42-day production period. This is clearly illustrated in Figure 3.1.This trial indicated that Br up to levels of 3 mg/L or an ingestion rate of 4.44 mg Br/L per day per bird significantly decreased the water intake of broilers. When the effect of weeks are taken into consideration it is seen in Figure 3.3 that at week 3 the 3 mg Br/L treatment broilers' water intake started to decrease, Treatment 4 (3 mg Br/L and 0.7 mg I/L) followed a week later (I can possibly be the cause of the delay) and Treatment 1 (1 mg Br/L) soon followed the downward curve. These statements are further supported by the overall trend observed in Table 3.2, Figure 3.1 and Figure 3.2 where the control group had significantly higher water consumption than Treatment 5 (3mg Br/L). When I was included in the comparison there were no longer significant differences within water consumption over the 42-day production period (Table 3.2), indicating that 0.7 mg I/L or that ingestion rates of 1.1 mg I/L per day per bird compensated for the loss in water consumption. The same observations were made at average daily water consumption as well as the cumulative water records. These observations prove the undesirable effect that the Br treatments had on the broilers' water intake over a 42-day production period. The two trends constantly observed throughout the 6 weeks were the significant adverse effect of Br on water intake and the significant alleviating effect of I.

Table 4.4 Water intake (ml/bird/day)

Week	Reference birds			Treatments					
	Aviagen (2002)	Mamabolo (2008)[1]	Mamabolo (2008)[2]	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
1	69	93	92	56.75	49.70	52.08	53.81	54.37	54.34
2	123	153	150	132.97	123.23	125.24	126.89	127.79	127.10
3	190	180	176	252.55	240.26	257.15	241.45	245.06	227.96
4	255	238	232	320.48	309.01	318.43	307.94	316.03	292.85
5	303	298	288	349.02	313.40	333.19	325.74	319.46	313.10
6	345	310	301	494.75	465.76	443.86	447.78	446.36	436.29

[1] *Control group (< 0.005 mg/L As, Br, Pb)*

[2] *Treatment 1 (As 0.1 mg/L; Br 1 mg/L: Pb 0.1 mg/L)*

When the water intake of the trial is compared to the reference groups a marked difference in water intake occurred from the third week onwards (Table 4.4). The treatment birds consumed more water than all of the reference birds. An external factor is involved in causing very high levels of water intake, this might be attributed to the fact that fresh water was provided daily or that the chicken density was much lower in the Br and I trial. When the control group and the treatment group of Mamabolo (2008) are compared to each other it is clearly visible that the high treatment group reported less water consumed than the control group. The same observation can be made for the Br and I trial; where the control group had consumed more water than the Br treated groups.

A decrease in water intake has a very unconstructive influence on broiler production systems as a short term reduction in water intake leads to the reduction in feed intake and a reduction in average daily gain (ADG) (Leeson & Summers, 2005); these adverse effects will be very prominent in a longer exposure period or with mature birds. Heat stress will probably occur when birds are exposed to these high Br treatments with a longer exposure period, or in a hot environment because water is crucial to relieve heat stress. Birds store heat and constantly undergo an increase in body temperature within a hot environment. This is mainly a result from the inability of birds to produce adequate evaporative cooling by panting (Bartholomew & Cade, 1963). The negative influence of high Br causing a decrease in water intake may not have reached its peak because the birds employed in this trial were not mature birds. The function of birds to lose water by method of evaporative water loss decreases as birds mature. The amount of water loss by a day-old-chick, expressed as a ratio of body weight, is appreciably higher than that of an adult (Casey *et al.*, 2001), thus mature birds need more water to cool their body temperature. This explains why the low water intakes due to Br administration will have an adverse effect on mature birds (lay hens or breeders).

A panel determined the taste threshold of I for humans to be 0.147 to 0.204 mg/L (Bryan *et al*., 1973). This observation might have had an influence on the water intake as Treatment 2 (0.7mg I/L) consumed less water than the control and reference treatments. A different element should also be tested to see if additional success in alleviation e.g. by making use of Cl, is possible. For Br the taste threshold for humans was set at 0.17 to 0.23 mg/L (Bryan *et al*., 1973). The question arises whether chickens are able to 'taste' Br and if this will explain the decrease in water intake for the high Br treatment group.

4.5 Feed intake

Figure 3.5 clearly illustrates the significant adverse effect Br had on the Br treated groups compared to the control group. Feed intake might even be sensitive to 1 mg Br/L. From Table 3.5 it is evident that Br, irrespective of I, did cause less feed to be consumed. Thus Br had a significant undesirable influence on feed consumption of broilers over a period of 42 days. I (0.7 mg/L) too had an adverse effect on feed intake (Table 3.5) but no trends were observed throughout the weeks. The favourable effect of low stocking density, short exposure period and a controlled environment may suppress the advance adverse effect Br may have on feed intake. Because Leeson and Summers (2005) found that as water intake decrease the feed intake decrease and then ADG will indicate a negative growth. If stocking density was higher or production occurred within an uncontrolled environment, the adverse effect of ADG would be apparent. When I was included in the comparison (i.e. Treatment 3 and Treatment 4) there were no increases in feed consumption (Table 3.4). Thus I did not compensate for the loss of feed intake during the 42-day exposure period. This can lead to further research to see if higher or even lower levels of I would compensate for the loss in feed intake.

Table 4.5 Average cumulative feed intake (mg/bird/day)

Week	Reference birds	Treatments					
	Aviagen (2007)	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
1	151	205.45	209.50	207.95	224.89	206.89	215.95
2	485	583.67	561.22	586.44	579.00	576.77	583.39
3	1065	1304.86	1270.43	1328.04	1281.02	1292.35	1303.90
4	1921	2367.18	2310.87	2365.26	2311.35	2336.43	2357.05
5	3039	3626.23	3494.02	3553.60	3482.26	3494.85	3568.07
6	4370	5052.80	4881.83	4921.65	4770.67	4821.86	4947.51

Table 4.6 Average Feed intake (mg/bird)

Week	Reference birds			Treatments					
	Mamabolo (2008)[1]	Mamabolo (2008)[2]	Aviagen (2007)	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
1	32.80	31.20	-	24.27	25.34	24.54	27.63	24.84	26.18
2	39.40	37.20	61	48.30	43.89	47.87	44.01	46.27	45.75
3	114.20	105.40	99	95.76	94.01	98.89	93.56	95.79	95.90
4	159.10	157.50	139	145.66	143.01	142.23	141.29	142.91	144.00
5	186.90	178.60	174	173.90	164.05	164.55	164.31	162.17	171.19
6	163.80	161.30	201	233.93	227.11	225.01	210.73	216.85	223.77

[1] *Control group (< 0.005 mg/L As, Br, Pb)*

[2] *Treatment 1 (As 0.1 mg/L; Br 1 mg/L: Pb 0.1 mg/L)*

From the third week a marked difference in feed intake occurred (Table 4.5), this observation could only be made in week 6 for the cumulative feed intakes (Table 4.6). Chicken density and fresh feed provision daily are most likely to be the explanation.

When the treatment and control groups of Mamabolo (2008) were compared the control group had a higher feed consumption than the treatment group. The same observation can be made within the Br and I trial between the control and Br treated (1and 3 mg Br/L) groups.

4.6 Live mass/ growth

Only the first week of production indicated significance, this can be explained by the chicks having to adapt to their new environment, since none of the other weeks indicated significant weight differences. The physiological explanation for this occurrence is described by (Casey *et al.*, 2001) as the result of marked respiratory loss due to rapid breathing of chicks during the first day. During the first day the nature of respiration of the chick changes from allantoic respiration to pulmonary respiration, which gives rise to relative anorexia. The definite decrease in average daily gain seen in week 5 of Figure 3.11 occurred throughout all the treatments and is due to an external factor. No severe effect on weight is reported, possibly due to a short exposure period.

For Table 4.7 and Table 4.8, the treatment groups had higher average daily gains than the reference birds, throughout all 6 weeks. Even the low ADG's of week 5 were still according to the Aviagen (2007) broiler standards. Table 4.7 only indicated significant lower average daily gains for week 1 at Treatment 1 (1mg Br/L) and Treatment 5 (3mg Br/L) but their average daily gain is still higher compared to the reference

birds. This again emphasises the fissure to be filled with regards to importance of these Br treatments' impact on rural or uncontrolled broiler production and high stocking density productions, as this trial were performed at highly positive conditions and might not indicate all the detrimental effects of Br. Loeber *et al.* (1983) and Jolles (1966) recorded a suppression of growth hormone release in male rats receiving sodium bromide (NaBr) enriched diets. On the other hand, retardation in growth of mice and chickens was related to Br deficient diets (Jolles, 1966). Where as in this trial neither depression nor induction of growth was observed, therefore increasing the necessity for further research on Br and its effect on livestock.

Table 4.7 Average daily gain (g/bird/day)

Week	Reference birds	Treatments					
	Aviagen (2007)	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
1	18.86	21.24	20.25	21.26	21.00	21.01	18.93
2	36.57	42.25	41.69	42.77	42.25	42.04	42.54
3	56.29	70.40	70.85	71.85	67.66	69.83	68.59
4	72.86	96.56	95.70	91.88	93.05	92.35	92.61
5	83.57	91.02	87.37	81.94	78.04	86.40	84.83
6	87.43	111.04	120.58	103.63	135.20	104.84	111.04

The Mamabolo (2008) trial indicated a slight growth improvement with the Br treated group but this was not significant (Table 4.8). No abnormal health-related cases were reported .However these results may indicate that the exposure period for this trial was too short, especially in the context of rural communal chicken farming, where chickens are raised under stressful conditions and are provided with poor drinking water, feed and environment.

Table 4.8 Live mass (g/bird)

Week	Reference birds					Treatments					
	Aviagen (2007)	NAMC (2007)[a]	NAMC (2007)[b]	Mama-bolo (2008)[1]	Mama-bolo (2008)[2]	0mgBr/L +0mgI/L	1mgBr/L	0.7mgI/L	1 mgBr/L +0.7mgI/L	3 mgBr/L +0.7mgI/L	3mgBr/L
1	0.17			0.30	0.30	0.19	0.19	0.19	0.19	0.19	0.18
2	0.43			0.64	0.63	0.49	0.47	0.49	0.48	0.49	0.49
3	0.82			1.11	1.12	0.95	0.97	1.00	0.96	0.98	0.97
4	1.33			1.69	1.73	1.66	1.64	1.64	1.61	1.62	1.62
5	1.92			2.23	2.33	2.30	2.25	2.21	2.15	2.23	2.21
6	2.53	1.76	1.78	2.90	2.90	3.07	3.09	2.94	3.10	2.96	2.99

[a]*The small farmer slaughters at 42 days of age.* [b]*The commercial farmer slaughters at 38 days of age*

[1]*Control group (< 0.005 mg/L As, Br, Pb)* [2]*Treatment 1 (As 0.1 mg/L; Br 1 mg/L: Pb 0.1 mg/L)*

4.7 T3 and T4 concentrations in the blood

For the T_3 levels it was observed that within week 6 where a significant difference was observed, Treatment 2 (0.7 mg I/L) measured the highest T_3 levels and the 1 and 3 mg Br/L treatments the lowest. The treatments including 0.7 mg I/L measured higher T_3 levels than the treatments where 0.7 mg I/L were absent. This observation indicates that I responds to higher T_3 levels. When T_3 levels were compared between weeks 4 and 6, T_3 levels decreased as the time exposure increased. High Br caused an elevated decrease in T_3 levels where 0.7 mg I/L and the control groups' decreases were very meek. Br irrespective of I had significantly influenced this decrease, but a longer exposure period is needed to guarantee this influence. Figures 3.19 and 3.20 clearly indicate that the factor of time exposure influenced T_3 production, thus a longer exposure period will present this research with more significant results.

No definite trend for the T_4 levels were reported, a bigger sample size and longer exposure period might assist in observing trends for the T_4 levels. As seen in Figure 3.33 it will be interesting to observe this trend with a longer exposure period, in order to make clear observations regarding the T_4 levels which were on the same level after 6 weeks but not T_3 levels.

The opposed reaction of T_3 to T_4 (as seen in Figures 3.26-3.31) is a normal reaction as reported by Darras *et al*. (1995) where T_3 and T_4 levels of *ad lib* fed chickens were compared. When the T_4 levels of the Br and I trial were analysed (Figure 3.15) it is clear that the 3 mg Br/L treatment constantly had the lowest levels measured and the 3 mg Br/L treatment with added 0.7 mg I/L (Treatment 4) measured with no difference to the control group, but at week 6 there were a significant difference between this treatment and the control group. Treatment 4 and 5 had the lowest T_4 quantities measured at 42 days. A significant increase in T_4 levels were seen with Treatment 2 (0.7 mg I/L). In other words as the I treatments increased the T_4 levels measured increased and T_3 decreased.

In Figure 3.14 again the 0.7 mg I/L treatment lies adjacent to the control group. A significant decrease in T_3 production is seen in the 3 mg Br/L group. Treatment 4 (3 mg Br/L and 0.7 mg I/L) is much closer to the control than the 3 mg Br/L group, this indicates the amelioration effect I has on Br. A longer exposure period and the measurement of TSH would provide with more data for better observations.

Where the effects of long term partial food restriction were studied in chickens, the plasma T_4 levels increased and the T_3 levels decreased (Darras *et al.*, 1995). As the Br treatments did lead to the reduction in T_3 levels but not the increase of T_4 levels, there are thus a correlation between high Br intakes and feed

restriction this was also seen at the significant lower feed intakes of the broilers in the high Br treatment group.

Table 4.9 Changes in human T_4 concentrations with age

Age	T_4 (pmol/L)
Foetus	
12-20 weeks	0-50
21-30 weeks	5-'12
31-40 weeks	12-'22
Infant	
1-4 days	28-68
1-4 weeks	12-'30
1-12 months	10-'23
Child	
1-5 years	10-'27
6-10 years	13-27
11-15 years	10-'26
16-20 years	10-'26
Adult	
21-50 years	12-32
51-80 years	12-32

(Fisher, 1996)

There were significantly lower T_4 levels measured at 4 weeks of age, within the chicks in the Br and I trial (5.52-6.50 pmol/L) than the T_4 hormone concentrations of human infants (12-30 pmol/L) of the same age as shown in Table 4.9. This big difference can be explained by the species difference within the two trials. Shen *et al* (2004) observed significant differences between the T_3 levels of female and male 4-and-50-week-old chickens of two different breed lines, where male birds' T_3 levels were noted to be significantly higher than female birds' levels. From the research done by Shen *et al.* (2004) it is noted that the T_3 levels of chickens decrease with age. When the minimum and maximum levels at 4 weeks of age T_3 levels in Br and I trial (3.33-4.60 pmol/L) were compared to the minimum and maximum levels of Table 4.10 (2.90-4.19 pmol/L) there were no big differences reported, which indicates no abnormality. An imperative improvement to the Br and I trial will be the sexing of birds before thyroid gland hormones in the blood be quantified.

Table 4.10 T_3 levels in 4- and 50-week-old chickens

Age and Hormone	Sex	Line 1 (pg/ml)	Line 2 (pg/ml)	Line 1 (pmol/L)[1]	Line 2 (pmol/L)[1]
4 Weeks of age					
T_3	Female	2.73	1.89	4.19	2.90
	Male	2.57	2.10	3.95	3.23
50 Weeks of age					
T_3	Female	0.28	0.28	0.43	0.43
	Male	0.53	0.41	0.81	0.63

[1] *Conversion according to Young (1987)*
(Adapted from Shen *et al.*, 2004)

Table 4.11 T_3 and T_4 levels in 42-day-old chickens

Hormone	Male (fmol/ml)	Female (fmol/ml)	Male (pmol/L)[1]	Female (pmol/L)[1]
T^3	3.14	2.50	3.14	2.50
T_4	8.04	8.67	8.04	8.67

[1] *Conversion according to Young (1987)*
(Adapted from Ma *et al.*, 2008)

10.92 pmol/L T_4 was measured for 2- week- old white leghorn chickens (not sexed) and at 5 weeks of age 13.2 pmol/L was measured (Wiley *et al.*, 2003), both levels are much higher than the Br and I trial levels. At 42-days of age the reference T_4 levels (8.04-8.67 pmol/L) are significantly higher than the T_4 levels at 6 weeks of age for the Br and I trial (5.10-6.00 pmol/L), but as explained above the T_3 levels were in range with the reference birds. This indicates that insufficient T_4 was possibly produced during the trial due to an external factor or the possibility of a defective sampling method occurs. Possible external factors are described as nonthyroid glandal illness or stressors by Fisher (1996): trauma, infection, cancer and metabolic diseases, where trauma seems to be the most possible case for T_4 hormones. TSH levels were not quantified due to financial restrictions, but should be included as a possible cause, to assist in quantifying the effect of Br on the thyroid gland hormones.

Fisher (1996) summarised the marked variations in thyroid function with age and gender. Thyroid function in the foetus matures progressively to maximum levels of thyroid hormone production and utilization. T_3, T_4 and TSH concentrations all peak during this period. T_4 utilisation rates decrease progressively with age; T_3 and TSH decrease more modestly (Fisher, 1996). In the domestic chicken the T_3 and T_4 plasma concentrations are inversely related and show rhythmic changes during the daily light and dark cycles (Klandorf *et al.*, 1978). Plasma T_3 concentrations increase with the light period and

decrease in the dark period, the inverse is true for plasma T_4. The nocturnal increase for plasma T_4 can be explained by an increase in TSH excretion during the dark period (Klandorf *et al.*, 1978). The birds within the trial did not reached maturity and they were not sexed, the various T_3 and T_4 results graphed with no definite pattern can be ascribed to the fact that the chickens were not sexed and that their adolescent age played a role. But the inverse relationship between T_3 and T_4 were clearly observed. Another contributor is the rhythmic changes during dark and light periods, as the representative plasma samples were sampled at different times during the day. T_3 levels within all the treatments decreased when week 4 is compared to week 6 plasma T_3 levels. The same observation is made for T_4 plasma levels.

Darras *et al.* (1995) also investigated *in vitro* hepatic outer ring deiodinating type 1 activity as well as *in vitro* hepatic inner ring deiodinating type 111 activity, it can be recommended that in future research these two parameters should be included to find changes within thyroid gland hormone production. The reason for this inclusion is that bromide is not oxidised by thyroid gland peroxidase and Br doesn't substitute I in thyroxine, but high levels of bromide decrease I uptake by the thyroid gland (NRC, 2005). Bromide interferes through ring substitution, and prevents I ions from entering the tyrosine ring and with this behaviour suppresses the synthesis of T_4 and T_3 (Loeber *et al.*, 1983). This effect will lead to the disorder hypothyroidism and cause the metabolism to slow down and reduce the sensitivity of tissues (NRC, 2005).

In summary the 3 mg Br/L treatment did measure less T_3 and T_4 levels than all the other treatments, but the differences were not significant. The T_4 levels measured are lower than the comparisons made to other studies. This can be explained by possible external factors; the influence of non-sexed use of trial birds, the difference in sampling time or defective sampling or analyses.

4.8 Thyroid gland, Liver and Kidney

For the thyroid gland, liver and kidney there were significantly higher levels of Br concentrations accumulated within the organs as the treatment of Br increased in quantity (Figures 3.34, 3.36 and 3.38). Jolles (1966) reported that animal tissue contain 0.001 to 0.009 mg Br/kg; the values are dependent on the diet of the animal as well as the time exposed since Br intake. Tables 3.18, 3.21 and 3.23 gave values ranging from 6 to 210 mg Br/kg. This is unquestionably higher than the values given by Jolles (1966) the animal species within the Jolles (1966) report is unknown. The long half-life of bromide, which is 14 to 94 hours for the thyroid gland, 88 to 235 hours for the liver and 22 to 197 hours for the whole body (Pavelka *et al.*, 1999). Frances *et al* (2003) reported a 10-day elimination half-life for Br in the blood. The prolonged half-life in the thyroid gland, liver, whole body and blood indicate why the vast quantities of

Br accumulation were seen in the three discussed organs. The I concentrations within all three of the tested organs did decrease as Br treatments increase but were insignificant (Tables 3.17, 3.20 and 3.22). A longer exposure period can possibly indicate significant I reduction within these 3 organs.

When the organ Br content found in this trial were compared to the organs of the study done by Casey and Meyer (2001) (0 mg Br/L with 12.04 mg/kg Br and 3 mg Br/L with 77.58 mg/kg Br measured), organ Br concentrations of 44 mg/kg up to 265 mg/kg were quantified with water Br measured levels of 0.024 to 2.140 mg/L, these results are a clear warning of how much Br can accumulate within rural chickens' organs compared to chickens within a controlled environment or chickens consuming water from an analysed source. Again the severe problem of PHCC was illustrated by Casey and Meyer (2001). It is clear that within rural and commercial production systems high levels of Br are a cause for concern because the main adverse effects may include weight loss and goitrogenic complications; and ration formulation may need to be adapted due to direct competition with I. Not only in the liver, but in breast and thigh muscle too, exceptionally high levels of Br were recorded by Casey and Meyer (2001). This implies that breast and thigh muscle should be included into future trials and should receive further attention as these cuts are consumed by humans, therefore these high Br levels recorded within these tissues are of great concern to the consumer. This leads to concerns for rural production systems where longer exposure times prior to consumption occur, due to the limitations on controlling the production environment. When the same drinking water for livestock containing the high levels of Br is also utilised by humans as drinking water source and for cooking, the risk is increased further.

Table 4.12 Mean liver bromine (Br) and iodine (I) (mg/kg) content of broilers

Element	Reference organs		Treatments					
	Mamabolo (2008)[1]	Mamabolo (2008)[2]	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
Br	10.61	14.89	12.04	32.11	6.18	17.75	77.58	95.59
I	0.25	0.25	0.27	0.35	0.26	0.33	0.21	0.28

[1] *Control group (< 0.005 mg/L Arsenic (As), Br, Lead (Pb))*

[2] *Treatment 1 (As 0.1 mg/L; Br 1 mg/L: Pb 0.1 mg/L)*

Table 4.13 Mean kidney bromine (Br) (mg/kg) content of broilers

Element	Reference organs		Treatments					
	Mamabolo (2008)[1]	Mamabolo (2008)[2]	0 mg Br/L + 0 mg I/L	1 mg Br/L	0.7 mg I/L	1 mg Br/L + 0.7 mg I/L	3 mg Br/L + 0.7 mg I/L	3 mg Br/L
Br	6.61	11.22	32.08	102.86	22.46	71.85	161.02	210.05

[1] *Control group (< 0.005 mg/L As, Br, Pb)*

[2] *Treatment 1 (As 0.1 mg/L; Br 1 mg/L: Pb 0.1 mg/L)*

When the reference organs of the Mamabolo (2008) trial are compared to the Br and I trial, the Br and I trial had a higher accumulation of Br in the liver (Table 4.13) and in the kidney (Table 3.14) than the Mamabolo (2008) trial. It can be assumed that the As and Pb included in Treatment 1 of the Mamabolo (2008) trial alleviated the accumulation of Br within the liver and kidney as these levels were lower than for the Br and I trial organs. The same observation for both of these trials are that the control groups had significantly less Br accumulated within the liver and kidney than the treatments which included Br.

When Tables 3.18, 3.21 and 3.24 are inspected it is clear that I did relieve the effect of Br; the treatments without I administration had higher Br accumulation than the I administered treatments. As a result the biological half-life of bromide can be decreased by the administration of surplus iodine ions (Langley, 1958). High levels of Br intake can influence the I metabolism of an animal in two ways: by decreasing the iodide accumulation in the thyroid gland and skin (and in the mammary glands of lactating dams) and increase the iodide excretion by the kidneys. By accelerating the renal excretion of iodide, excessive bromide can also influence the amount of exchangeable iodide in the thyroid gland (Pavelka *et al*., 2002). Baker *et al.* (2003) studied the I x Br interaction and came to the three possible mechanisms by which Br decreases the I concentration. The three likely possibilities are that Br; 1) reduces intestinal absorption of I; 2) enhances urinary excretion of I or; 3) reduces I uptake by the thyroid gland. I concentration did decrease with an increase of Br inclusion levels, but was insignificant within the thyroid gland.

There's also an overall trend within all 3 organs where Treatment 5 (3mg Br/L) had the highest Br accumulation and Treatment 2 (0.7mg I/L) had the lowest. Treatment 3 (1mg Br/L and 0.7mg I/L) had lower Br content than Treatment 1 (1mg Br/L); furthermore Treatment 4 (3mg Br/L and 0.7 mg I/L) had lower Br content than Treatment 5 (3mg Br/L). It is thus clear that the 0.7mg I/L administered did decrease the Br accumulation within all three of the discussed organs. Whereas I had relatively constant levels throughout the thyroid gland and liver, in the kidney Treatment 2 (0.7mg I/L) and Treatment 5 (3mg Br/L) had significantly lower I levels than Treatment 3 (1mg Br/L and 0.7 mg I/L). The kidney is the primary organ responsible for Br elimination. A possible reason for the kidney having very high levels of Br is that the kidney excretes Cl proportionately to Br (Palmer & Clarke, 1932). Jolles (1966) also reported that the kidney reabsorbs Br preferentially to Cl. This observation is supported by the I concentrations within the kidney. The I concentration for the kidney were less than for the other two organs, indicating the ameliorating effect of I on the kidney. This observation can be explained by Pavelka *et al*. (2002) as high levels of Br intake influence the I metabolism of the animal by increasing the iodide excretion by the kidneys. As I is the only trace element required for the synthesis of hormones involved in embryogenesis, differentiation and cognitive development (Underwood &Suttle, 1999) of a

female animal in gestation, a lower level of I, as reported here, will have detrimental effects on embryo development due to high Br level intake. Fortunately for a 42-day production period I did alleviate the effect of Br not to the expense of I, with a longer exposure period the above mentioned developmental processes can be adversely affected, when the I content of the body decrease due to high Br level excretion.

When Tables 3.18, 3.21 and 3.24 were compared, the kidney had the highest accumulation of Br, the liver second and the thyroid gland third. Jolles (1966) reported that after 2 hours of Br administration the proportional amounts of Br accumulated within the thyroid gland was 1, for the kidney it was 0.5 and the liver 0.4. This indicates that the highest accumulation of Br should have occurred within the thyroid gland but in this trial the highest accumulation occurred within the kidney. On the other hand Winnek and Smith (1937), Abelin and Poretti (1952), Mack and Shipley (1952), Bosshardt *et al.* (1956) and Huff *et al.* (1956) could demonstrate no preferential uptake by the thyroid gland. Cole and Patrick (1958) measured the kidney and intestines as the most active organs to have taken up ^{82}Br after a 2 hour intraperitoneal administration. Hellerstein *et al.* (1960) reported that Br accumulation does not occur in any particular organ or tissue. Winnek and Smith (1937) and Abelin and Poretti (1952) through chemical analyses, showed that supplemented dietary Br increased Br levels in the blood, liver, kidney, adrenals, muscle, spleen and the brain. Golomb (1999) also reported that Br, because of slow excretion of bromide through the kidneys, is given the opportunity to accumulate in the body when continuously ingested. Masoud *et al*. (1973) has reported acute renal failure cases leading to death by a direct nephrotoxic effect of the bromate ion. This emphasise the problem with Br in water when Br reacts with ozone and bromate is formed.

A significant observation for this trial was not only that the broilers employed in this trial receiving elevated selected elements in the drinking water accumulated these elements within organs at a significantly higher concentration than did their counterparts, but that the concentration attained within a short production period did exceed the MAC for Br in poultry production destined for human consumption (the MAC for Br is 1mg/L). The I levels were the highest for the thyroid gland and less for the liver and kidney. This is a normal observation as reported by Perlman *et al.* (1941). The thyroid gland has 100 times the ability of the liver and the kidney to retain I.

When the histopathology results of the three organs were reviewed it was noted that there was explicit damage to the livers that received the elevated Br treatments; severe damage in the form of hepatocllular hypertrophy and vacuolar degeneration were reported. It is also clear in Table 3.25 that the alleviator element (I) compensated for the damage caused by Br, by comparing the mild damage in Treatment 4 and

the more severe damage in Treatment 5 (3 mg Br/L). At 6 weeks of age (Table 3.26) the damage has augmented and a new symptom, intracytoplasmic fatty change was reported for both of the treatment groups, where the elevated treatment group was moderately damaged and the alleviator group mildly damaged. It is clearly reported within these two tables that the factor of time did play a role in the degeneration of the histopathology of the organs receiving high Br treatments. Although the highest Br concentration was found to be within the kidney, the liver was most severely damaged according to the histopathology report. A longer exposure period might have indicated histopathological severity to the thyroid gland and the kidney. A study by Velicky *et al*. (2004) noted the adverse effect Br had on the ultra structure of rat thyroid glands. As 10, 50 and 100 mg Br/L were administered for 16 or 60 days, marked hypertrophy and hyperplasia of the thyroid gland, micro follicular rearrangement and lower colloid volume were reported. Sub-clinically Br had a detrimental effect on the chickens that might be observed clinically after an elongated exposure period.

The first observation that can be made from this study is that high Br content in the mentioned areas might have been caused by fertilizers used high in Br content or the geology of the area consists of labradorite (the highest Br containing mineral) or any other mineral mentioned in the discussion of Br (section 1.7). The exact cause can be identified by making use of geological expertise or by surveying farmers on their fertilizer or pre- and post harvesting methods.

From the discussed points it is apparent that 3 mg Br/L administered to broiler chickens over a production period of 42 days within the drinking water does affect the production parameters of broilers. The significantly lower water and feed intakes of broilers after receiving 3 mg Br/L water support this observation. The symptoms were very robustly observed sub-clinically rather than clinically. The concern with this attribute is that the lack of aesthetic adverse effects can create the impression to the producer and consumer that the water has no possible hazardous effect. The same applies for animal products for human consumption, as very high levels of Br accumulated within the three observed organs, the thyroid gland, liver and kidney. The histopathology report also confirmed this statement with the severe damage caused by the high treatment level of Br. Thus 3 mg Br/L cannot be included as the MTL for Br. It will be very interesting to measure the level of bromate formed within the water including organs when 3 mg Br/L is administered and to determine whether I will compensate for bromate formation within this 3 mg Br/L treatment group.

The third observation which can be derived from this broiler trial is that the administration of 0.7 mg I/L water did alleviate the severe damaging effect of high Br administration. This was highly confirmed sub-

clinically where the concentrations of I and Br were quantified within the three primary organs as well as within the histopathology report.

A possible fourth observation that can be made is that if the trial exceeded the production period of 42 days or until the birds reached maturity or even after maturity, many more clinical observations would have been apparent. The endocrinology effect may have been evident. This observation leads to the question of how many animals or humans have or had subclinical symptoms due to the consumption of these high Br levels within the drinking water.

These observations confirm the importance of identifying the occurrences of natural groundwater containing very high and possible hazardous levels of Br in South African production units. A very high frequency of occurrences is reported by Casey and Meyer (2001); the need to include this element in the "South African Water Quality Guidelines. Agricultural use: Livestock watering" is highly recommended.

The fifth observation is that the high levels of Br did not significantly interfere with the levels of I within the organs nor with the production of T_3 and T_4 hormones. Thus there was no I deficiency observed for this short exposure period, but a tendency towards I disturbance and T_3 and T_4 hormone production was noted and this will possibly be reported in an extended Br exposure period.
Broiler meat exports expand dramatically in a declining stage (2002) and overturn totally when the Rand strengthens in value (2003) (Figure 4.2) (NAMC 2007). Export market development takes time to be established as a supplier in the world market, but overturns suddenly as domestic prices cannot compete when currency strengthens (NAMC, 2007). Good manufacturing practices should be followed for producers to be considered for the exporting of meat to the European Union (EU). Good manufacturing also includes water quality. A definite concern is water fitness for use according to EU standards. These high levels of Br in the groundwater will not comply with export standards.

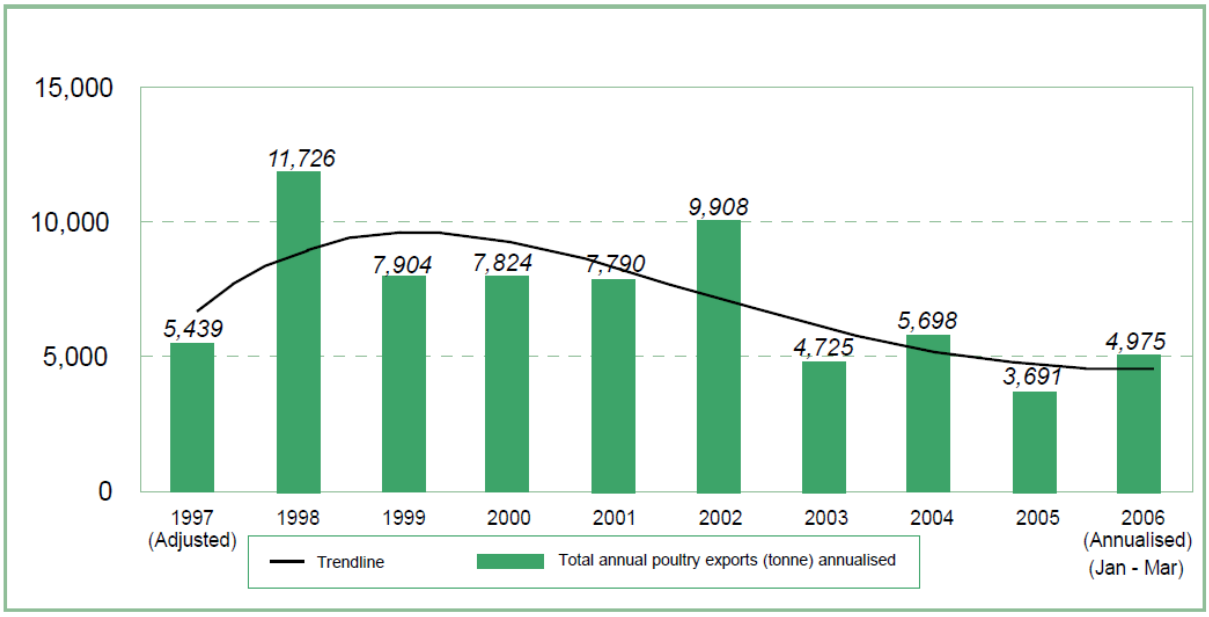


Figure 4.2 South African annual broiler meat exports (NAMC, 2007)

The financial feasibility of small-scale poultry farming and the involvement of workers in these operations make them ideal candidates to contribute towards food security for the very poor. As these enterprises grow, they could contribute towards the alleviation of poverty. With the high level of Br within the groundwater, the above contribution towards poverty alleviation or food security cannot be feasible. Not only health but economy is adversely affected by these naturally high levels of Br in groundwater.

One environmental friendly observation is that apart from the hazards of excessive exposure to probable chronic toxicity, the additional load these high Br levels lay on environmental loading from animal production system waste is also of concern. There is a growing worldwide concern over the accumulation of potential hazardous elements in the environment. Br occurs as residue in the soil and ground water, as bromide is excessively used as fumigation of soils prior to planting and after harvesting agricultural products. The availability of Br from plant sources vs. animal sources should also be considered an important contributor to the concern over the potential hazardous elements in the metabolism of this element within living organisms. Possible Br accumulator plants may expose previously unavailable forms of Br. These may be readily available for plant uptake and may lead to the environmental accumulation of Br. Pollution of the air, soils and surface water may also result in greater environmental Br levels.

An imperative conclusion is that Br inclusion levels of 1 and 3 mg/L in the drinking water might only have revealed sub-clinical hazardous effects in this trial, but the water quality concern in relation to bromate relates to its carcinogenic effect when Br reacts in synergy with the disinfectants namely ozone and Cl and the end-product bromated is formed (Magazinovic *et al.*, 2004).

It would therefore not be advisable to implement intensive broiler production in areas with natural high concentration of Br in the ground water, as adverse effects could be detected over 42 days and consumer health hazards were evident in the case of organ consumption. Due to the incidence of paradoxic hypothyroidism with exposure to excess I, the contribution via water to variable total I intake with concurrent variable Br intake suggests that a more detailed site specific approach is required to accurately formulate rations, diagnose production problem causes, and investigate I deficiency disorders in community health investigations.

The implication of the results obtained for water quality guideline formulation is that ingestion rates need to be calculated, species tolerance needs to be considered and the production system and exposure time must be taken into account in order to fully utilise the water source. This trial highlighted the importance of a second trial sought to assess these potential hazards within a harsher, less environmentally controlled production system, similar to conditions found in rural community household production systems. A trial of this nature will reveal the importance of temperature on elevated intakes, geophagia contributors and a longer exposure period and reduced level of nutrition on elevated tissue concentrations of PHCC. The need for adequate trace element nutrition to be applied within the context of exposure to PHCC with poorer diet provided was because adverse effects extended not only to product quality but also essential trace element nutrition.

The central departure point remains a comprehensive water quality investigation in which the relevant norms for the proposed use of water are investigated, followed by which site-specific risk factors should be assessed in order to most appropriately utilise and manage the available water. From the current results it is clear that the current water quality guidelines are not too restrictive, but need to take production criteria and especially exposure time into account.

5 CHAPTER 5: CONCLUSION

This trial confirmed that concentrations of PHCC (Br in this trial) observed in the drinking water of the poultry production unit can accumulate to concentrations that exceed the maximum acceptable concentration, 1 mg Br/L (MAC) for animal tissues used for human consumption regardless of a short exposure period. Significantly lower water and feed intakes were induced by 1 and 3 mg Br/ L in the drinking water or inclusion levels of 1.59 and 4.44 mg Br/L per bird per day. The observation that an alleviator treatment of 0.7 mg I/ L in the drinking water or the intake of 1.1 mg I/L per bird per day, may be used without adverse effects, at least over a six week period, may assist in attempting to decrease the risk to communities that may be exposed to a high Br water source. Explicit histopathology damage occurred to the broiler livers receiving the elevated Br treatment (3 mg Br/L). Histopathology evidently observed the ameliorative effect of I and that time did play a role in the degeneration of the histopathology of the organs receiving high Br treatments. Production and physiological parameters of broilers that were not significantly influenced by Br over this relatively short term exposure period and low stocking density were, average daily gain or growth, FCR, free T_3 and T_4 hormone concentrations. The long term effects of these parameters and exposure of mature animals are yet to be quantified, since the high presence of Br in the thyroid gland, liver and kidney may have a detrimental effect on the broilers as well as the consumers of these animal products.

6 CHAPTER 6: CRITICAL EVALUATION, FUTURE RESEARCH AND RECOMMENDATIONS

6.1 Critical evaluation

- Broilers were slaughtered at 4 weeks of age and again at the end of the trial (6 weeks of age). Due to financial constrains not all of the sampled tissues were analysed. If a representative sample of each treatment could be slaughtered weekly, valuable information could have been acquired concerning the rate of Br deposition in the soft tissue. Histopathological changes could have been described as they occur.
- The exposure period was too short; a longer exposure period would have provided more information on the retention of elements directly related to rural communal farming and other species as well as the effect on humans.
- The stocking density in each pen was not equivalent to either rural or commercial production: 30 chickens were placed per pen (10.76 birds per square metre (bird/m^2) in a commercial production system) (Aviagen, 2002).
- If the weights of the sampled organs were recorded it might have indicated significant physiological differences at specific periods.
- By recording the carcass weight, a percentage yield of dressed carcasses of Br versus ameliorator treated carcasses could be used as another critical parameter.
- For many of the results obtained in this trial the SD's were very high, a larger number of samples have to be analysed in order to have a lower SD. More repetitions included in the trial design are also necessary. Owing to the financial implications of sample analyses and production costs of broilers, a lower number of samples were analysed and only three repetitions of each treatment were included in the trial.
- Blood is often the most sampled indicator of the mineral status of an animal due to its comparatively simple sampling methods and its relative reliability. This is one critical parameter, blood Br concentration levels, and was not included in the trial due to financial restrictions.
- The quantification of bromate concentrations within all Br treatments should be measured, as high levels of Br within the drinking water, do have the potential for bromate formation. Bromate poses a carcinogenic threat to humans and animals. (Kempster *et al.*, 1980).
- Cl and F quantification need to be included as Br, I Cl and F are halogens.

- According to Jolles (1966) the relative ratio of Cl^-: Br^-: I^- in animals are 1 000: 1: 0.01, therefore Cl, Br and I should be quantified in the organs being studied in order to study the relationship of Br on Cl and I within livestock, disturbances in the ratio can reveal important information.
- Accumulation of Br within muscle was also reported by Winnek and Smith (1937). Levels of Br should also be quantified within the breast and thigh muscles (meat for consumption). These tissues' Br concentrations were quantified in a study done by Casey and Meyer (2001) and indicated very high hazardous levels of Br accumulated within these tissues.
- The inclusion of the spleen and thymus as research organ or gland in the study. Jolles (1966) reported a decrease in the thymus size and the deformation of the spleen, when rats were administered NaBr through their diet for several months.
- As urine I concentration cannot be quantified within chickens, the I concentration of the excreta should be measured as Br causes a decrease in I levels by renal excretion (Baker *et al.*, 2003). The elimination of Br also occurs through renal excretion (Jolles, 1966); the excreta should be tested for Br concentration in order to determine the Br excretion rate of broilers.

6.2 Future research recommendations

It may be of importance to supplement the study field of Br and its relevant ameliorators with regards to their interactions and effect on water quality, animal production, animal health and reproduction parameters. The consequent effect of high Br concentrations in ground water on the environment as well as on humans may be of significance for future research.

Suggested areas of future research are:

- The primary key risk events pertaining to cultural cooking practices using the same water source, drinking water as well as water used for preparing food. Water safe for household use (cooking and drinking etc.) should be identified.
- Effect of high Br concentrations in drinking water and I as a possible ameliorator on the physiological parameters of laying hens, other livestock and even humans. Evaluation of the long- and short-term exposure on the hatchability as well as egg, milk and meat quality. Pavelka *et al*. (2003) reported a drop in milk production due to high Br levels administered to rat dams through drinking water, Jolles (1966) reported a drop in milk fat and glucose concentration when Br was administered through the diet, what will the effect be on lactating ewes /cows or dairy cows or goats?
- The impact of high bromide intake in dams on I and Br transfer to sucklings and offspring or eggs. I- containing hormones are involved in embryogenesis (Underwood & Suttle, 1999), does high Br inclusion levels adversely affect embryogenesis?
- Pharmacokinetic properties of Br in chickens after oral administration of single dosages. Similar studies were done in dogs (Trepanier & Babish, 1995) and rats (Pavelka *et al*., 1999) where the mean apparent elimination half-life after oral administration, the mean total body clearance, the mean apparent volume of distribution and oral bioavailability of bromide were determined. This will definitely increase the available knowledge on Br metabolism of which there is a lack (Jolles, 1966).

  Cl, TDS and F, all possible alleviators for Br, are suggested investigations. Keeping in mind that the administration of an alleviator treatment, for animal management and sustainable resource management, should not adversely affect the managerial focus of the manager.
- The physiological effect of Br exposure on the reproduction of livestock. Abnormal head shape of spermatozoa, flagella degeneration (Linder *et al*., 1994), decreased spermatogenesis in testes, decreased prostate activity in males and a reduction in corpora lutea quantities found in the ovaries of females were observed in rats (Loeber *et al*., 1983) exposed to bromoacetic acid and NaBr in the diet. In humans, abortions in maternal exposure and a mild degree of

spermatogenesis suppression and impaired reproductive performance follow paternal exposure to Br vapour (Potashnik *et al.*, 1992).

- Further adverse effects of excessive Br exposure may be a reduction in the efficacy of vaccines administered via the drinking water, as this was not researched in this trial. Given that poultry raised under typical rural systems may be under more stress (nutritional or heat), failure for adequate health care to be maintained could have severe financial implications.
- The detailed histopathological effect of exposure to Br. Microscopic evaluations revealed hepatocellular hypertrophy laminated cytoplasmic inclusions, foamy cytoplasm, margination of basophilic cytoplasm and an increase in lipid droplets (Lee *et al.*, 1975) and hepatocellular degeneration (Hurt *et al.*, 1987). Important differences in the nature of cellular responses to Br may facilitate studies on the mode of actions.
- Pavelka (2004) and Jolles (1966) reported that Br is excreted via urine and faeces. The effect of animal manure for the fertilisation of crops in areas with high Br concentrations in their drinking water, cumulative effects of possibly high Br concentrations in both the manure and irrigation water on the crops and the ultimate consumers of the end product could be studied. The physiological effect of the inclusion of chicken manure with a high Br content, due to chickens consuming water with a high Br content, in diets for ruminants as a source of low-cost protein
- The effect of high Br concentrations in groundwater on the environment:
  The application of the manure for crop fertilization, where these crops are used for livestock and human consumption.
  Further research may be warranted to evaluate the availability of Br to be taken up by plants as Br is excreted by the kidneys in the urine and ends up in the soil and is easily filtered through the soil into ground water.
  The ease of an alleviator treatment for animal management and sustainable resource management without adversely affecting the managerial focus of the manager needs attention.
- In areas with prevalent goiter (I deficiency) in humans or livestock; is goiter caused by high Br concentrations within the water source?
- Analyse endocrine activity of adolescent chickens consuming Br within the drinking water, in order to study the effects on the immune and thyroid functions, as the birds employed in this trial had not reach maturity.
- The distribution of labelled Br to determine the distributions as well as organs of accumulation within the body, as there are still different results from the research obtained.

- As a complete reversal of induced growth retardation was obtained by Huff *et al*. (1956) with Br supplied as NaBr in the diet of rats, research on the administration of Br when growth retardation needs to be inverted at broilers should be done.
- Jolles (1966) and Pavelka (2004) found that saliva eliminates excess Br and counteracts the effect of Br retention by the kidney; the extent of saliva compensation as well as the effect on livestock should be researched.
- The virtue of the toxic effects of bromide on the thyroid gland and mechanisms of its interference with the thyroid gland hormones has not been completely explained to a large extent necessitate more research concentration.
- Masoud *et al*, (1973) reported renal failure when Br toxicity occurred as cause of death. Within this trial 3 mg Br had a distinct effect on the kidney; within what time period will this Br concentration cause renal failure and what level of administration via drinking water will lead to acute renal failure?
- Cl and total dissolved solids (TDS) data can be successfully used to estimate Br concentrations in Australian water. This provides a useful tool of estimating possible bromated formation level for water to be treated with disinfectants as ozone and Cl. As similar relationships between Cl and Br, and Cl and TDS to those calculated using data from specific studies (Magazinovic *et al.*, 2004).
- The possibility of a linear relationship between Br and Cl, and Br and TDS in South African water should be studied in order to alleviate the bromated quantification in water as well as the possible prevention of the carcinogenic consequence of bromated water.

7 CHAPTER 7: REFERENCES

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8 CHAPTER 8: APPENDIXES

APPENDIX A

Water samples analyses Meyer (2005 a-e)

Water for livestock use

Sample area or number	Observed Br level (mg/L)
REPORT: 022005/01/54	
Attempt2	0.395
Basderpad	0.197
Beaty	0.339
Bergval	0.403
Botchdish	0.301
Botchskop	2.113
Cradock	0.383
de End	0.614
DeBeershoop	0.674
Dissapointment	0.219
Elberfield	1.57
Friendship	0.39
Goedgevonde	2.322
Good Hope	1.265
Grafrenet	0.271
Groothoop	2.276
Harrysmit	0.497
Immelman	3.123
Juno	0.699
Kauletsi	0.582
Kitty	0.303
Klipspruit	0.736
Kokstad	0.177
Kromkloof	0.304
Lamonsite	3.942
Lusiand	0.393
Lutherdale	0.268

Water for domestic use

Sample area or number	Observed Br level (mg/L)
REPORT:022005/01/54	
Attempt2	0.395
Basderpad	0.197
Beaty	0.339
Bergval	0.403
Botchdish	0.301
Botchkop	2.113
Cradock	0.383
de End	0.614
DeBeershoop	0.674
Dissapointment	0.219
Elberfield	1.57
Friendship	0.39
Goedgevonde	2.322
Good Hope	1.265
Grafrenet	0.271
Groothoop	0.227
Harrysmit	0.497
Immelman	3.123
Juno	0.699
Kauletsi	0.582
Kitty	0.303
Klipspruit	0.736
Kokstad	0.177
Kromkloof	0.304
Lamonsite	3.942
Lusiand	0.393
Lutherdale	0.268

Mabopane	0.847
Nekar	0.267
Nelly	0.528
Olifantdrift	0.264
Olimpies	1.007
Osbourne	0.217
Paulatsy	0.559
Raadslid	2.407
Registrasie	0.276
Rietfontein	0.253

Mabopane	0.847
Nekar	0.267
Nelly	0.528
Olifantdrift	0.264
Olimpies	1.007
Osbourne	0.217
Paulatsy	0.559
Pinkie	0.097
Raadslid	2.487
Registrasie	0.276

Sample area or number	**Observed Br level (mg/L)**
REPORT: 022005/01/54	
Rob-Roy	0.715
Rustervley	0.313
Schoongelegen	1.335
Schurmanshoop	0.176
Selena	0.169
Semoneng	4.712
Sour Apple Tree	0.659
St Cards	0.299
Taung	0.716
Thornsland	0.569
Tibane	0.417
Uitkyk2	0.669
Wembly	1.538
Witfontein	1.788
Zoetendalsvlei	9.129

Sample area or number	**Observed Br level (mg/L)**
REPORT:022005/01/54	
Rietfontein	0.253
Rob-Roy	0.715
Rustervley	0.313
Schilpadskraal	0.042
Schoongelegen	1.335
Schurmanshoop	0.176
Selena	0.169
Semoneng	4.712
Sour apple Tree	0.659
St Cards	0.299
Taung	0.716
Thornsland	0.569
Tibane	0.417
Uitkyk	0.669
Wembly	1.538
Witfontein	1.788
Zoetendalsvlei	9.129

REPORT: 032005/02/26

Burgersford Groothoek	0.179
Burgersford Groothoek	0.214
Burgersford The Shelter	0.126
Dresden Camp 3	0.11
Groblersdal Herfort Grobler	0.115
Groblersdal Kitty Loskop Suid	0.251
Klipport	0.133
Rockliffe	0.148
Somshoek	0.229
	0.099
Thionville Camp 5	
Thionville K1	0.247
Twyfelaar	0.152
Uitval	0.178
Vergelegen	0.208
Viljoenshoop Camp 3	0.134

Sample area or number	Observed Br level (mg/L)

REPORT: 032005/02/26

Burgersford Groothoek	0.179
Burgersford Groothoek	0.214
Burgersord The Shelter	0.126
Dresden Camp 2	0.085
Dresden Camp 3	0.11
Fairview	0.013
Forrest Hill Kgoete	0.052
Groblersdal Herfort Grobler	0.115
Groblersdal Herford Loskop	0.076
Groblersdal Kitty Loskop Suid	0.251
Klipport	0.133
Limehill	0.062
Ncanakazi	0.013
Rockliffe	0.148
Sehlakoana Digokgela Veg	0.04
Setlhakwane Paardefontein	0.019
Somshoek	0.229
Thionville Camp 3	0.052
Thionville Camp 5	0.099

Sample area or number	Observed Br level (mg/L)
REPORT: 032005/02/26	
Thionville K1	0.247
Twyfelaar	0.152
Uitval	0.178
Vergelegen	0.208
Viljoenshoop	0.047
Viljoenshoop Camp 3	0.134

REPORT: 062005/04/90	
W723(DOA1122)	0.155
W724(DOA1124)	0.099
W725(DOA1127)	0.087
W726(DOA1128)	0.506
W727(DOA1129)	0.391
W728(DOA1130)	0.5
W729(DOA1132)	0.14
W730(DOA1133)	2.037
W731(DOA1134)	0.319
W732(DOA1135)	0.399
W733(DOA1137)	0.331
W734(DOA1138)	0.346
W735(DOA1141)	0.126
W736(DOA1143)	0.55
W737(DOA1145)	0.144
W738(DOA1146)	0.188
W739(DOA1147)	0.032
W740(DOA1150)	0.076
W741(DOA1151)	2.328
W742(DOA1152)	0.595
W743(DOA1153)	0.524
W744(DOA1154)	0.216
W745(DOA1155)	0.228
W746(DOA1156)	0.24
W747(DOA1157)	0.21
W748(DOA1159)	0.36
W749(DOA1161)	0.089
W750(DOA1163)	0.056
W751(DOA1165)	1.826
W752(DOA1167)	0.174
W753(DOA1168)	0.426
W754(DOA1169)	0.737

REPORT: 062005/04/90	
W723(DOA1122)	0.155
W724(DOA1124)	0.099
W725(DOA1127)	0.087
W726(DOA1128)	0.506
W727(DOA1129)	0.391
W728(DOA1130)	0.5
W729(DOA1132)	0.14
W730(DOA1133)	2.037
W731(DOA1134)	0.319
W732(DOA1135)	0.399
W733(DOA1137)	0.331
W734(DOA1138)	0.346
W735(DOA1141)	0.126
W736(DOA1143)	0.55
W737(DOA1145)	0.144
W738(DOA1146)	0.188
W739(DOA1147)	0.032
W740(DOA1150)	0.076
W741(DOA1151)	2.328
W742(DOA1152)	0.595
W743(DOA1153)	0.524
W744(DOA1154)	0.216
W745(DOA1155)	0.228
W746(DOA1156)	0.24
W747(DOA1157)	0.21
W748(DOA1159)	0.36
W749(DOA1161)	0.089
W750(DOA1163)	0.056
W751(DOA1165)	1.826
W752(DOA1167)	0.174
W753(DOA1168)	0.426
W754(DOA1169)	0.737

Sample area or number	Observed Br level (mg/L)
REPORT: 062005/04/90	
W755(DOA1170)	0.79
W756(DOA1172)	0.315
W757(DOA1176)	0.459
W758(DOA1177)	0.083
W759(DOA1179)	0.241
W760(DOA1180)	0.301
W761(DOA1182)	4.158
W762(DOA1183)	2.454
W763(DOA1184)	0.475
W764(DOA1185)	0.31
W765(DOA1186)	0.189
W766(DOA1187)	0.182
W767(DOA1188)	0.138
W768(DOA1189)	0.378
W769(DOA1191)	0.134
W770(DOA1192)	0.382
W771(DOA1195)	1.109
W772(DOA1197)	0.215
W773(DOA1198)	0.231
W774(DOA1200)	0.309
W775(DOA1203)	0.166
W776(DOA1204)	1.117
W777(DOA1205)	0.228
W778(DOA1206)	1.003
W779(DOA1207)	0.142
W780(DOA1208)	0.382
W781(DOA1209)	0.057
W782(DOA1210)	0.248
W783(DOA1211)	0.05
W784(DOA1214)	0.114

Sample area or number	Observed Br level (mg/L)
REPORT: 062005/04/90	
W755(DOA1170)	0.79
W756(DOA1172)	0.315
W757(DOA1176)	0.459
W758(DOA1177)	0.083
W759(DOA1179)	0.241
W760(DOA1180)	0.301
W761(DOA1182)	4.158
W762(DOA1183)	2.454
W763(DOA1184)	0.475
W764(DOA1185)	0.31
W765(DOA1186)	0.189
W766(DOA1187)	0.182
W767(DOA1188)	0.138
W768(DOA1189)	0.378
W769(DOA1191)	0.134
W770(DOA1192)	0.382
W771(DOA1195)	1.109
W772(DOA1197)	0.215
W773(DOA1198)	0.231
W774(DOA1200)	0.309
W775(DOA1203)	0.166
W776(DOA1204)	1.117
W777(DOA1205)	0.228
W778(DOA1206)	1.003
W779(DOA1207)	0.142
W780(DOA1208)	0.382
W781(DOA1209)	0.057
W782(DOA1210)	0.248
W783(DOA1211)	0.05
W784(DOA1214)	0.114

W785(DOA1215)	0.048
W786(DOA1216)	0.198
W787(DOA1218)	0.387
W788(DOA1219)	0.271
W789(DOA1221)	0.356
W790(DOA1222)	0.393
W791(DOA1223)	0.248
W792(DOA1224)	0.475
W793(DOA1225)	0.513
W794(DOA1227)	0.871

Sample area or number	Observed Br level (mg/L)
REPORT: 062005/04/90	
W795(DOA1228)	1.687
W796(DOA1231)	0.536
W797(DOA1232)	0.086
W798(DOA1233)	0.121
W799(DOA1236)	0.129
W800(DOA1238)	0.113
W801(DOA1239)	0.183
W802(DOA1241)	0.296
W803(DOA1244)	0.394
W804(DOA1245)	0.864
W805(DOA1247)	0.359
W806(DOA1248)	0.241
W807(DOA1250)	0.077
W808(DOA1251)	0.301
W809(DOA1252)	0.107
W810(DOA1254)	0.173
W811(DOA1254B)	0.241
W812(DOA1255)	0.235

REPORT: 082005/06/18

W785(DOA1215)	0.048
W786(DOA1216)	0.198
W787(DOA1218)	0.387
W788(DOA1219)	0.271
W789(DOA1221)	0.356
W790(DOA1222)	0.393
W791(DOA1223)	0.248
W792(DOA1224)	0.475
W793(DOA1225)	0.513
W794(DOA1227)	0.871

Sample area or number	Observed Br level (mg/L)
REPORT: 062005/04/90	
W795(DOA1228)	1.687
W796(DOA1231)	0.536
W797(DOA1232)	0.086
W798(DOA1233)	0.121
W799(DOA1236)	0.129
W800(DOA1238)	0.113
W801(DOA1239)	0.183
W802(DOA1241)	0.296
W803(DOA1244)	0.394
W804(DOA1245)	0.864
W805(DOA1247)	0.359
W806(DOA1248)	0.241
W807(DOA1250)	0.077
W808(DOA1251)	0.301
W809(DOA1252)	0.107
W810(DOA1254)	0.173
W811(DOA1254B)	0.241
W812(DOA1255)	0.234

REPORT: 082005/06/18

Chenzi	1.014
Esikhaleni	1.024
Hlaindlela	1.032
Ingindzini	0.779
Latha	0.611
Mfangosi	1.145
Mlangana	0.027
Mlumba	0.059
Nkandla Village	1.106
Nogejane	3.352
Nqulwana	0.053
Siphetwini	0.886

Chenzi	1.014
Esikhaleni	1.024
Hlaindlela	1.032
Ingindzini	0.779
Latha	0.611
Mfangosi	1.145
Mlangana	0.027
Mlumba	0.059
Nkandla Village	1.106
Nogejane	3.352
Nqulwana	0.053
Siphetwini	0.886

REPORT: 082005/07/87	
W1924(DOA280)	0.082
W1925(DOA622)	0.123
W1926(DOA623)	0.142
W1927(DOA624)	0.129
W1928(DOA628)	0.11
W1929(DOA629)	0.131

REPORT: 082005/07/87	
W1924(DOA280)	0.082
W1925(DOA622)	0.123
W1926(DOA623)	0.142
W1927(DOA624)	0.129
W1928(DOA628)	0.11
W1929(DOA629)	0.131

Sample area or number	**Observed Br level (mg/L)**
REPORT: 082005/07/87	
W1930(DOA632)	0.108
W1931(DOA633)	0.121
W1932(DOA634)	0.119
W1933(DOA635)	0.116
W1934(DOA636)	0.114
W1935(DOA639)	0.107
W1936(DOA1660)	0.115
W1937(DOA1661)	0.056
W1938(DOA1662)	0.065
W1939(DOA1663)	0.101

Sample area or number	**Observed Br level (mg/L)**
REPORT: 082005/07/87	
W1930(DOA632)	0.108
W1931(DOA633)	0.121
W1932(DOA634)	0.119
W1933(DOA635)	0.116
W1934(DOA636)	0.114
W1935(DOA639)	0.107
W1936(DOA1660)	0.115
W1937(DOA1661)	0.056
W1938(DOA1662)	0.065
W1939(DOA1663)	0.101

W1940(DOA1672)	0.101
W1941(DOA1674)	0.1
W1942(DOA1675)	0.115
W1943(DOA1676)	0.11
W1944(DOA1677)	0.116
W1945(DOA1678)	0.104
W1946(DOA1680)	0.114
W1947(DOA1681)	0.104
W1948(DOA1682)	0.125
W1950(DOA1691)	0.127
W1951(DOA1692)	0.13
W1952(DOA1695)	0.122
W1953(DOA1696)	0.119
W1954(DOA1686)	0.129
W1955(DOA1693)	0.119
W1956(DOA1698)	0.076
W1957(DOA1699)	0.092
W1958(DOA1701)	0.105
W1959(DOA1702)	0.115
W1960(DOA1703)	0.103
W1961(DOA1704)	0.118
W1962(DOA1707)	0.121
W1963(DOA1709)	0.124
W1964(DOA1718)	0.126
W1965(DOA1729)	0.114
W1966(DOA1735)	0.117
W1967(DOA1739)	0.123
W1968(DOA1751)	0.12
W1969(DOA1756)	0.13

Sample area or number	Observed Br level (mg/L)
REPORT: 082005/07/87	
W1970(DOA1759)	0.13

W1940(DOA1672)	0.101
W1941(DOA1674)	0.1
W1942(DOA1675)	0.115
W1943(DOA1676)	0.11
W1944(DOA1677)	0.116
W1945(DOA1678)	0.104
W1946(DOA1680)	0.114
W1947(DOA1681)	0.104
W1948(DOA1682)	0.125
W1950(DOA1691)	0.127
W1951(DOA1692)	0.13
W1952(DOA1695)	0.122
W1953(DOA1696)	0.119
W1954(DOA1686)	0.129
W1955(DOA1693)	0.119
W1956(DOA1698)	0.076
W1957(DOA1699)	0.092
W1958(DOA1701)	0.105
W1959(DOA1702)	0.115
W1960(DOA1703)	0.103
W1961(DOA1704)	0.118
W1962(DOA1707)	0.121
W1963(DOA1709)	0.124
W1964(DOA1718)	0.126
W1965(DOA1729)	0.114
W1966(DOA1735)	0.117
W1967(DOA1739)	0.123
W1968(DOA1751)	0.12
W1969(DOA1756)	0.13

Sample area or number	Observed Br level (mg/L)
REPORT: 082005/07/87	
W1970(DOA1759)	0.13

W1971(DOA1760)	0.129	W1971(DOA1760)	0.129
W1972(DOA1762)	0.117	W1972(DOA1762)	0.117
W1973(DOA1764)	0.126	W1973(DOA1764)	0.126
W1974(DOA1766)	0.128	W1974(DOA1766)	0.128
W1975(DOA1768)	0.128	W1975(DOA1768)	0.128
W1976(DOA1769)	0.127	W1976(DOA1769)	0.127
W1977(DOA1770)	0.126	W1977(DOA1770)	0.126
W1978(DOA1774)	0.129	W1978(DOA1774)	0.129
W1979(DOA1775)	0.128	W1979(DOA1775)	0.128
W1980(DOA1861)	0.126	W1980(DOA1861)	0.126
W1981(DOA1862)	0.111	W1981(DOA18662)	0.111
W1982(DOA1863)	0.103	W1982(DOA1863)	0.103
W1983(DOA1864)	0.095	W1983(DOA1864)	0.095
W1984(DOA1865)	0.081	W1984(DOA1865)	0.081
W1985(DOA1868)	0.063	W1985(DOA1868)	0.063
W1986(DOA1871)	0.115	W1986(DOA1871)	0.115
W1987(DOA1872)	0.107	W1987(DOA1872)	0.107
W1988(DOA1874)	0.122	W1988(DOA1874)	0.122
W1989(DOA1875)	0.128	W1989(DOA1875)	0.128
W1990(DOA1876)	0.103	W1990(DOA1876)	0.103
W1991(DOA1877)	0.116	W1991(DOA1877)	0.116
W1992(DOA1878)	0.123	W1992(DOA1878)	0.123
W1993(DOA1880)	0.117	W1993(DOA1880)	0.117
W1994(DOA1881)	0.129	W1994(DOA1881)	0.129
W1995(DOA1882)	0.113	W1995(DOA1882)	0.113
W1996(DOA1883)	0.128	W1996(DOA1883)	0.128
W1997(DOA1884)	0.131	W1997(DOA1884)	0.131
W1998(DOA1885)	0.124	W1998(DOA1885)	0.124
W1999(DOA1886)	0.129	W1999(DOA1886)	0.129
W2000(DOA1888)	0.11	W2000(DOA1888)	0.11
W2001(DOA1889)	0.131	W2001(DOA1889)	0.131
W2002(DOA1890)	0.129	W2002(DOA1890)	0.129
W2003(DOA1895)	0.125	W2003(DOA1895)	0.125
W2004(DOA1896)	0.127	W2004(DOA1896)	0.127

W2005(DOA1898)	0.129
W2006(DOA1900)	0.123
W2007(DOA1903)	0.11
W2008(DOA1906)	0.129

Sample area or number	Observed Br level (mg/L)
REPORT: 082005/07/87	
W2009(DOA1907)	0.129
W2010(DOA2250)	0.122

W2005(DOA1898)	0.129
W2006(DOA1900)	0.123
W2007(DOA1903)	0.11
W2008(DOA1906)	0.129

Sample area or number	Observed Br level (mg/L)
REPORT: 082005/07/87	
W2009(DOA1907)	0.129
W2010(DOA2250)	0.122

Summary of statistics for Br content in the water samples:

REPORT: 022005/01/54	
Average (mg/L)	1.05
Standard Deviation (mg/L)	1.501
Median (mg/L)	0.544
Minimum (mg/L)	0.169
Maximum (mg/L)	9.129
Relative guideline (mg/L)	0-0.01

REPORT: 032005/02/26	
Average (mg/L)	0.168
Standard Deviation (mg/L)	0.051
Median (mg/L)	0.152
Minimum (mg/L)	0.099
Maximum (mg/L)	0.251
Relative guideline (mg/L)	0-0.01

REPORT: 062005/04/90	
Average (mg/L)	0.468
Standard Deviation (mg/L)	0.613
Median (mg/L)	0.2595
Minimum (mg/L)	0.032
Maximum (mg/L)	4.158

REPORT: 022005/01/54	
Average (mg/L)	0.977
Standard Deviation (mg/L)	1.48
Median (mg/L)	0.457
Minimum (mg/L)	0.042
Maximum (mg/L)	9.129
Relative guideline (mg/L)	0-0.01

REPORT: 032005/02/26	
Average (mg/L)	0.119
Standard Deviation (mg/L)	0.074
Median (mg/L)	0.115
Minimum (mg/L)	0.013
Maximum (mg/L)	0.251
Relative guideline (mg/L)	0-0.01

REPORT: 062005/04/90	
Average (mg/L)	0.468
Standard Deviation (mg/L)	0.613
Median (mg/L)	0.2595
Minimum (mg/L)	0.032
Maximum (mg/L)	4.158

Relative guideline (mg/L)	0-0.01

REPORT: 082005/06/18

Average (mg/L)	0.924
Standard Deviation (mg/L)	0.876
Median (mg/L)	0.95
Minimum (mg/L)	0.027
Maximum (mg/L)	3.352
Relative guideline (mg/L)	0-0.01

REPORT: 082005/07/87

Average (mg/L)	0.116
Standard Deviation (mg/L)	0.016
Median (mg/L)	0.119
Minimum (mg/L)	0.056
Maximum (mg/L)	0.142
Relative guideline (mg/L)	0-0.01

Relative guideline (mg/L)	0-0.01

REPORT: 082005/06/18

Average (mg/L)	0.924
Standard Deviation (mg/L)	0.876
Median (mg/L)	0.95
Minimum (mg/L)	0.027
Maximum (mg/L)	3.352
Relative guideline (mg/L)	0-0.01

REPORT: 082005/07/87

Average (mg/L)	0.116
Standard Deviation (mg/L)	0.016
Median (mg/L)	0.119
Minimum (mg/L)	0.056
Maximum (mg/L)	0.142
Relative guideline (mg/L)	0-0.01

Norms adversely affected: Health (Toxic), Product quality

APPENDIX B
ISCW: Water analyses quantification results

APPENDIX C
Histopathology results

APPENDIX D
T_3 and T_4 serum concentrations

APPENDIX E
ISCW: Br and I quantification organ results

APPENDIX F
Selected raw data