Evaluation of pH Levels or High Content of Calcium, Magnesium and Sulphate in Drinking Water on Production Performance, Egg Quality, Bone Quality and Mineral Retention of Laying Hens

By

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ABSTRACT

A water quality survey and 3 laying hen production trials were conducted to evaluate extremes in water pH and minerals found in Canadian egg production units. Two water mineral trials using waters with high contents of Ca, Mg and SO_4 were conducted from 33-69 weeks and from 7-46 weeks of age. In the first trial, up to 487 ppm Ca, 234 ppm Mg and 1317 ppm SO_4 were supplied in water. During the second trial, up to 786 ppm Ca, 562 ppm Mg and 1988 ppm SO_4 concentrations were tested. High contents of Ca, Mg and SO_4 did not have effects on production, egg quality, bone quality or mineral balance of Lohmann-Lite laying hens. pH treatments of 6, 6.5, 7.9 (unadjusted) and 8.2 were assessed for 4 replicates for hens at 66-69 weeks of age. PH 8.2 had negative impact on hen production performance while other pH levels did not.

LIST OF ABBREVIATIONS AND SYMBOLS USED

Alberta Agricultural Coordinating Committee ..... AACC

Alberta..... AB

Albumen height..... AH

American Public Health Association ..... APHA

Analysis of variance..... ANOVA

Association of Official Analytical Chemists ..... AOAC

Australian and New Zealand Environment and Conservation-

Committee ..... ANZECC

Bicarbonate ..... HCO_3

British Colombia ..... BC

Calcium ..... Ca

Carbonate ..... CO_3

Centimeter..... cm

Chloride..... Cl

Copper..... Cu

Degrees centigrade..... °C

Feed consumption ..... FC

Food and Agriculture Organization Statistics..... FAOSTAT

Gram ..... g

Hydroxide ..... OH

Iodine I

Iron..... Fe

Kelvin degree..... K

Kilogram ..... Kg

Light: Dark..... L: D

Litre..... L

Magnesium ..... Mg

Manganese ..... Mn

Manitoba ..... MB

Milligram ..... mg

Millilitre ..... mL

New Brunswick ..... NB

Newfoundland and Labrador ..... NL

Nitrate ..... NO_3

Nitrite ..... NO_2

Nova Scotia ..... NS

Ontario ..... ON

Parts per million ..... ppm

Percent ..... %

Phosphorus ..... P

Potassium ..... K

Prince Edward Island ..... PE

Quantitative Computed Tomography ..... QCT

Quebec ..... QC

Saskatchewan ..... SK

Specific gravity ..... SG

Standard error of the mean ..... SEM

Sulphate ..... SO_4

Water consumption ..... WC

Weeks ..... wks

Zinc ..... Zn

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CHAPTER 1 INTRODUCTION

Water is an important nutrient for laying hens and is involved in many vital metabolic functions in poultry (Solomon et al. 1995). Poor water quality may negatively affect hen performance, even when a well-balanced diet is supplied (Leeson and Summers 2008). Thus, an adequate supply of high quality water is essential for optimum performance of hens.

Water quality can vary from place to place, depending on the dissolved substances in the water (Zimmermann 1998). Types and levels of minerals may change in water bodies, including surface and ground waters, due to changes in soil composition or bedrock types associated with the ground water sources (US-Environment Protection agency (USEPA) 2012). Leaching from soil and surface runoff with rain water can lead to accumulation of these substances in ground water and surface water bodies.

High levels of dissolved minerals in water can negatively affect poultry performance or health (NRC 1974), but effects of minerals in water have not been well studied and documented. Information about the effects of minerals in water on laying hen performance or egg quality, other than sodium chloride (NaCl), is scarce. Elevated levels of Ca, Mg and SO_4 are often found in hard waters in different parts of the world (USEPA 2012). High levels of Ca and Mg have caused poor absorption of many essential minerals in animals (McDowell 1992). Excess SO_4 can cause diarrhoea, which may lead to poor absorption of nutrients (Carter and Sneed 1996).

Water pH, which is a measure of acidity of water, may affect hen performance and egg quality (Carter and Sneed 1996). Available information on the effects of different pH levels

in water for poultry has been limited to antibacterial effect on the digestive tract of poultry (Chaveerach et al. 2004; Açıkgöz et al. 2011). The preferred range of water pH for laying hens is not discussed in any peer reviewed literature.

Commercial laying hens have a high metabolic demand for egg production due to their high production rate. To be profitable, these birds need to be supplied with optimum nutrition throughout their production cycle. Water quality cannot be ignored in layer nutrition. Since there is a lack of scientific information on the effects of different pH levels and levels of common minerals including Ca, Mg and SO_4, on production performance and egg quality, this study was planned to fill these knowledge gaps in laying hen nutrition. Since, the composition of drinking water supplied to commercial hens in different areas can vary, it is important to know the pH and mineral composition of water, currently being used for hens in commercial egg production in Canada as a starting point for evaluating water quality. A survey of waters used in laying hen production need to be conducted. Based on these findings studies on the effects of water pH and mineral content can be planned and conducted.

CHAPTER 2 LITERATURE REVIEW

2.1 Egg production in the world and Canada

Eggs are an important source of high quality protein for humans (Stadelman 1995). In 2012, global hen-egg production was about 66.4 million tonnes. Major contributors were China (37%), United States of America (8%), India (5%), Japan (4%) and Mexico (3%) (FAOSTAT 2014a). The estimated number of layers in the world were 4.93 billion (International Egg Commission 2014). Canada produced 0.4 million tonnes of hen-eggs in 2012. There were 1,016 registered egg farms in Canada and the average flock size was 20,241 hens in 2012 (Agriculture and Agri-Food Canada 2013). Eggs were mainly sold as table eggs (70%) and the remainder were used for manufacturing value added products (Agriculture and Agri-Food Canada 2013). The worldwide use of egg products has increased recently (International Egg Commission 2014). The estimated average egg consumption per Canadian increased from 11 kg to 12 kg from 2000 to 2011, while world per capita consumption rose from 8.1 to 8.9 kg per person during that period (FAOSTAT 2014b). Water quality in different areas of the world where laying hens have been raised, could have an impact on egg production. Hens need to consume good quality water for effective egg production.

2.2 Water for poultry production

Water is considered an essential nutrient for poultry (National Research Council (NRC) 1994). Water comprises about 55 to 75% of animal body weight and more than 65% of an egg (Ewing 1963). In an animal body, water is essential for many vital functions, such as regulating body temperature, growth, reproduction, digestion, metabolism, excretion, and

regulation of mineral homeostasis (Schlink et al. 2010). Animals can produce some metabolic water during nutrient metabolism in the body (Doreau et al. 2012). However, most water is obtained through drinking water and consumption of feed.

Laying hens only consume water from fresh sources opposed to salt water. The global fresh water supply is made up of snow and ice (68.7%); groundwater (30.9%) and rivers or lakes (0.4%) (Environment Canada 2013). It has been estimated that 70% of the total annual fresh water used for global agricultural purposes was for irrigation and livestock production. During 2008 to 2012, Canada used 12% of the total fresh water drawn annually for agricultural purposes (World Bank 2014), while livestock used only 10% of that (Kulshreshtha and Grant 2007).

In many parts of the world, underground water supplies are utilised as sources of drinking water for laying hens (Balnave and Yoselewitz 1987). These waters are a vital source of water for rural agricultural activities across Canada (van der Kamp and Grove, 2001). In AB, MB and SK, the largest proportion of sources used for livestock production is ground water (Environment Canada 2013). In Prince Edward Island, 100% of water requirements are fulfilled by ground water sources. These well waters are known to exceed (20-40% of rural wells) at least one or two aspects of esthetic quality or health quality guidelines (van der Kamp and Grove 2001). Well water can be poor in quality due to its increased mineral content (Koelkebeck et al. 1999) as compared with municipal water supplies.

2.2.1 Water quality for poultry

Water is a compound that consists of hydrogen and oxygen and it is difficult to find water in its pure form naturally. Any compound dissolved in water can change its quality. The definition of water quality may vary from situation to situation and where it is applied, such

as for municipal use or for agricultural purposes. High drinking water quality for poultry can be defined as being free of substances which could negatively affect water acceptability and performance of the birds (Schlink et al. 2010).

Water quality indicators are measured as total dissolved solids (TDS), pH, hardness, alkalinity, mineral content, bacteria numbers and esthetic factors, such as color, taste, odor and turbidity (USEPA 2012). Conductivity can be used to determine TDS in water, since it estimates the total dissolved ions in water (Singh and Kalra 1975). Water quality aspects such as extremes in mineral concentration, pathogenic bacterial count or other harmful contaminants can affect poultry performance (NRC 1994). Most information found in the literature on water quality effects on laying hens was based on field observations, rather than controlled experiments (Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC) 2000).

The composition of ground water can vary from place to place (US-Environment Protection agency (USEPA 2012). When water passes through rocks and soils, minerals, such as Mg, Ca and Cl, may be dissolved in the water (USEPA 2012). There may be natural contaminants in the ground water, such as microbes, mineral ions, heavy metals and nitrates and nitrites (USEPA 2012) while human activities also contribute these contaminants.

TDS consists of all inorganic salts dissolved in water. The TDS usually consists of Ca, Mg, Na, K, CO_3, HCO_3, Cl, SO_4 and NO_3 (USEPA 2012) and non-mineral particulate matter. The solids can be added to water from soil, wastes from human activities and agricultural chemicals such as fertilizers (USEPA 2012). High level of TDS (more than 3000 ppm) could make water unpalatable and may cause diarrhoea, especially when high contents of

Na, Mg and SO_4 are present (University of Saskatchewan 1965). However, the maximum tolerable levels of the minerals were not mentioned. High concentrations of specific ions in water can cause poor performance or health implications to the hens (NRC 1994), but information on mineral effects on hen performance or egg quality is limited. Knowledge on effects of specific mineral ions would be useful to evaluate water quality for hens.

Water acidity or alkalinity level is measured by pH. Acidity level of water can be changed due to many reasons. The dissolved mineral content can affect the acidity of the water. Acidic water consists of more dissolved trace minerals than alkaline water (Sullivan et al. 2005). The most common mineral compound that affects water pH is calcium carbonate ($CaCO_3$). When it dissolves in water, it contributes hydroxyl ions (OH), which increase the pH. The pH of water from carbonate or silicate containing rocky areas is usually above 7 (Sullivan et al. 2005). Water with a pH above 7 is known as alkaline water. Since carbonate and silicates are rich in soil and rocks, ground water sources are mostly alkaline in nature. When iron sulphide, which is also a mineral in the earth's crust, dissolves in water, it can produce sulphuric acid and the water becomes acidic. Burning fossil fuels produces sulphur dioxides that will dissolve in water and produce sulphuric acid. Acidic water has a pH of less than 7. Surface and shallow ground water can became acidic when atmospheric carbon dioxide dissolves in water and produces hydrogen ions, as occurs in lake acidification caused by acid rain (Sullivan et al. 2005). Fertilizers or pesticides applied to the soil or plants can contribute to water sources through leaching or surface runoff and could change water acidity levels (Sullivan et al. 2005). Therefore, pH of the water can change from source to source. The effects of water pH have not been studied on hen performance or egg quality and no information was found in the peer reviewed literature. However, there are

some studies with adding organic acids for broilers which mostly focused for bacterial control (Chaveerach et al. 2004; Açıkgöz et al. 2011).

Hardness indicates the levels of Ca and Mg present in water in either a HCO_3 or SO_4 form (Blake and Hess 2001). It is measured as the concentration of $CaCO_3$ in water. Ca and Mg can be found in increased levels in water (Sengupta 2013). These two minerals are high in areas with limestone and dolomites and make water hard. Hardness of water is not detrimental to birds unless the ions are present at toxic levels. The available recommendations for poultry, which are discussed later in this chapter, do not well define the safe levels of hardness. There is considerable variation in the hardness of water supplies, so Ca, Mg and SO_4 obtained from water by birds will vary (Atteh and Leeson 1983a). Effects of these three minerals on layer performance and egg quality was not well evaluated.

The capacity of water to neutralize acidity is known as alkalinity (USEPA 2012). This is also known as the buffering capacity of water. It is a measure of CO_3, HCO_3 and OH ion levels of water (USEPA 2012). These anions can react with hydrogen ions in water and increase pH. Alkalinity can be affected by the soil minerals dissolved in water and chemical substances added by human activities. Alkalinity of 170 ppm can cause diarrhoea in animals (Manitoba Department of Health 1973).

Nitrate/Nitrite-N (NO_3/NO_2-N) is another indicator of water quality. High content in water causes negative effects in poultry (Adams et al. 1966). For human drinking water, the level should be less than 10 ppm (USEPA 2012). However, poultry can tolerate more than the human recommended level. Adams et al. (1966) found that laying hens can tolerate 300 ppm nitrate-nitrogen (NO_3-N) or 200 ppm nitrite-nitrogen (NO_2-N) without affecting

performance if the diet contains adequate vitamin A, as high levels of NO_3/NO_2-N can affect vitamin A metabolism. NO_3/NO_2 can be added to water in many ways. Inorganic and organic fertilizer application in agriculture can lead to leaching of NO_3 or NO_2 into the ground water or accumulation in surface waters through runoff. Leakage from sewage systems and septic tanks could also increase the levels in ground water (USEPA 2012). In this project, alkalinity and NO_3/NO_2-N will not be evaluated.

2.2.2 Guidelines to evaluate water quality for poultry

There are no well established guidelines to evaluate poultry drinking water quality that are based on experimentation on poultry. The ones that do exist are mostly based on human standards or recommendations for other livestock species (Blake and Hess 2001). The use of human standards for animal drinking water evaluation may not be appropriate since animals can tolerate higher levels of minerals and their ability to tolerate dissolved substances may be different than for humans (South Dakota State College 1959). Lack of scientific knowledge has been the challenge to provide recommendations for livestock drinking water, including poultry (Alberta Agricultural Coordinating Committee (AACC) 1972). According to Weltzien (2002), many of the available guidelines for poultry drinking water have been based on mortality and not on performance.

South Dakota State College (1959) established some guidelines for total dissolved solids for livestock drinking water. To develop a criteria for the evaluation of water quality, experiments were conducted with different animal species, including rats, swine, cattle and laying hens. Laying hen studies were conducted with different high levels of sodium NaCl in water (4000, 7000 and 10000 ppm). Water consumption increased with the NaCl level and diarrhoea was observed. Egg production and body weight were affected at 10,000 ppm.

The effects on rats, cattle and swine were also evaluated for chlorides and sulphates of Na, Mg and Ca cations, using the same levels provided in laying hen studies in water. The studies concluded that dissolved salts at more than 10,000 ppm could be toxic to the animals. Animals had reduced weight gain and developed diarrhoea at the higher levels of minerals in water. The authors suggested that 0 to 999 ppm dissolved solids in water was ideal for livestock consumption, while 1000 to 3999 ppm was good. Concentration from 4000 to 6999 ppm was satisfactory, while more than 7000 ppm was not appropriate. Further, the importance of evaluation of alkalinity, NO_3 level and Fe levels in water was mentioned. However, tolerable levels were not suggested, since experimental work was not conducted at that time.

AACC (1972) developed recommendations, especially for poultry drinking water. The recommendations were based on studies conducted to evaluate different NaCl levels in water in poultry at that time (Kare and Biely 1948; Bigland 1950; Krista et al. 1961; Roblee and Clandinin 1961; Adams et al. 1966). The effects of other minerals and their interaction effects at different levels were not investigated. Based on the research findings, some general recommendations for poultry were suggested by the committee. TDS content in water was used to evaluate the suitability of water for poultry, since no information was available on individual mineral effects or their interaction effects at different concentrations. TDS content should be less than 1500 ppm for poultry less than 3 weeks of age, based on reported edema and ascites incidents in young poultry given higher levels of salt in water.

Therefore, the TDS content from 0 to 1500 ppm was recognised as suitable for all poultry species of all ages, while 1500 to 3000 ppm was safe only for poultry more than 3 weeks

of age. Concentrations from 3000 to 4000 ppm were recommended only for laying hens and turkeys, but not for young poultry. The levels from 4000 to 7000 ppm may cause diarrhoea in laying hens and turkeys, while more than 7000 ppm dissolved solids was not recommended for the poultry drinking water.

The Canadian Council of the Ministers of Environment (CCME) (1993) developed maximum desirable limits for the some physico-chemical parameters in livestock water in 1987 (Table 2.1). The criteria were based on 4 major factors: 1) tolerable daily intake rates of the contaminant; (2) daily water intake rates; (3) body weights and; (4) bioaccumulation in livestock products (CCME 1993). There were no recommendations for Mg, Cl, P, Cu, Fe, Mn ions or conductivity levels.

Table 2.1. The Canadian Council of the Ministers of Environment guidelines for the livestock drinking water

Parameter	Maximum desirable limit (ppm)
Calcium	1000
Magnesium	No data
Chloride	No data
Nitrate/Nitrite-N	100
Sulphates	1000
Phosphorus	No data
Copper	No data
Iron	No data
Manganese	No data
Boron	5
Cadmium	0.08
Zinc	50
TDS	3000
Conductivity	No data

Source: CCME (1987)

Weltzien (2002) also suggested guidelines for poultry drinking water. However, the research supporting these guidelines was not mentioned. According to those suggestions, the maximum desirable limit for Mg and Ca ions in poultry drinking water should be less than 350 ppm and 600 ppm, respectively. However, Fairchild and Ritz (2012), reported the maximum acceptable content for Mg and Ca as 125 ppm and 500 ppm, respectively, based on field research findings. Based on their broiler studies, the authors reported that poultry can tolerate 600 ppm Fe, 20 ppm Mn and 600 ppm nitrate in drinking water, (Batal et al. 2005; Fairchild et al. 2005).

The available information on SO_4 in drinking water was variable. For livestock species, the maximum safe limit for SO_4 was 1000 ppm in the CCME guidelines (1987). However, for poultry species, more than 50 ppm SO_4 was found to negatively affect performance, if the Mg concentration was high in water (Weltzien 2002). The maximum desirable limit for SO_4 was reported as 250 ppm (Carter and Sneed 1996; Weltzien 2002; Alberta Agriculture and Rural Development 2007; Fairchild and Ritz 2012).

Based on available information in different agriculture extension publications, the maximum acceptable limits of minerals for all poultry species in their drinking water are summarised in Table 2.2. The recommended levels varied from source to source and the original research data were not discussed or linked in these popular press articles. Therefore, the information on effects on hens could not be found.

Table 2.2 Guidelines for the maximum acceptable mineral concentrations for poultry drinking water

Parameter[1]	Carter and Sneed (1996)	Weltzien (2002)	Fairchild and Ritz (2012)
Calcium	No data	600	500
Chloride	250	250	250
Copper	0.6	No data	0.6
Iron	0.3	3	0.03
Magnesium	125	350[2]	125
Manganese	No data	No data	0.05
Nitrate	25	(Nitrate-N = 10)	25
Phosphorus	No data	No data	0.1
Potassium	No data	No data	500
Sodium	No data	300[2]	50
Sulphate	250	250	250
Hardness	180	1500	110
TDS	No data	3000	No data
pH	6.8-7.5	6.5-8.5	5-6.8

[1]All concentrations were in parts per million (ppm).
[2]If sulphate concentration is more than 50 ppm, bird may develop diarrhoea at 125 ppm (Weltzien 2002).

2.2.3 Effect of water pH on laying hen performance

Information regarding the effects of water pH on laying hens is scarce in the literature. Neither Canadian water quality guidelines (CCME 1987) nor NRC (1994) have suggested a preferred pH range for livestock. Carter and Sneed (1996) and Weltzien (2002) reported that water pH below 6 and 6.5 respectively, could negatively affect digestion and lead to poor performance in poultry. Fairchild and Ritz (2012) also suggested that pH below 6 would not be suitable for poultry. According to Blake and Hess (2001), low pH water is considered to be less palatable and may have negative effects on poultry performance. However, peer reviewed literature was not found to describe the effects of different pH levels on laying hen performance or egg quality. Information was found in poultry extension publications.

There was some information regarding water acidification effect on broiler performance. Water acidification is used as an approach to reduce pathogenic bacterial count in the gut of broilers today. Watkins et al. (2004) assessed water pH from 3 to 8 on broiler performance. There was no effect of the pH levels in water on body weight or feed conversion efficiency. Water consumption per gram of body weight was also not affected by the pH levels. However, Açıkgöz et al. (2011) found reduction in body weight when birds were supplied with pH 4.5 water, when compared to control water pH 7.4, at both 21 and 42 days of age. However, feed intake, feed conversion ratio or mortality were not affected by pH 4.5. Both studies reported that gizzard pH did not significantly change with the pH levels of the water. Chaveerach et al. (2004) did not find body weight change in broilers supplied with water which had pH from 3.9 to 6.9. Neither feed intake nor water intake was measured, but Pesti et al. (2004) found an increase in body weight when hens were supplied with acidified water, compared to the control. These findings on water pH effect on broiler performance were variable from study to study. Therefore, applying these findings to evaluate pH effect on laying hens would be inaccurate.

pH in drinking water may have an effect on mineral absorption in the gut of hens and consequently, on homeostasis. Diets with low pH improved nutrient digestibility including, minerals in swine and broilers. Dibner and Buttin (2002), in their review, discussed the possible mechanisms of improved performance of poultry and swine when supplied with acidified diets (supplement with organic acids). One possible mechanism was enhanced digestion of nutrients, including minerals, by lowering the gut pH. Reduced pH in digesta would improve the action of digestive enzymes and the retention time, which would allow more opportunity for digestion. Yesilbag and Çolpan (2006) found that acidified diet

improved egg production, while there were no effects on feed consumption, body weight or egg quality measurements. The pH of acidified diets were 5.5, 5.7 and 5.8 while the control diet pH was 6.2, without acidification. Protein and lipid metabolism was evaluated by using serum indicators. Protein metabolism was improved by low pH diets, while no effect was found on lipid metabolism. Mineral metabolism was not evaluated. However, findings from past broiler studies did not demonstrate pH changes in the gut due to water pH changes from pH 3 to 8. No layer studies were found in the literature that conducted an evaluation of drinking water pH effect on nutrient metabolism.

Although available guidelines did not recommend water below 6 or 6.5 for all poultry, the above studies showed that broilers can tolerate a wide range of pH in water, as low as 3 and as high as 8. No studies were found that evaluated pH above 8 in poultry. No information was found on different water pH levels on nutrient metabolism, including minerals. Mineral metabolism is critical for laying hen productivity. Therefore, it is useful to conduct studies to evaluate different pH levels on laying hen production performance, egg quality and mineral balance.

2.2.4 Effect of high mineral content in water on laying hen performance

A number of studies have been conducted on the effect of NaCl on laying hen egg quality and its adverse effect on egg shell quality (Yoselewitz et al. 1988; Yoselewitz and Balnave 1989a, b; Pourreza et al. 1994; Brackpool et al. 1996; Balnave and Zhang 1998).

The effect of high NaCl (up to 600 ppm) in water on 60 week old hens was studied by Balnave and Yoselewitz (1987). The authors found a significant increment of shell defects within 2 days after treatment initiation, when between 200 to 600 ppm NaCl was added to

water. Daily feed intake and egg production was not affected during the 5 week treatment period. Although egg weight was not affected, shell breaking strength, shell thickness, shell weight and shell percentage values were significantly decreased with the high level of NaCl. Significant incremental shell damage occurred, compared to the control water treatment and persisted for 5 weeks after the treatment was ended. These experiments were conducted at a room temperature of 26^0C.

Further, Yoselewitz et al. (1988) studied the effect of up to 2000 ppm NaCl in drinking water on laying hen performance. More than half of the eggs from 80 to 95 week old hens exhibited defective shells. They concluded that Ca metabolism was permanently affected, since the shell defects were still observed after hens were supplied with normal water for 5 weeks. In 1989a, Yoselewitz and Balnave found an increased production of poor quality eggshells when high NaCl water was given to laying hens at 40 weeks of age, when compared to hens at early production. The older hens seemed to be more sensitive to the higher salt level in the water.

Pourreza et al. (1994) found reduced eggshell thickness when 2000 ppm NaCl in drinking water was supplied to hens, but eggshell thickness was not affected by 1000 ppm. However, Chen and Balnave (2001) did not find any significant effect of 2000 ppm NaCl in drinking water on production performance measures or egg quality measures over a 40 weeks study. Feed intake, egg production, egg weight or feed conversion efficiency were not affected by NaCl, nor were shell quality measures. Shell breaking strength, shell weight or shell thickness were not affected by high salt content. Damron (1998) did not find any effect on shell quality with 2000 ppm NaCl in white leghorn layers.

The studies which showed positive effects were mainly those conducted in Australia with locally developed strains (White Leghorn × New Hampshire, White Leghorn x Australorp) that might have been more sensitive to high NaCl, unlike the ISA brown hens produced in the USA (Chen and Balnave 2001).

Information on the effects of high Ca and Mg in water on poultry was older and no current data was found in the most recent literature. Heller (1933) reported that Rhode Island Red hens tolerated NaCl and magnesium sulphate ($MgSO_4$) up to 1500 ppm in water without affecting body weight, while calcium chloride ($CaCl_2$) reduced body weights at 1500 ppm inclusion. Egg production was significantly reduced at 2% NaCl in white leghorn hens. But effects of $MgSO_4$ or $CaCl_2$ were not determined for white leghorn hens. In 1975, Adams and co-researchers evaluated the effect of high content of $MgSO_4$ in the water of hens. The authors observed significant reduction in egg production, feed and water consumption when 4000 ppm sulphate (as $MgSO_4$) was given to laying hens. However, there was no effect on body weights of the birds. When 16,000 ppm $MgSO_4$ was supplied, 100% mortality occurred within seven days. Sulphate content had no effect on egg weight, Haugh unit or shell thickness. However, these findings did not give exact threshold levels for the hen performance. There may be negative impacts at lower levels than 4000 ppm of $MgSO_4$. The effects of lower levels should also be assessed in order to find a maximum tolerable level for hens, where performance or egg quality is not affected. Moreover, the effects were evaluated in hens that produced fewer eggs during that era. Modern commercial lines are bred to produce high number of eggs per year and these findings on older breeds may not be appropriate to evaluate sensitivity of today's commercial hens to higher levels of minerals in their drinking water.

High content of minerals in drinking water may affect mineral metabolism of hens (NRC 1994). Minerals in the digestive tracts of animals can affect absorption of other minerals (Georgievskiĭ et al. 1981). The negative effects of high Ca on other divalent mineral ions has been reported in livestock species. High Ca can reduce the absorption of Mg, Fe, Cu, Mn, Zn and I (Georgievskiĭ et al. 1981). Mg can affect the absorption of Mn and P, while SO_4 can affect Cu and Se absorption. High concentrations of these minerals in drinking water may affect absorption of minerals in the digestive tract of hens. Since mineral homeostasis is critical for laying hen egg production, eggshell quality and bone quality (Hurwitz and Bar 1966), any effects on absorption in the gut may negatively affect these functions. It was found that during low intake of Ca, bone reserves, especially the medullary bones, are utilized to maintain eggshell quality (Hurwitz and Bar 1966). Prolonged insufficiency mobilizes structural bone Ca, which can cause bone weakness (Cheng and Coon 1990). Since no evaluation has been carried out on mineral metabolism in hens supplied with drinking water minerals, it would be useful to conduct a balance study with hens supplied with different levels of minerals in water. Further, bones should be analysed to examine any effect on bone mineral content.

2.2.5 Effect of high mineral content in water on other poultry

In other poultry species besides laying hens, the effects of high NaCl in water have been determined. Kare and Biely (1948) studied the effect of high NaCl in water, on day-old chicks. A NaCl level of 3000 ppm did not show a significant impact on chicks. Mortality occurred at 9000 ppm NaCl. Krista et al. (1961) reported that day-old chicks given 4000 ppm NaCl developed diarrhoea and low feed intake. At 7000 ppm, a higher mortality rate

was observed. These levels were higher than the level (2000 ppm) where laying hen eggshell quality was affected (Yoselewitz and Balnave 1989a).

The impact on broilers, of high Mg and Ca in drinking water was assessed by Atteh and Leeson in 1983(a). Production performance, combined with the mineral content in their bones and plasma, were evaluated in day-old broiler chicks, which were given one of 15 water treatments with different Mg and Ca concentrations for 3 weeks. Deionised distilled water was used as the control. There were no significant effects from high concentrations of Ca (up to 100 ppm in water) on feed and water consumption, weight gain, or mortality in broiler chickens, when compared to the control. However, high Mg up to 100 ppm decreased feed intake and increased number of bone deformations including tibia shortening and hocks swelling. The bone ash concentrations of Mg and P increased with water that was high in Mg. However, the concentrations of Ca and Mg used in that study were well below the guidelines described for poultry (Table 2.2). Although up to 100 ppm of Ca did not show harmful effects, natural drinking water given to poultry may contain higher levels and may have negative impacts.

2.2.6 Effect of high mineral content in water on other livestock

Numerous studies have been conducted on swine and cattle performance related to high mineral content in water. A study with young weanling pigs with high sulphate (2392 ppm) drinking water and with 6000 ppm TDS exhibited scouring during the first week of the experiment and increased water consumption (Anderson and Stothers 1978). However, no significant effects on feed consumption, weight gain or feed to gain ratio were observed, compared to the control group. Paterson et al. (1979) observed that water consumption and

excreta moisture content was higher in pigs supplied with water having 3000 ppm sulfate content, but no effect on reproduction or weight gain was found.

A study on cattle, treated with high SO_4 water, showed that a concentration of 3200 ppm reduced the feed, water intake and body weight (Weeth and Hunter 1971). Loneragan et al. (2001) reported that an SO_4 concentration of more than 583 ppm in water had negative effects on daily weight gain and gain: feed ratio of cattle.

The results of these studies suggested that high SO_4 in water could induce diarrhoea and cause poor performance in livestock animals, but these levels were higher than the maximum level of 250 ppm suggested for poultry by Carter and Sneed (1996).

2.2.7 Integration of literature on laying hens

Water minerals or other quality parameters need to be evaluated specifically for laying hens due to incompleteness of the available data. When considering the results of these studies on poultry species, studies on laying hens mainly focused on high NaCl in water. The information on Ca and Mg in water for laying hens dates back to the 1970's or before (Heller 1933; Adams et al. 1975) and may not be accurate if compared with modern high producing commercial laying hens which have higher metabolic demands. Effects on egg quality were not evaluated in hens that were supplied with high mineral water, other than NaCl. Mineral metabolism and bone quality have not been studied in hens supplied with high mineral content water. Different pH levels were not assessed for production performance, egg quality or mineral metabolism of the hens. Water quality guidelines for poultry were mainly found in popular press articles produced by agricultural extension services and not in peer reviewed literature. Therefore, evaluation of different levels of pH

and minerals, such as Ca, Mg and SO_4, in water for high producing commercial laying hens would provide valuable data, applicable to today's birds.

2.3 The avian egg

Nutritional changes can affect egg production and egg quality. Therefore, it is important to understand the egg formation process in laying hens. An avian egg is formed to produce a chick and contains all the essential nutrients for the growth of an embryo. A newly hatched female chick already has more than 3000 undeveloped ova in its ovary (Stadelman 1995).

2.3.1 Structure and formation of the egg

The reproductive cell (blastoderm) of the female chicken is surrounded by layers of yolk deposited in the ovary (North and Bell 1990). There are 3 stages in the yolk formation process; 1) during embryonic development, 2) slow growth from hatching to about ten days before ovulation then 3) accelerated growth about 10 days prior to ovulation (Stadelman 1995). It is assumed that about 10 days is required to mature the ovum and after a day or two, a second ovum starts to develop. Ovulation is the process of releasing a mature ovum or yolk into the oviduct, where the egg albumen and shell components are deposited. The second ovulation is stimulated by the laying of the previous egg and follows within 15 to 40 minutes of the event (Stadelman 1995).

The infundibulum, which is a funnel shaped structure in the oviduct closer to the ovary, catches the released mature ovum and passes it into the next section, the magnum. The magnum is the longest part of the oviduct and is responsible for the deposition of the elastic, semisolid egg white, taking about 3 hours (North and Bell 1990). The egg white or albumen is deposited in the oviduct and it comprises about 60% of the egg weight. The major

minerals in egg white are sodium (0.15%), potassium (0.14%), calcium (0.013%), magnesium (0.01%), sulphur (0.2%), and chloride (0.13%) of the total egg white. There are four layers in egg white: chalaziferous layer (inner thick white), inner thin white, outer thick white and outer thin layer (Stadelman 1995). The proportion of each layer depends on the strain and age of the hen and age of the egg (Stadelman 1995). Generally, the proportions of the four layers are 2.7%, 17.3%, 57%, 23%, respectively (North and Bell 1990).

Then egg white is surrounded by two membranes with 0.01-0.02 mm thickness: the inner and outer membranes (Hamilton 1982). Egg shell membranes are deposited in the isthmus, the next part of the oviduct, and it takes around 75 minutes to deposit the protein fibers (Robinson 1987). Eggshell membranes consist of about 1% ash and 95% protein, 1% carbohydrates and 3% fat on ash-free basis (Wedral et al. 1974). At the broader end of the egg, the separation of two eggshell membranes creates the "air cell" and the size is comparatively small in freshly laid eggs (Robinson 1987). Romanoff and Romanoff (1949) noted that during storage, the size of the air cell increased when the egg contents became dehydrated.

The eggshell is important as a protective barrier against physical damage to the egg. Parsons (1982) noted that the eggshell is a complex structure where all the components contribute to produce good quality shell. Over the membranes, eggshell components are deposited within the 'uterus" or shell gland of the oviduct (Nys et al. 2004). The eggshell consists of about 9 to 12% of the total egg weight and is mainly made of calcium carbonate (94%). Other than calcium carbonate, magnesium carbonate (1%), calcium phosphate (1%), and proteins (4%) are important compounds in the shell structure (Stadelman 1995).

There are three layers in a true eggshell: the mammillary knob layer, the spongy or palisade layer and the surface crystal layer (Solomon 1997). The palisade layer mainly contributes eggshell strength and thickness. During crystal growth, most of the cubic crystals are fused but some are not. This results in the formation of numerous pores (Solomon 1997). The cuticle, a waxy organic layer, is deposited over the true eggshell and contains mainly proteins and some carbohydrates and lipids (Parsons 1982). The cuticle seals the pore openings with thickened plugs which prevent microbial invasion. Once the eggshell is formed, the egg is passed to the vagina and after a few minutes, the egg is laid down (ovi position).

Ca for eggshell formation mainly comes from dietary calcium sources supplied through the blood to the shell gland lumen (Nys et al. 2004). The medullary bones act as readily available calcium reservoirs for eggshell calcification when the dietary calcium supply is limited, especially during the dark period (Farmer and Roland 1986). They observed that even if enough dietary calcium was supplied, at least 15% calcium in the shell originated from the bone reservoirs and the greater the requirement of bone calcium, the more shell defects were observed.

Carbonate ions for the formation of $CaCO_3$ are supplied through the blood and the shell gland itself secretes a portion (North and Bell 1990). Carbonic anhydrase (CA) enzyme activity in the shell gland has been related to eggshell quality, as discussed in the literature. Pearson et al. (1977) and Nys and de Laage (1984) found higher CA activity in laying hens than in non-laying hens and harder eggshell producing hens vs shell-less egg producing hens, respectively. CA was found to catalyze the first step of carbonate ion production from the carbonic acid in tissues (Hansson 1967).

Acid-base balance in plasma is important in eggshell quality (Keshavarz and Austic 1990). Balance between cations and anions in the diet is important for maintaining blood pH (Mongin 1981). High dietary cations (Na, K, Ca, Mg) can cause alkaline pH in blood while high anions (Cl, SO_4, hydrogen phosphate (HPO_4) can cause acidic pH (Mongin 1981). In normal conditions, blood pH is maintained around 7.4 (Mongin 1981). Keshavarz and Austic (1990) found that low blood pH (7.1) caused by high P and Cl in the diet reduced the eggshell quality measurements including eggshell thickness, eggshell weight, specific gravity and percent eggshell. Yoselewitz and Balnave (1989a) found that high NaCl (2000 ppm) in water reduced bicarbonate (HCO_3) ion concentration in shell gland fluid, which caused high percentage of damaged shell and lower breaking strength, eggshell weight and eggshell thickness. However, high Na and Cl ions in water did not change blood pH or blood HCO_3 ion concentration when compared to city water (control treatment). Literature on the effects of high Ca, Mg or sulphate ions in water on eggshell quality was not found.

2.3.2 Egg quality

Eggs are used as a human food worldwide since they comprise high quality protein (Stadelman 1995). The quality assessment considers internal and external measurements. Internal egg quality accounts for albumen quality and yolk quality, while external quality deals with the eggshell (Roberts 2004).

2.3.2.1 Eggshell quality

Eggshell quality is vital in terms of reducing egg breakage losses from farm to table, as well as for consumer acceptability. It was estimated that losses due to breakage was around 7 to 8% of total egg production (Hamilton 1982). Roland (1988) estimated egg losses due

to shell problems as, 12.9% of total eggs at the farm and the processing plant, including collectable and uncollectable egg losses. The egg size, specific gravity, eggshell weight, eggshell percentage, eggshell thickness and eggshell breaking strength are widely used eggshell quality measures (Roberts 2004).

There are many factors which affect eggshell quality. Genetics of the birds, age, production rate, nutrition, disease, and husbandry and environment factors such as temperature are the major factors that govern eggshell quality (Roberts 2004). An inadequate supply of calcium and carbonate ions to the shell gland of hens is the primary reason for poor eggshell quality (Balnave et al. 1992)

Minerals in drinking water can affect eggshell quality. There were a number of studies that found a negative effect of high NaCl in drinking water (Balnave and Yoselewitz 1987; Yoselewitz et al. 1988; Yoselewitz and Balnave 1989a, b; Pourreza et al. 1994) on shell quality. Although the effect of high NaCl in drinking water on eggshell quality has been well studied, the effects of other minerals in water has not.

2.3.2.2 Eggshell quality measurements

There are different methods for evaluating eggshell quality and they can be separated into two groups, as direct and indirect methods (Hamilton 1982). The eggshell quality simply means the eggshell strength, which can be defined as the ability to withstand external force related to breakage (Hamilton 1982). Quasi-static compression force, impact fracture force and puncture force are considered as direct measurements of eggshell quality or eggshell strength. Specific gravity, non-destructive deformation, eggshell weight, and eggshell thickness are considered as indirect measurements of the eggshell quality (Roberts 2004).

Specific gravity is the most commonly used method to evaluate eggshell quality because it is rapid, inexpensive and practical (Hamilton 1982). Specific gravity of the eggs is a measure of shell in relation to the egg size. Therefore, eggshell strength and thickness are expected to increase when specific gravity is high in an egg. The specific gravity of the whole egg can be measured by the immersion method or by using Archimedes' principle (Hamilton 1982). In the floatation method, the eggs are immersed in a series of salt solutions with known specific gravity values, in an increasing order and the solution in which the eggs first float is considered to be the specific gravity of the egg. Usually, salt solutions from 1.060 to 1.102 with increments of 0.004 have been used (Hamilton 1982). The specific gravity of the salt solutions was adjusted using a hydrometer. Since specific gravity of the solutions are temperature dependent, it is important to maintain eggs at the same temperature as the solutions to minimize error caused by the temperature effect (Hamilton 1982).

Eggshell strength is a function of material and structural strength of the eggshell (Hamilton 1982). Material strength is determined by the type and association of mineral and organic materials in the eggshell, while structural strength is dependent on egg size, shape, and distribution of eggshell and thickness of the eggshell. Eggshell breaking strength is mostly measured using quasi-static compression fracture force, where the egg is compressed between flat parallel surfaces until the shell cracks or breaks (Hunton 1987). The minimum force required to break the eggshell is recorded as eggshell breaking strength.

2.4 Bone structure of laying hens

Laying hen mineral nutrition is highly associated with the bone metabolism (Whitehead 2004). For the modern high producing laying hens, bone calcium reserves are a very

important source of calcium during eggshell formation. When blood calcium was limited for eggshell formation, bone calcium was mobilized resulting in blood calcium levels being maintained in appropriate levels (Whitehead 2004). Once the calcium intake was sufficient, the bone calcium were replenished. Therefore, high mineral intake through drinking water might have an effect on bone mineral metabolism of these hens.

Bones are structures of calcium phosphate crystals over a matrix of collagen fibers (Whitehead and Fleming 2000). There are three types of bones in laying hens. The cortical and trabecular (cancellous) bones are primarily important for structural support. Those types of bones are developed during initial growth. The third is the medullary bone, a non-structural bone which is formed at sexual maturity of the hen by the influence of hormones. Osteoclasts and osteoblasts are two types of cells in the bone tissues are important for bone reabsorption and deposition of new bones, respectively (Whitehead and Fleming 2000). This process is called remodelling and it constantly occurs in the bone tissues.

The importance of the medullary bone in layers has been widely discussed (Whitehead 2004). It acts as a readily available source of calcium for the eggshell calcification process (Whitehead 2004) which is found within the bone marrow cavities (Whitehead and Fleming 2000). The leg bones have higher content of medullary bone. However, in some birds, medullary bones are also present in the humerus, which is a pneumatic bone, not containing marrow (Whitehead 2004). The rate of medullary bone formation is higher in the early laying stage, decreasing over time.

The amount of cortical bone decreases while medullary bone grows during the laying period (Whitehead 2004). Therefore, it has been suggested that the total bone content does not change during the laying period. A medullary bone is weaker than a structural bone

due to its uneven arrangement of collagen fibrils in the bone matrix and its isolated nature in the marrow cavity (Whitehead 2004). Therefore, laying hens are more susceptible to bone breakages. However, once they stop laying eggs, structural bones again start to grow.

Naturally, a normal hen recovers structural bone losses during the incubation period after the production of egg clutches. However, in modern high producing laying hens, bone quality problems, such as osteoporosis, are prominent because of prolonged egg production without sufficient break to recover its bone losses. Based on survey results, Fleming (2008) reported that 30% of layers in cages have at least one lifetime bone fracture.

There was limited information found in the literature on the impact of high mineral content in drinking water on bone quality measurements of laying hens. Merkley (1981) reported that the breaking strength and percent ash were higher in hens supplied fluoride rich water (100 ppm) when compared to well water received hens at 45 weeks of age. The effects of other mineral ions including Ca, Mg or SO_4 which can be found in higher levels in water are not well known.

2.5 Mineral balance and retention in laying hens

The mineral balance in livestock species is quite important and imbalances may cause clinical disorders (Suttle 2010). The balance between major anions and cations is vital in regulating acid base balance in an animal. Na, K, Ca and Mg are known to be alkali producing cations and Cl and SO_4 are known as acid producing anions (Mongin 1981). Excess mineral intake may be harmful to animals but available information on mineral toxicity or tolerance remains limited (NRC 1974). The maximum tolerable limits of

minerals in drinking water is not well documented and must be considered as a source of total dietary intake.

Minerals are ingested into the digestive tract through the animals feed, water and endogenous secretions (Annenkov 1981). Feed minerals are derived from animal and plant feed ingredients as well as from mineral supplements in organic or inorganic forms. A large amount of minerals are excreted through the body secretions into the digestive tract of animals (Annenkov 1981). The minerals in the diet are solubilized in the proventriculus and gizzard by acid secretions (Guenter and Sell 1973) and then absorbed in regulated pathways in the intestine to the blood and through the blood into organs or other tissues. Sufficient amounts of minerals must be supplied to animals to fulfill their requirements for growth, reproduction and losses through the production of eggs and other body functions (Suttle 2010). Although mineral distribution of body tissues is not uniform, approximately 46% Ca, 29% P, and 25% S, Na, Cl and Mg and essential trace metals, are present in the body (McDowell 1992). Further, bones act as major storage sites of minerals (Whitehead 2004). Approximately 99% of total body Ca is associated in the skeleton as hydroxyapatite crystals. The bones primarily consist of Ca, P and Mg salts. Around 60-70% of body Mg is in the skeletal system (Sell and Fontenot 1980).

Mineral retention can be estimated using a balance study technique (Georgievskiĭ et al. 1981). The fecal mineral content includes not only the unused dietary minerals but also endogenous minerals which are excreted into the gut (Brink et al. 1992). The retention of minerals in the body can be calculated by subtracting minerals in excreta (fecal and urinary) and eggs from the intake of minerals by chickens (Clunies et al. 1992). Chickens have a cloaca, which is a common opening for the both urinary and digestive tracts, thus

colostomization is needed to separate urine from the feces in chickens if needed (Georgievskiĭ et al. 1981).

High minerals in water may affect mineral absorption in the digestive tract of hens. Higher levels of Ca in the diet can affect absorption of other minerals, such as Fe, I, Mg, Mn, P and Zn (McDowell 1992). High Mg and SO_4 are known to cause diarrhoea in birds, which can lead to poor nutrient absorption (Carter and Sneed 1996). Therefore, evaluation of mineral balance in hens supplied with water high in mineral content may be useful to the egg industry.

2.6 Focus of literature

Water plays an important role in poultry performance. Water quality measurements could vary from source to source depending on natural and man-made factors. Limited information is available on the quality characteristics of the water provided to laying hens in Canada. Knowledge of water composition for layers would be a useful starting point for a study on water quality.

The effects of different water pH levels have not been researched for laying hens. The suggested pH range from 6.5 to 8.5 for poultry was not evaluated in experiments evaluating the performance of layers or on the mineral retention and balance when different pH waters are provided. The available information on water pH effect on chickens mainly discussed the antimicrobial effect at low pH levels when organic acids are used to adjust the pH. Therefore, it is important to evaluate water pH levels on laying hen performance, egg quality and mineral metabolism.

High contents of Ca, Mg and SO_4 in water are known to reduce livestock performance. The tolerance limits of minerals may be different for different species and for different ages. The available water quality guidelines for poultry were not specific to laying hens but for all species in all ages. The high level of productivity and high metabolic demands during the laying phase may cause unique impacts of high TDS water.

The effects of minerals other than NaCl in water in laying hens are not well known. The available information on Ca, Mg and SO_4 minerals were mostly based on the popular press articles published from the different agricultural extension services. The information found in the articles were dated back to 1970s or earlier. The hens from that era were different metabolically than today's hen producing significantly more eggs. Therefore, this data may not be applicable to high producing laying hens today.

The present study will evaluate the water quality profile being used for the commercial egg production in Canada to establish a bench mark. Based on the survey results from water samples collected across Canada, the effects of different pH and high levels of Ca, Mg and SO_4 in drinking water on laying hen performance will be investigated. Snapshot evaluation through mineral balance studies at different production stages will be conducted to identify changes due to hen day production level and metabolic state. Effects will be assessed at different ages of hens in order to elucidate any age effect in mineral retention.

CHAPTER 3 EVALUATION OF DRINKING WATER QUALITY PROVIDED TO LAYING HENS ACROSS CANADA

3.1 Abstract

Drinking water quality profile of commercial laying hens can be highly variable from farm to farm. To evaluate the nature of waters being provided to laying hen in Canada, water samples were collected from volunteer egg farmers across Canada. Eighty seven samples were received from farmers representing all provinces except Ontario and British Colombia. Fifty percent of total expected samples were received. Physico-chemical parameters of water including, conductivity, pH, total hardness, total alkalinity, and individual mineral ion concentrations of Ca, Mg, Cl, K, P, Na, SO_4, Cu, Fe, Mn, Zn and NO_3/NO_2-N. Data were analyzed using Minitab 17 software. Data were summarized for each province and for Canada as a whole. Ground water was the major source (73%) of water currently being used by Canadian egg producers. Surface (18%) and municipal water (9%) were the other major water sources. The pH varied from 5.97 to 9.20 in water. The pH of 6% of the total farms were not in the pH range of 6.5 to 8.5, considered acceptable for poultry. The conductivity ranged from 58 to 5090 µmhos/cm. The highest conductivity of 5090 µmhos/cm was reported from Saskatchewan. The highest concentrations of SO_4 and NO_3/NO_2-N resulted in samples from Prince Edward Island. The maximum content of Ca, Mg, Na, Cu, Fe, Mn, Zn were 231, 103, 1151, 3, 2, 2, 0.1 ppm, respectively among all waters. Ca or Mg did not exceed the maximum acceptable limits of 600 ppm and 125 ppm respectively in any water sample. Fifty percent of samples received from Saskatchewan contained more than 300 ppm Na, which was considered the maximum acceptable limit for poultry. For the anions of Cl, SO_4, NO_3/NO_2-N, the maximum values obtained were 519 ppm, 2703 ppm and 22 ppm respectively among all waters. SO_4 concentration of 13% of farms was more than 250 ppm, which was accepted as maximum desirable limit for poultry. Alkalinity of the waters ranged from 6 to 956 ppm. The hardness ranged from 3 to 786 ppm in the waters. Both Ca and Mg ions were high in water with higher hardness. The water quality was highly variable from source to source.

Key words: Canada, drinking water, laying hens, minerals, pH

3.2 Introduction

A supply of adequate amount of good quality water is a critical factor for the productivity and well-being of any livestock species. Without a supply of good quality water, performance of laying hens may be affected even though birds were given diets with adequate nutrients (Leeson and Summers 2008). Quality of water can be changed by the dissolved substances in it. Chemical substances such as mineral ions and biological substances such as bacteria affect water quality. When these substances are present in high concentrations in poultry drinking water, bird performance could be negatively affected (Youssef et al. 2009). Water quality can be a major factor that can affect performance of poultry in different areas (Youssef et al. 2009).

Canada has ample good quality fresh water sources. According to CCME (1987), 7.6 % of Canada land mass is covered by fresh water which is the major source of water for agriculture including animal husbandry.

Since water quality profile can be vary with geographical region (Zimmermann et al. 1993), it is important to evaluate water quality profiles in different areas in order to establish the scope of quality of waters provided to laying hens in different areas. There were 1,016 registered egg farms in Canada and the average flock size was 20,241 hens in 2012 (Agriculture and Agri-Food Canada 2013). However, there was limited information on the water quality, which is being used by the egg producers in Canada. A database on the drinking water quality profile of commercial egg laying hens across Canada would be beneficial. An objective of the present study was to evaluate the water quality, currently being used for egg production across Canada. From results of the survey, further studies could be designed to evaluate the water quality hens were receiving.

3.3 Objectives

The objective of this survey was to determine the water quality profile that is currently being provided to laying hens by the egg producers across Canada.

3.4 Hypothesis

It is hypothesized that the quality of drinking water supplied to laying hen vary across Canada.

3.5 Materials and Methods

3.5.1 Water sample collection

Water samples (200 mL) were requested from volunteer egg producers across Canada including the provinces of Alberta (AB), British Colombia (BC), Manitoba (MB), New Brunswick (NB), Newfoundland and Labrador (NL), Nova Scotia (NS), Ontario (ON), Prince Edward Island (PE), Quebec (QC) and Saskatchewan (SK) with the help of provincial egg boards and the Egg Farmers of Canada (EFC). Water samples from Atlantic Poultry Research Centre (APRC), Truro, NS, where the experimental trials were conducted and Truro municipal water (TMW) were also collected. Each producer was supplied with a 200 mL water bottle and a questionnaire form (Appendix 1). Information of water source and water testing history was collected through the questionnaire. Water samples were received via mail and refrigerated at 4℃ until analysis. The number of samples requested and received from each province and calculation of % participation by province were reported (Table 3.1). Twenty samples from each province except Atlantic Canada provinces were requested. For the Atlantic Canada provinces (NB, NL, NS and PE), one

water sample from each farm was requested. Total of 87 water samples (50% of requested samples) were received all across Canada.

Table 3.1. The number of water samples requested and received for the water quality survey from each province from January 2013 to January 2014

Province	Number of samples Received	Number of samples Requested	% of participation
AB	3	20	15
BC	None	20	0
MB	20	20	100
NB	12	17	71
NL	6	7	86
NS	9	23	39
ON	None	20	0
PE	4	8	50
QC	20	20	100
SK	13	20	65
Total	87	175	50

3.5.2 Analysis of water quality measures

Water samples were analysed at Nova Scotia Department of Agriculture feed testing laboratory, Harlow Institute, Truro, NS for the water quality. Parameters measured included minerals and other physico-chemical measurements (Table 3.2).

Table 3.2. Physicochemical parameters evaluated in the water samples collected from egg farmers across Canada

Macro minerals	Micro minerals	Other parameters
Calcium (Ca)	Copper (Cu)	pH
Chloride (Cl)	Iron (Fe)	Conductivity
Magnesium (Mg)	Manganese (Mn)	Hardness
Potassium (K)	Zinc (Zn)	Alkalinity
Phosphorus (P)		
Sodium (Na)		
Sulphate (SO_4)		
Nitrates/Nitrite-N (NO_3/NO_2 -N)		

Conductivity of water was measured with a conductance meter using Standard Methods of the Examination of Water and Wastewater (SMEWW) method 2510B (American Public Health Association (APHA) 1995). Conductivity is an indirect measurement of total dissolved solids in water. Conductance of water was measured using inert electrodes and conductivity was calculated.

Alkalinity was determined by flow injection colorimetry using the modified method USEPA method 310.2 (USEPA 1986). Methyl orange, which was the indicator, and pH 3.1 buffer solution was used to determine alkalinity. Colour change of the indicator was directly proportionate to the alkalinity of the water. pH was measured using pH meter (SMEWW 4500-H+B) (APHA 1995).

Major mineral ions and trace minerals were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) using modified SMEWW methods 3120 B (APHA 1995). In this technique, atomic emissions of elements are measured by an optical spectrometry. Samples were digested with nitric-hydrochloric acid mixture and then filtered. Sample aerosol was produced by a nebulizer and spray chamber in the Inductively Coupled Plasma (ICP) source. The high temperature (6000 to 8000 K) in ICP produce excited ions by dissociating molecules. Ionization of atoms produce ionic emission spectra, which then direct to a polychromater. Dispersed spectra using the polychromater, is then directed to photomultiplier tubes, in which the intensity of specific spectrum was measured simultaneously. The concentrations were determined for each mineral ions present in water samples. Concentrations of Ca, Mg, SO_4, K, Na, Cu, Mn, Zn and Fe were determined using this method. Based on the concentration of Ca and Mg from ICP-OES, hardness of water was calculated as $CaCO_3$ ppm. (SMEWW method-2340B) (APHA 1995).

NO_3/NO_2-N content in water was analysed by flow injection colorimetry (USEPA method 353.4) (USEPA 1986). NO_3 was reduced to nitrite and then a color azo dye was produced. By measuring the absorbance, NO_3/NO_2-N concentration was determined.

Cl ion concentration was determined by flow injection colorimetry (USEPA method 325.2) (USEPA 1986). Cl ions was reacted with mercuric thiocyanate to liberate thiocyanate ions. The thiocyanate ions was reacted with ferric ions. The concentration of ferric thiocyanate was directly proportional to the chloride ions. Fluoride in water was determined by flow injection analysis-ion selective electrode method (USEPA method 340.2) (USEPA 1986).

3.5.3 Data analysis

The analysis reports of water samples were summarised according to the province of origin. Data were analysed using MINITAB versions 17 software (Minitab 17 Statistical Software. 2010. State College, PA: Minitab, Inc.). Mean, standard deviation, maximum and minimum values of each water parameter were determined for each province of Canada and summarised for the country as a whole.

3.6 Results and Discussion

The information gathered through questionnaire was summarised for each province (Table 3.3). The number of samples received, source of water, water testing history, and any other comments regarding water supply were included for each province contributing.

The total number of water samples received was 88 from the provinces. Except the Truro municipal water sample, 87 of samples were received from commercial egg producers including APRC. That represented 50% of the total requested samples.

Table 3.3. Survey information gathered from egg producers from each province of Canada

Province	Sample number	Water source	Water analysis history	Comments
Alberta	1	Surface	Yes	River water
	2	Deep well	Yes	
	3	Deep well	Yes	
Manitoba	1	Deep well	Not mentioned	Untreated
	2	Deep well	yes	Untreated
	3	Surface	yes	river water
	4	Shallow well	Not mentioned	Treated
	5	Surface	yes	river water
	6	Deep well	Not mentioned	
	7	Deep well	Not mentioned	
	8	Deep well	yes	
	9	Deep well	Not mentioned	
	10	Surface	Not mentioned	
	11	Deep well	yes	not treated
	12	Deep well	yes	
	13	Municipal	Not mentioned	
	14	Deep well	Not mentioned	
	15	Deep well	Not mentioned	
	16	Municipal	Not mentioned	
	17	Deep well	Not mentioned	
	18	Shallow well	yes	
	19	Deep well	yes	
	20	Deep well	yes	Reverse osmosis
Newfoundland and Labrador	1	Shallow well	No	
	2	Shallow well	No	
	3	Shallow well	No	
	4	Municipal	No	
	5	Deep well	No	
	6	Surface	No	
Nova Scotia	1	Deep well	yes	
	2	Deep well	Not mentioned	
	3	Deep well	No	
	4	municipal	yes	
	5	Deep well-APRC	yes	
	6	Municipal-Truro	Surface water	
	7	Deep well	yes	
	8	Deep well	yes	
	9	Deep well	yes	
	10	Deep well	yes	
Prince Edward Island	1	Municipal	yes	
	2	Deep well	No	
	3	Deep well	No	
	4	Deep well	Not mentioned	

Table 3.3. con't….

Province	Sample number	Water source	Water analysis history	Comments
New	1	Shallow well	yes	
Brunswick	2	Deep well	yes	
	3	Deep well	yes	
	4	Deep well	No	
	5	Deep well	yes	
	6	Deep well	yes	
	7	Deep well	yes	
	8	Deep well	yes	49 m deep
	9	Deep well	Not mentioned	
	10	Deep well	yes	
	11	Deep well	No	
	12	Deep well	yes	
Quebec	1	Deep well	yes	
	2	Deep well	yes	
	3	Deep well	yes	
	4	Deep well	yes	
	5	Deep well	yes	
	6	Municipal	yes	
	7	Deep well	yes	
	8	Deep well	yes	
	9	Deep well	yes	
	10	Municipal	yes	
	11	Deep well	yes	
	12	Surface	yes	
	13	Deep well	yes	
	14	Deep well	yes	
	15	Deep well	yes	
	16	Surface	yes	
	17	Deep well	yes	
	18	Deep well	Not mentioned	
	19	Municipal	yes	
	20	Deep well	yes	

Table 3.3. con't….

Province	Sample Number	Water source	Water analysis history	Comments
Saskatchewan	1	Deep well	yes	
	2	Deep well	yes	
	3	Deep well	yes	52 m deep
	4	Deep well	yes	
	5	Surface	yes	
	6	Deep well	yes	
	7	Deep well	yes	21 m deep
	8	Deep well	yes	
	9	Deep well	yes	
	10	Deep well	yes	
	11	Surface	No	
	12	Deep well	yes	
	13	Deep well	Not mentioned	

No samples were received from BC and ON. The highest farmer participation (100%) was observed from MB and QC, where all 20 samples requested were received. From SK and NB, 13 and 12 samples were received, respectively. Eight samples were received from NS egg farmers while 2 other samples were collected from the APRC deep well and Truro municipal water source (surface water). NL and PE supplied 6 and 4 samples, respectively. Only 3 samples were received from AB.

Summary of the data collected indicated the majority of egg farms (73%) used ground well water sources for the laying hens. All of the egg production units from NB being supplied well water for their birds. Deep wells were more commonly used than shallow wells. Half of the NL farms who participated for the survey being used shallow wells as the water source. The depth of the wells were not reported in all samples received. Other water sources (27%) included surface water (18%) and municipal water (9%).

Sixty seven percent of farms surveyed had a water analysis history for their waters while 14% did not analyze the water samples. Nineteen percent of farmers did not mentioned a

history of water analysis. There were 2 farms in MB that treated their water before supplying it to the hens. One farm used reverse osmosis to treat water and other farm did not specially mentioned the method of treatment. Reverse osmosis is a process that used for desalination of water. Since the water from that farm contained high NaCl concentration, reverse osmosis might be used to reduce salinity of the water. None of the other farms in any province mentioned water treatments.

The water analysis results were summarised by province (Table 3.4). The mean, maximum and minimum values of each parameter were identified for the samples received per province and across Canada. The mineral concentration was reported as ND-not detected when the concentrations of the given mineral was below the detection limit of analysis or equipment used.

The composition of drinking water of hens was quite variable. The mineral ion composition highly variable from farm to farm in a province and province to province across Canada. Moreover, other parameters including pH, alkalinity, hardness, and conductivity of waters were also quite variable. Natural water chemical composition is mainly affected by dissolving soil and rock minerals and synthesis and degradation of the organic matter such as proteins, fat and carbohydrates (Sullivan et al. 2005). Mineral richness and variations in soil in different geological regions, cause water composition to be quite variable (Sullivan et al. 2005). Water from rain either can accumulate in the surface water bodies such as lakes, rivers, ponds or can percolate through the soil particles or accumulated as ground water sources. The ground water sources are known as ‘aquifers’.

Table 3.4. The mean, maximum and minimum values of water quality measures of drinking water for laying hens on a province basis and whole data base summary

	Alberta				Manitoba				New Brunswick			
Parameter[1]	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
No.of samples[2](n)	3				20				12			
pH	7.69	0.47	7.3	7.6	7.87	0.29	6.90	8.22	7.93	0.76	6.70	9.20
Conductivity	1238	575	595	1414	898	338	541	1743	452	160	109	668
Chloride	11	1	11	11	68	114	3	424	34	25	5	93
Sulphate	211	229	20	148	84	87	0.3	290	21	16	7	52
Magnesium	22	12	11	36	32	20	2	91	6	5	1	18
Calcium	38	9	31	48	60	34	2	154	42	31	4	98
Sodium	219	169	26	294	89	91	14	283	47	38	6	111
Copper	0.01	-	ND	0.01	0.2	0.3	ND	1	0.1	0.2	ND	0.5
Iron	0.1	-	ND	0.1	0.3	0.6	ND	2	0.2	0.3	ND	0.7
Manganese	0.2	0.3	ND	0.4	0.1	0.1	ND	0.3	0.2	0.2	ND	0.3
Potassium	ND	ND	ND	ND	-	-	ND	ND	-	-	ND	ND
Zinc	0.01	-	ND	0.01	0.04	0.03	ND	0.1	0.03	0.01	ND	0.04
Nitrate/Nitrite-N	-	-	ND	ND	9	11	ND	17	3	2	ND	6
Hardness	186	73	122	266	297	140	56	575	129	88	11	270
Alkalinity	456	336	146	814	279	127	8	463	156	63	23	246

[1]Units: pH-pH units; conductivity- µmhos/cm; Cl, SO_4, Mg, Ca, Na, Cu, Fe, Mn, K, Zn, nitrate/nitrite-N, hardness, alkalinity measured in ppm.
[2]Total for province.
ND indicates the concentration below detectable limit of the analysis method.
SD=Standard Deviation; Min. = Minimum; Max. = Maximum.

Table 3.4.con't…

Parameter[1]	Newfoundland and Labrador				Nova Scotia				Prince Edward Island			
	Mean	SD	Min.	Max.	Mean	SD	Min	Max.	Mean	SD	Min.	Max.
No. of samples[2]	6				10				4			
pH	6.97	0.20	6.76	7.20	7.65	0.49	6.45	8.10	7.95	0.09	7.82	8.03
Conductivity	103	83	66	271	353	134	170	574	701	422	306	1074
Chloride	11	5	4	15	23	17	5	65	171	178	15	333
Sulphate	5	4	1	12	21	13	6	42	1346	1545	5	2703
Magnesium	1	1	1	4	5	3	ND	10	4	2	2	6
Calcium	9	15	2	39	45	19	ND	76	54	8	49	65
Sodium	8	3	2	11	23	19	5	64	82	86	8	157
Copper	0.01	-	ND	0.01	0.04	0.05	ND	0.03	0.03	0.01	ND	0.04
Iron	0.02	0.02	ND	0.06	0.02	-	ND	ND	-	-	ND	ND
Manganese	-	-	ND	ND	-	-	ND	ND	-	-	ND	ND
Potassium	-	-	ND	ND	-	-	ND	ND	-	-	ND	ND
Zinc	-	-	ND	ND	0.03	0.04	ND	0.09	0.01	-	ND	0.01
Nitrate/Nitrite	6	-	ND	6	11	7	ND	22	7	1	6	9
Hardness	28	43	7	115	131	55	ND	225	150	17	122	169
Alkalinity	32	48	ND	103	106	42	25	154	118	11	102	126

[1]Units: pH-pH units; conductivity- µmhos; Cl, SO_4, Mg, Ca, Na, Cu, Fe, Mn, K, Zn, nitrate/nitrite-N, hardness, alkalinity measured in ppm.
[2]Total for province.
ND indicates the concentration below detectable limit of the analysis method.
SD=Standard Deviation; Min. = Minimum; Max. = Maximum.

Table 3.4.con't…

	Quebec				Saskatchewan			
Parameter[1]	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
No.of samples[2]	20				13			
pH	7.49	0.80	5.97	9.19	7.82	0.38	6.93	8.34
Conductivity	415	284	58	1295	1767	1360	465	5090
Chloride	20	17	4	68	130	174	5	519
Sulphate	47	124	0.4	569	318	476	0.4	1652
Magnesium	6	5	1	24	23	30	0.3	103
Calcium	41	50	2	231	47	50	1	145
Sodium	40	58	1	227	319	361	4	1151
Copper	0.1	0.1	ND	0.2	0.2	-	ND	2.6
Iron	0.2	0.2	ND	0.7	0.3	0.5	ND	1.5
Manganese	0.3	0.8	ND	2.2	0.1	0.1	ND	0.3
Potassium	-	-	ND	ND	-	-	ND	ND
Zinc	0.02	0.01	ND	0.04	0.03	0.03	ND	0.1
Nitrate/Nitrite-N	5	5	ND	12	3	-	ND	3
Hardness	128	144	16	677	211	246	3	786
Alkalinity	154	112	18	438	422	260	95	956

[1]Units: pH-pH units; conductivity- µmhos/cm; Cl, SO_4, Mg, Ca, Na, Cu, Fe, Mn, K, Zn, nitrate/nitrite-N, hardness, alkalinity measured in ppm.
[2]Total for province.
SD=Standard Deviation; Min. = Minimum; Max. = Maximum.
ND indicates the concentration below detectable limit of the analysis method.

Table 3.4 con't….

Parameter[1]		Canada wide			
		Mean	SD	Min.	Max.
No. of samples[2]	88				
pH		7.70	0.60	5.97	9.20
Conductivity		746	762	58	5090
Chloride		57	104	3	519
Sulphate		153	451	0.3	2703
Magnesium		15	20	0.3	103
Calcium		45	39	1	231
Sodium		99	180	1	1151
Copper		0.2	0.5	ND	3
Zinc		-	-	ND	0.1
Nitrate/Nitrite-N		7	5	ND	22
Hardness		175	160	3.0	786
Alkalinity		222	185	6.0	956

[1]Units: pH-pH units; conductivity- µmhos/cm; Cl, SO_4, Mg, Ca, Na, Cu, Fe, Mn, K, Zn, nitrate/nitrite-N, hardness, alkalinity measured in ppm.
[2]Total for province.
SD=Standard Deviation; Min. = Minimum; Max. = Maximum.
ND indicates the concentration below detectable limit of the analysis method.

The minerals in soil and rock can dissolve in water when it passes through them. There are many minerals found in soil and rock such as silica (Si), aluminum (Al), iron (Fe), calcium (Ca), sodium (Na), magnesium (Mg), and potassium (K), titanium (Ti), hydrogen (H), phosphors (P), manganese (Mn), fluorine (F), barium (Ba),strontium (Sr), sulfur (S), carbon (C), zirconium (Zr), vanadium (V), and chlorine (Cl) (Sullivan et al. 2005) which can be dissolved in the water. These minerals can be dissolved in different concentrations in water. Minerals present are either anions or cations. Agricultural activities such as crop and livestock production can add chemical substances such as nitrates into water sources (Sullivan et al. 2005). Wastes from house hold and industries can accumulate heavy metals in water (USEPA 2012).

The average provincial pH of the water samples ranged from 6.97 to 7.95 (Table 3.4). The minimum reported pH in a water sample was 5.97 from a QC sample. The maximum pH was 9.20 in a sample from NB. There were no recommended pH range for the livestock drinking water in the Canadian water quality guidelines for the agriculture use (CCME 1987). Weltzien (2002) suggested that pH 6.5 to 8.5 is preferred for poultry and this range was similar to the human recommendation. The pH of 6% or 5 samples of the total samples were outside of this range. Carter and Sneed (1996) recommended pH 6.8 to 7.5 as ideal range for poultry. Fourteen samples or 16% of samples were within this range while 84% of the samples were not. Fairchild and Ritz (2012) suggested pH from 5 to 6.8 as the preferred range. Only 8 samples or 9% was within this range while 91% were not. Therefore, a considerable number of waters were not within the recommendations. The effects of different pH levels in water on laying hens are not well known. The studies conducted with broilers showed that no effects on gut pH of broilers when supplied water

with pH from 3 to 8 (Watkins et al. 2004; Açıkgöz et al. (2011). The information on effects of different water pH on broiler performance were variable from study to study (Chaveerach et al. 2004; Pesti et al. 2004; Watkins et al. 2004; Açıkgöz et al. 2011). In some of the studies it has been found that body weight of the broilers improved when supplied water with low pH, while in the other studies did not. No information available to discuss water pH effects on laying hen performance or egg quality. Therefore, it is important to determine effects on laying hens supplied with different pH in water. According to the survey data, the laying hen drinking water pH ranged from 5.97 to 9.20, so it would be useful to test different pH in this range on laying hen performance and egg quality.

The capacity of water to neutralize the acidity is known as alkalinity (USEPA 2012). It was a measure of CO_3, HCO_3 and OH ions in water (USEPA 2012). Average alkalinity of the waters ranged from 32 to 457 ppm (Table 3.4). The sample with the highest alkalinity was from AB (956 ppm), while lowest was from NL. Watkins (2008) suggested 300 ppm as the maximum acceptable limit of alkalinity for poultry drinking water since above 300 ppm water can be bitter in taste and may reduce the water intake of birds. Sixty six percent of SK farms had more than 300 ppm alkalinity. 45% of samples from MB, 10% samples from QC and 66% of samples from AB exceeded this alkalinity limit. High alkalinity make it difficult to reduce water pH (Watkins 2008). The information on the effect of alkalinity on laying hens may be important in assessing water quality. Elevated levels of alkalinity due to high concentrations of CO_3, HCO_3 and OH ions affect bird performance (USEPA 2012). It would be beneficial to determine the impacts of these ions as a measure of alkalinity on laying hen performance when present in excess amounts in drinking water.

Total dissolved solids (TDS) contains all inorganic minerals dissolved in water. The TDS usually consists of Ca, Mg, Na, K, CO_3, HCO_3, Cl, SO_4 and NO_3. The CCME (1987), recommended 3000 ppm as the maximum safe limit for the TDS in livestock water. NRC (1974) and Weltzien (2002) reported the desirable range of TDS as 1000 to 3000 ppm to avoid negative effects on performance or health of poultry, although watery droppings can be expected at higher levels within this range.

Conductivity is a measures of total dissociated ions present in water (Singh and Kalra, 1975). This includes the electrically charged inorganic anions and cations, which contribute to total dissolved solids. Therefore, conductivity is an indicator of total dissolved solids content in water (Singh and Kalra 1975). The difference between TDS and conductivity is the material without an electric charge. Geology of the area is a major factor that affect conductivity (USEPA 2012). Water which flows through the areas with granite will have low conductivity because granite has more inert materials. Conductivity is high when water flows through clay soils, because it has more materials that can be ionised (USEPA 2012).

The conductivity of the water was below 3000 µmhos/cm in all farms except one from SK (5090 µmhos/cm) (Table 3.4). In that sample SO_4 (1652 ppm) and Na (1151 ppm) contents were high. The average conductivity ranged from 103 to 1843 µmhos/cm. The conversion factor for the TDS calculation from conductivity of natural waters range from 0.5 to 0.9 (Singh and Kalra 1975). The factor can be varied by the type and concentration of ions present in water. Therefore, the TDS of that sample could range from 2545 to 4580 ppm. If these conversion factors used for conversion of conductivity to TDS, only one sample had a TDS more than 3000 ppm, which is from SK. The percent of samples which had more than 1000 ppm TDS from AB, MB, NB, NL, NS, PE, QC and SK were 60%, 20%,

0%, 0%, 0%, 0%, 10% and 50%, respectively. All waters from Atlantic Canada egg producers were below 1000 ppm TDS. AB and SK waters consisted higher TDS contents.

TDS in the 3000 to 5000 ppm range is unsuitable for poultry drinking since it may cause watery droppings, reduce growth and increase mortality (NRC 1974). Higher conductivity (more than 1000 ppm) in the water samples in any province were mainly because of high Na ions with either SO_4 or Cl anions according to the water analysis results. In some cases high Ca was also present in the water. The conductivity and anion and cation concentrations were lower in all samples received from NL. The lower conductivity of those samples could be due to the bedrock type that associate with the ground sources. About 55% population in NL rely on ground water sources, either shallow or deep wells. There are several bedrock types associated with well water in NL including granite, gneiss, sandstone, shale, quartzite, ironstone and limestone (Newfounland- Ministry of Environment, Labor and Justice (NMELJ) 2013), but most wells are associated with granite. The general chemistry of well water when granite predominates is slightly acidic and of low mineral content. Therefore, the low conductivity, low mineral content, low pH, low hardness and alkalinity in the water samples were expected (NMELJ 2013).

The nitrate/nitrite-N (NO_3/NO_2-N) content in waters ranged from 0 to 22 ppm. The average concentrations of NO_3/NO_2-N in each province of Canada ranged from 0 to 11 ppm (Table 3.4). High NO_3 content of water can originate from fertilizers and animal waste and sewage systems (WHO 2011a). The higher values obtained for the three samples (21.98, 12.34 and 10.5 ppm) in Nova Scotia farms were not known. The NO_3/NO_2-N content of all PE water samples ranged from 5.52 to 8.59. All samples had more than 5 ppm NO_3/NO_2-N. High level of nitrates in wells in PE could be due to the substantial use of nitrogen fertilizers for

the intensive potato cultivation (Jiang et al. 2011). The population of PE mainly depend on ground water sources for drinking purpose and for the majority of industrial purposes (NMELJ 2013). Fertilizers such as ammonium sulphate, magnesium sulphate, and ammonium orthosulphate contributes higher amount of sulphate into water (Health Canada 2012). NO_3 are highly soluble and can easily penetrate through soil into the ground water sources. The Canadian water quality guideline for livestock reported the maximum acceptable limit for NO_3/NO_2-N as 100 ppm. Nova Scotia Department of Environment (2012) reported that 23% of wells monitored were above the 10 ppm NO_3-N, which is the recommended level for human drinking water by Canadian water quality guidelines. The maximum concentration reported was 39 ppm for across Canada. According to the report, high use of agriculture fertilizers and manure can cause elevated levels of NO_3-N in water. However, Adams et al. (1966) reported that laying hens can tolerate 300 ppm NO_3-N or 200 ppm NO_2-N without affecting performance if the diet contains adequate vitamin A. Therefore, the levels in these waters may not have negative impact on laying hens, but different concentrations of NO_3 or NO_2 ions in water have not been studied for modern high producing commercial laying hens.

There are no recommended limits for Na, Mg, K, Fe, Mn and Cl in the Canadian water quality guidelines for poultry (CCME 1987). The highest Na content was 1151 ppm in a SK sample which exceed the recommendation proposed by Weltzein (2002) of 300 ppm. Sixty six percent of samples received from SK exceeded this limit (Table 3.4). In SK, saline soil is abundant which contains high concentrations of sodium sulphate (Glauber's salts), magnesium sulphate (Epsom salts) and calcium sulphate (Gypsum) (Saskatchewan Agriculture 2008). For AB, MB, NB, NL, NS, PE and QC, the highest Na concentrations

were 294, 283, 111, 11, 64, 157 and 227 ppm respectively. Koelkebeck et al. (1999) found low egg production in laying hens that received well water with high Na (190 ppm) content than city water source. Balnave and Yoselewitz (1987) found that 600 ppm NaCl in water reduced the shell quality. High NaCl can negatively affect activity of carbonic anhydrase enzyme, which is important in shell formation (Yoselewitz and Balnave 1989a). However, the maximum limits of these minerals for poultry vary from source to source. K was below the detectable limit in all the samples. The average concentrations of major cations and anions in the samples were summarised (Table 3.4).

Ca content in waters ranged from undetectable levels to 231 ppm. The highest was from a QC sample. Highest Ca concentrations for AB, MB, NB, NL, NS, PE and SK were 48, 154, 98, 39, 75, 65 and 145 ppm respectively. The highest Mg content (103 ppm) was observed from a SK sample, where SO_4 content was 625 ppm. The highest Mg contents for AB, MB, NB, NL, NS, PE and QC were 35, 91, 17, 4, 10, 6 and 24 ppm, respectively. The high Mg and Ca contents were associated with high SO_4 contents in water. All samples received were below 125 ppm and 500 ppm Mg and Ca levels respectively, which were the maximum suggested for poultry (Carter and Sneed 1996; Fairchild and Ritz 2012). So none of the samples (0%) received from egg production units across Canada exceeded the current recommendations for Ca and Mg in poultry drinking water.

Cl and SO_4 are two major anions found in water. Cl concentration of water was highest in SK (519 ppm). The highest concentrations for AB, MB, NB, NL, NS, PE and QC were 11, 424, 93, 15, 65, 333 and 68, respectively. Koelkebeck et al. (1999) found low egg production in laying hens that received well water with high Cl (210 ppm) content than city water source. High levels of Cl (as in 600 ppm NaCl) in drinking water can cause poor

shell quality (Balnave and Yoselewitz 1987) by negatively affecting carbonic anhydrase enzyme activity (Yoselewitz and Balnave 1989a). The maximum recommended level of Cl was 250 ppm (Carter and Sneed 1996; Weltzein 2002; Fairchild and Ritz 2012). Ten percent of all water samples exceeded this limit of Cl. The farmers whose waters that higher in Cl ions, should pay their attention on the eggshell quality.

The highest concentration of SO_4 of all waters was 2703 ppm, which was from a PE sample. There were 2 samples from PE, which contained more than 2600 ppm SO_4. The SO_4 content in water could be higher because of high fertilizer use for crop cultivation (Health Canada 2012). The maximum SO_4 concentrations in water for the AB, MB, NB, NL, NS, QC and SK were 148, 290, 52, 12, 42, 569 and 1651 ppm, respectively. Thirteen percent of samples contained SO_4 beyond the 250 ppm concentration, which was the current suggested level for poultry by Carter and Sneed (1996), Weltzein (2002) and Fairchild and Ritz (2012). Six samples (7%) contained more than doubled and two samples (2%) had more than 10 times higher SO_4 contents. These levels could be toxic, or affect performance of hens. The effects of higher levels of SO_4 on laying hens is not still well known. So that, different levels from 250 to 2700 ppm should be tested in order to determine the possible impacts on production performance, egg quality and mineral metabolism of the laying hens.

The range of hardness was 0 to 786 ppm as $CaCO_3$. This range was well below the maximum desirable limit that has been proposed by Weltzien (2002). However, the average hardness was higher in some provinces than 180 ppm that have been suggested by Carter and Sneed (1996). Average hardness levels in all provinces, except NL, were higher than 110 ppm, which was suggested by Fairchild and Ritz (2012). According to the results

hardness was increased with both higher Ca and Mg content in the water (Table 3.4). The average hardness ranged from 28 to 297 ppm. Ca and Mg are the major cations that make water hard while some other minerals also can contribute to the water hardness such as aluminium, barium, strontium, iron, zinc, and manganese (Sengupta 2013). CO_3, HCO_3, SO_4, Cl and OH are the anions which associate with the cations in hard water (Sengupta 2013). Cl ions did not increase with the hardness of water. Therefore, Ca and Mg seemed to be associated with other anions such as SO_4 in these hard waters. The highest hardness was from a sample from SK (786 ppm). The Ca, Mg and SO_4 content of that sample was 145, 103 and 673 ppm respectively. When SO_4 content higher than 50 ppm in water, birds could develop diarrhea at low levels of Mg as 125 ppm (Weltzien 2002).

3.7 Summary of the survey results

The majority of egg farms in Canada (73%) use ground well water sources while others used surface water sources (18%) such as rivers and municipal water (9%) for supplying laying hens. Sixty seven percent of farms surveyed had a water analysis history for their waters while 14% did not analyse the water. Nineteen percent of farmers did not provide information about previous water analysis.

The water pH of 6% of the samples were outside of pH 6.5 to 8.5 suggested by Weltzien (2002) as being acceptable. Eighty four percent of samples did not fall within the range of pH 6.8 to 7.5 suggested by Carter and Sneed (1996). Only 9% of samples were within pH from 5 to 6.8 which has been suggested by Fairchild and Ritz (2012) as being acceptable.

The conductivity of all water samples across Canada was satisfactory. SO_4 concentration in the water was not satisfactory and exceeded current maximum recommended value of 250 ppm by several folds. Ca and Mg levels were good when compared to the maximum

suggested values of 600 and 125 ppm, respectively, but negative impacts could be occurred at these levels when SO_4 ion concentration is high in water (Carter and Sneed 1996). Hardness of water was not satisfactory compared to the limit (110 ppm) suggested by Fairchild and Ritz (2012). The maximum NO_3/NO_2-N content was 39 ppm in all waters from across Canada. Adams et al. (1966) did not find negative impacts of 300 ppm NO_3-N in laying hen drinking water. Na level in water is satisfactory for all provinces except SK and these waters needs to be monitored for salinity. Alkalinity level was not satisfactory when compared to the recommendations for poultry drinking water.

3.8 Conclusions

Based on the available recommendations for poultry, water pH, hardness, alkalinity, and SO_4 ion content is not satisfactory in all drinking waters provided to laying hens across Canada. Higher levels of Ca and Mg in the water which is associated with water hardness, would not be satisfactory when SO_4 concentration of that water is high, according to the current recommendations for poultry drinking water. Therefore, further studies are necessary to evaluate water pH levels, excess amounts of Ca, Mg, SO_4 and alkalinity on laying hen performance.

CHAPTER 4 EFFECT OF HIGH CALCIUM, MAGNESIUM AND SULPHATE IN DRINKING WATER ON LAYING HEN PRODUCTION PERFORMANCE, EGG QUALITY AND BONE QUALITY

4.1 Abstract

The mineral composition of natural water is highly variable from place to place and may have impact on performance of laying flocks in different geological areas, but effects were not well known. This study evaluated the effect of high Ca, Mg and SO_4 content in drinking water on production performance, egg quality and bone quality of laying hens. Five water treatments with high Mg, Ca and SO_4 were given to Lohmann LSL-Lite white hens in two trials. The experimental design for both trials was completely randomized experiment with 6 replications. During the first trial, hens were randomly assigned to water treatments including 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, 625 $MgSO_4$ plus 1417 ppm $CaSO_4$ and 1250 $MgSO_4$ plus 708 ppm $CaSO_4$ and control (well water) from 33-69 weeks of age. Feed consumption (FC), hen-day egg production (HD) and feed conversion ratio were not affected by water treatments. Water consumption (WC) was lower for 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, and 625 $MgSO_4$ plus 1417 ppm $CaSO_4$ treatment groups than control ($P<0.05$). Body weight (BW) of the 1250 ppm $MgSO_4$ group was lower than 1250 $MgSO_4$ plus 708 ppm $CaSO_4$ ($P<0.05$). Egg weight, specific gravity, shell %, albumen %, albumen height, shell thickness, and breaking strength and bone quality measures were not affected by water treatments ($P>0.05$). The second trial was conducted with 2100 ppm $MgSO_4$, 2600 ppm $MgSO_4$, 2100 ppm $CaSO_4$ and 2600 ppm $CaSO_4$ and control treatments. Data were collected from 7-46 weeks of age. During the pullet stage (7-18 weeks), high mineral water did not affect BW or WC. FC of the 2600 ppm $MgSO_4$ group was lower than other groups during 15-18 wks. During the laying stage, BW, FC, HD, WC or egg quality were not affected by water treatments ($P>0.05$). Therefore, the mineral content of water containing Ca, Mg and SO_4 up to 786, 562 and 1988 ppm, respectively, did not negatively affect pullet grower and developer production performance while levels up to 732 ppm, 508 ppm and 1818 ppm, respectively, did not negatively affect laying hen production performance and egg quality. Bone quality of the laying hens at the end of production cycle was not affected by Ca, Mg and SO_4 up to 487 ppm, 234 ppm and 1317 ppm, respectively, in water. Hens at all stage of production tolerated concentrations of these minerals that exceeded the recommendations for poultry under the given experimental conditions.

Key words**:** bone quality, egg quality, high mineral water, laying hens

4.2 Introduction

Water is important in many body functions in animals including digestion, absorption, maintenance of ionic balance, excretion of waste materials from the body, and providing media to transport nutrients, heat regulation and metabolism of nutrients (Schlink et al. 2010). However, less attention has been paid to water as a nutrient in poultry nutrition than to feed ingredients (Atteh and Leeson 1983a). For laying hens, a supply of good quality water is important for a profitable egg production since poor quality water can reduce optimum productivity of the hens (NRC 1994). Increased levels of minerals, which often make poor quality water, can be undesirable to birds, and reduce the performance (NRC 1974). But, effects of high levels of minerals in the water on poultry performance including laying hens are not well documented (Atteh and Leeson 1983a).

Water for poultry production can originate from ground or surface water sources or municipal water supplies (Chapter 3 section 3.6). SO_4, which is a divalent anion in the water, can be highly variable in natural water sources including ground and surface water sources (USEPA 2012). Concentration of SO_4 can be higher than 250 ppm in water (Weeth and Hunter 1971; Chapter 2 section 3.6), which was recommended as maximum desirable limit for poultry (Carter and Sneed 1996; Weltzien 2002; Fairchild and Ritz 2012).

Ca and Mg can be found in increased levels in water (Sengupta 2013). These two minerals are high in areas with limestone and dolomite soils and make water hard, hence unsuitable for drinking purpose of animals (WHO 2011b). Elevated levels of Ca and Mg in water can cause a laxative effect on hens if sulphate content is more than 50 ppm (Carter and Sneed 1996) and consequently reduce nutrient absorption. High content of SO_4 in the digestive tract will increase water retention due to an osmotic effect (Cassidy 1999), which results

in flushing of nutrients producing watery excreta. However, the levels that has been exert these effects were not discussed.

High Ca and Mg in the digestive tract can negatively affect absorption of many essential minerals including Fe, Zn, Cu, Mn and I (Georgievskiĭ et al. 1981) and may lead to deficiencies. Ca is the major mineral found in eggshells (Solomon 1997), and bones (Whitehead 2004). Prolonged exposure to waters high in Mg might reduce available Ca in body pools which could affect eggshell and bone quality.

Excess intake of Ca and Mg due to increased levels in water may affect acid-base balance in blood and body fluids (Mongin 1981), which can cause metabolic disturbances. However, the effects of these minerals at high levels in drinking water on the hens have not been elucidated.

There are research reports on the use of water with elevated levels of NaCl for laying hen production. Saline water, which contained 2000 ppm NaCl was found to reduce eggshell quality and increase the number of damaged eggs by reducing carbonic anhydrase activity in the shell gland in a study by Yoselewitz and Balnave (1989a). The pH in the shell gland fluid increased while bicarbonate and Ca ion were reduced with increasing Na and Cl ion concentrations. However, no information was reported on the effects of other minerals in water on eggshell quality.

Recent information on effects of high Mg in water on poultry is limited with most reported during the 1960-1975 time period (Krista et al. 1961; Adams et al. 1975). High concentrations (4000 ppm) of $MgSO_4$ negatively affected egg production, and feed consumption but not water consumption and body weight (Adams et al. 1975).

Additionally, these findings from decades ago may not be applicable to the current high producing commercial lines of hens with high metabolic demand.

Therefore, evaluation of the effects of different levels of Ca, Mg and SO_4 in drinking water on laying hen production performance, egg quality and bone quality will fill the knowledge gaps. Two trials were conducted to evaluate the effects of Ca, Mg and SO_4 in laying hen drinking water. The treatments and mineral levels evaluated in the first trial for the later stage of laying were based on the results of a water quality survey conducted across Canada (Chapter 3 section 3.6). The second trial was planned to study the effects of levels of Ca, Mg and SO_4 higher than the first trial and expand the testing from early pullet stage through the complete laying cycle of hens.

4.3 Objectives

4.3.1 Trial 1

To evaluate the effects of 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, 625 $MgSO_4$ plus 1417 ppm $CaSO_4$ and 1250 $MgSO_4$ plus 708 ppm $CaSO_4$ in drinking water compared to a control (well water) treatment on:

a) Laying hen production performance measurements including: feed consumption, water consumption, hen day egg production, body weight, and feed conversion ratio from 33 to 69 weeks of age.
b) Egg quality measurements including: egg weight, specific gravity, breaking strength, albumen height, percent albumen, yolk and shell, and shell thickness from 33 to 69 weeks of age.
c) Bone quality measurements of tibia and humerus including: length, width, weight, mineral density, breaking strength, percent ash at 70 weeks of age.

4.3.2 Trial 2

To evaluate the effects of 2100 ppm $MgSO_4$, 2600 ppm $MgSO_4$, 2100 ppm $CaSO_4$ and 2600 ppm $CaSO_4$ in drinking water compared to a control (well water) treatment on;

a) Production performance measurements including: feed consumption, water consumption, hen day egg production, and body weight of hens from early pullet stage (7 weeks) to 46 weeks of age.

b) Egg quality measurements including: egg weight, specific gravity, breaking strength, albumen height, percent yolk and shell, and shell thickness from 19-46 weeks of age.

4.4 Hypothesis

4.4.1 Trial 1

a) It is hypothesized that the addition of high levels of Ca, Mg and SO_4 in water will negatively affect production performance measures of laying hens including; feed consumption, water consumption, body weight and hen day egg production when compared to the well water control from 33 to 69 weeks of age.

b) It is hypothesized that the addition of high levels of Ca, Mg and SO_4 in water will negatively affect egg quality measures including: egg weight, specific gravity, breaking strength, albumen height, percent albumen, yolk and shell, and shell thickness of laying hens when compared to the well water control from 33 to 69 weeks of age.

c) It is hypothesized that the addition of high levels of Ca, Mg and SO_4 in water will negatively affect bone quality measures of tibia and humerus including: length, width, weight, mineral density, breaking strength, percent ash when compared to the well water control at 70 weeks of age.

4.4.2 Trial 2

a) It is hypothesized that addition of high levels of Ca, Mg and SO_4 in water will negatively affect production performance measures of laying hens when compared to the well water control from 7-46 weeks.

b) It is hypothesized that the addition of high levels of Ca, Mg and SO_4 in water will negatively affect egg quality measures of laying hens when compared to the well water control from 19-46 weeks.

4.5 Materials and Methods

4.5.1 Trial 1

This study occurred during the laying phase of the hen's production cycle from 33-69 weeks of age.

4.5.1.1 Experimental design and hen management

The trial spanned a period of 36-weeks using 300, 33-week old Lohmann LSL- Lite white laying hens. The hens were housed within 60 battery cages with 5 birds in each (50 × 60 × 44 cm; length × width × height). Two adjacent cages were considered an experimental unit. Therefore, there were 30 experimental units and each treatment had 6 replicates. The experiment was completely randomised. The hens were provided with standard layer diet and water *ad libitum* throughout the study. Hens were fed in three phases with diet changes at 46 and 66 weeks of age (Table 4.1). Feed samples from each phase were analysed for nutrient composition at the Department of Agriculture feed analysis lab in Truro, Nova Scotia. Water was supplied through 2 nipple drinkers per cage. Hens were supplied a 16L: 8D photoperiod throughout the trial. Room temperature was kept between 22 and 24°C and

checked twice a day. Mortality was recorded as it occurred and all birds that died were necropsied by a veterinary pathologist. All animals were managed in accordance with the Dalhousie University Animal Care and Use Committee guidelines that follow the Canadian Council on Animal Care Codes of Practice (2009).

Table 4.1.The ingredients and calculated nutrient compositions of diets used in water mineral study (trial 1) *

	Phase of diet changes		
	Phase I (33-45 wks)	Phase II (46-65 wks)	Phase III (66-69 wks)
Ingredients	---------- --- % of Diet (as fed)----------		
Soybean meal	25.90	20.85	1.34
Corn	51.81	57.41	50.41
Wheat	10.00	10.00	10.00
Canola meal	-	-	23.18
Animal/vegetable fat [1]	1.31	0.68	3.82
Vitamin–mineral premix[2]	0.50	0.50	0.50
Mono-Dicalcium phosphorus	0.10	0.11	0.16
Iodide Salt	0.33	0.33	0.26
Methionine premix[3]	0.36	0.29	0.21
Shell mix[4]	2.42	2.45	2.41
Limestone	4.84	4.90	4.82
Biophytase[5]	0.01	0.01	0.01
Oyster shell	2.42	2.45	2.41
Lysine 98 %	0.00	0.01	0.47
Total	100.00	100.00	100.00
Calculated Composition			
Metabolizable energy(kcal/kg)	2820	2820	2820
Protein (%)	18	15	15
Methionine (%)	0.5	0.4	0.3
Methionine+Cystine (%)	0.8	0.7	0.5
Phosphorus available (%)	0.4	0.4	0.3
Calcium (%)	4	4	4

Expected feed intake at different phases of production Phase I, II and III were 105,115 and 110 g/hen/day respectively. **[1]Animal vegetable fat** contained free fatty acids 15%, moisture 1%, insoluble matter 0.15%, unsaponifiables 2.5%. S.F. Rendering Ltd. NS, Canada. **[2]Layer premix** (Amount per tonne of feed): Vitamin A ($650x10^6$ IU kg^{-1}), 12 g; Vitamin D3 premix ($50x10^6$ IU kg^{-1}), 50 g; Vitamin E ($5x10^5$ IU kg^{-1}), 40 g; Vitamin K (33%), 9 g; Riboflavin (95%), 8 g; DL Ca-pantothenate (45%), 16 g; Vitamin B12 (1000 mg kg^{-1}), 12 g; Niacin (99%), 31 g; Folic acid (3%), 22 g; Choline chloride (60%), 117 g; Biotin (0.04%), 400 g; Pyridoxine (990000 mg kg^{-1}), 4 g; Thiamine (970000 mg kg^{-1}), 220 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 100 g; Copper sulfate (25%), 100 g; Selenium premix (675 mg kg^{-1}), 14.85 g; Ethoxyquin (50%), 100 g; Wheat Middlings, 2189 g; Ground limestone (38%), 500 g. **[3]Methionine Premix:** 50% Wheat middlings and 50% DL methionine.**[4]Shell mix**: $CaCO_3$ 97.5%, $MgCO_3$ 0.3%, Ca 39%,Mg 0.1%, Silica(SiO2) 1.4%, Ferric Oxide (Fe_2O_3) 0.2%,Alumina (Al_2O_3) 0.2% Total S 0.01%.Graymont (QC)Inc.,QC,Canada.**[5]Biophytase**: 5000 phytase units per g. Canadian Bio-Systems Inc., Calgary, Alberta.

4.5.1.2 Water treatments and preparation

Five water treatments including APRC (Atlantic Poultry Research Centre) - well water (Control), for the remaining water treatments well water plus, 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, 625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$ and 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$ were used as treatments (Table 4.2). $MgSO_4.7H_2O$ (Giles Chemical, Waynesville, NC) was used as the $MgSO_4$ source and $CaSO_4.2H_2O$ (EMSURE®, Merck KGaA, Darmstadt, Germany) was used as the $CaSO_4$ source. Water treatments were prepared in 200 L plastic containers and pumped into individual 10 L containers attached to each experimental unit connected to nipple drinkers in each cage. Water treatments were prepared every 2 weeks. Digital water flow meters were attached to each experimental unit to measure water consumption. Treatments were allocated randomly to the experimental units. Water samples (200 mL) were taken monthly and sent to Nova Scotia Department of Agricultural lab, Harlow institute, Truro, NS for mineral and other water quality measures as described in Chapter 3. Total dissolved solids (TDS) of the water treatments was analysed in the nutrition laboratory, Haley Institute, Dalhousie Agricultural campus monthly by evaporation method (SMEWW 2540 C) (APHA 1999). A water sample of 100 mL from each treatment was measured into a pre-weighed beaker and dried at 180°C for 24-36 hours in a drying oven (Iso temp 300 series, Fisher Scientific Company, Ottawa, Ontario, Canada). Water was analysed in duplicates for each treatment.

The TDS (ppm) was calculated as follows.

$$\text{TDS} = \frac{(\text{A} - \text{B}) \text{ x } 1000}{\text{Sample volume (mL)}}$$

Where, A= Weight of residue + beaker (mg), B= Weight of the beaker (mg).

Table 4.2. Description of water mineral levels given to laying hens from 33-70 weeks of age in water mineral study (trial 1)

Description	Level of test mineral	Total dissolved solids[1]
Control	Mg - 9 ppm, Ca - 56 ppm SO_4 - 31 ppm	321
Low Mg[2]	625 ppm $MgSO_4$	1035
High Mg[2]	1250 ppm $MgSO_4$	1648
Low Mg Ca[2]	625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$	2436
High Mg Ca[2]	1250 ppm $MgSO_4$ plus 807 ppm $CaSO_4$	2421

[1]Analysed by drying at 180 °C; units: ppm
[2]The levels added to the control water.

4.5.1.3 Production performance measurements

Body weight (BW), feed consumption (FC), water consumption (WC) and hen-day egg production of the birds were determined for each 28-day period. Hens were group weighed on the last day of each 28-day period for each experimental unit. Body weight per hen was determined for each 28-day period. Feed was weighed and added daily to each experimental unit (two cages). On the last day of each 28-day period and as mortality occurred, feed remaining in the feeder was measured. Daily feed consumption (g) per hen was determined. Feed conversion ratio (FCR) was calculated per experimental unit for each 28-day period as the amount of feed consumed in kg to produce one kg of egg using the following equation.

$$\text{FCR} = \frac{\text{total feed consumed in 28 day period}}{\text{average egg weight x total number of eggs in 28 day period}}$$

The water meter reading of each experimental unit was recorded weekly. The water consumption was calculated in mL/hen/day basis using the difference of initial and final readings. At the last day of each 28-day period, water meters were reset. Egg production per day was recorded from each experimental unit. Hen day egg production (HD) was

calculated using number of marketable eggs from each experimental unit using following equation.

$$\text{HD egg production } \% = \frac{total\ number\ of\ eggs\ produced\ in\ 28\ day\ period}{number\ of\ birds\ alive\ \times\ 28} \times 100$$

4.5.1.4 Egg quality analysis

Egg quality was analysed at the end of every 28-day period. On the last day of each period, 8 eggs per experimental unit were collected and numbered. Eggs were analysed for specific gravity, egg weight, albumen height, yolk weight, shell weight, shell breaking strength and shell thickness.

Specific gravity was measured by flotation of the eggs in a graded series of saline solutions ranging from 1.066 to 1.102 in increments of 0.004 (Hamilton 1982). Before floating, eggs were kept 24 hrs at room temperature to equate the egg temperatures to salt solutions temperatures. Eggs were allowed to air dry after floating, weighed and egg breaking strength was determined using a TA.XT Plus texture analyzer (Texture technologies Corp. Scarsdale, New York, USA). A 30 kg load cell and flat, cylindrical acrylic probe were used to measure the breaking strength. The blunt end of each egg was oriented upwards to apply force. Albumen height was measured using an albumen height gauge (OCD™, Technical services and supplies, Chessingham Park, Dunnington, York, England). Yolk was separated from albumen and weighed. Egg shells were washed with water and air dried for 2-3 days. Eggshells were weighed and eggshell thickness was measured using the TA.XT Plus texture analyzer. A flat small piece of eggshell was taken at the equator of an egg to measure the eggshell thickness using TA.XTPlus texture analyzer with a stainless steel

conical probe. The texture analyser was calibrated for height (2 mm) prior to take measurements.

4.5.1.5 Bone sample collection and preparation

Two hens per experimental unit were euthanized by cervical dislocation at the end of trial-1 at 70 weeks of age. Bones were collected in two groups as A and B. Bones from group-A hens were collected for the bone mineral density analysis and bones from group B hens were collected for ash determination. Right tibia and right humerus were collected from each hen and placed in Whirl-pak bags and sealed. Samples were kept on ice during collection and then refrigerated at 4°C until cleaned. Bone samples were cleaned of soft tissues using scalpel blades and scissors.

4.5.1.6 Bone density measurements

The cleaned tibiae and humeri for mineral density analysis were placed in whirl-pak bags and falcon tubes respectively and covered with 10% phosphate buffered formalin (Sigma-Aldrich Canada Co., Oakville, ON) for 4 weeks at room temperature in a fume hood. Then bones were removed from formalin and rinsed with distilled water and placed individually in clean whirl-pak bags. A few distilled water soaked cotton balls were put into each bag to prevent drying out. This set of bones was shipped to University of Alberta for mineral density analysis by Quantitative Computed Tomography (QCT). Waste formalin was discarded safely. Bones for ash determination were frozen at -20°C until further analysis. Six bones per treatment were analysed for mineral density at the Department of Agricultural, Food and Nutritional Sciences at the University Alberta (Edmonton, Alberta, Canada) using Stratec XCT Scanner (model 922010, Norland Medical Systems Inc., Fort Atkinson, WI) with XMENU software version 5.40C, according to the method of Korver

et al. (2004). Cross sectional X-ray pictures were taken at the 30% and 50% positions from the proximal end of the bone. A longitudinal scan was initially done to set the positions. The cross sectional pictures were then analysed using XCT software and total, cortical and trabecular bone densities and areas were measured. Mineral content of a 1 mm longitudinal slice of total, cortical and trabecular bone was calculated by multiplying density and area measurement of each bone type (Saunders-Blades et al. 2009).

4.5.1.7 Bone breaking strength measurement

Bone breaking strength was determined for both density analysis and ash analysis groups of bones using TA.XTplus Texture Analyzer (Texture technologies Corp., Scarsdale, NY) at the Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University, Truro. Bones for ash determination were removed from the freezer and thawed for 24 hrs. Bone weight and length were measured in grams and mm respectively. Midpoint of each bone was marked once the length was measured. Bone diameter was measured at the midpoint of the bone using a micrometer (Central Scientific Co., Chicago, Ill). A 50 kg load cell, a three point bend rig and a standard sheer plate were used for breaking strength analysis of bones. Each bone was placed across the two supports which are 40 mm apart facing same side of the bone every time. The sheer plate descended perpendicular to break the bone at its midpoint and force required to break each bone was recorded automatically as hardness in kilogram force (kgf). The standard sheer plate descended at the speed of 0.5 mm/sec for a distance of 20 mm. All fragments of bones were collected after breaking in order to determine bone ash content.

4.5.1.8 Ash analysis of bones

Tibiae and humeri were ashed to determine percent ash. Bones were first dried at 103°C for 24 hrs in an oven (Iso temp 300 series, Fisher Scientific Company, Ottawa, Ontario, Canada), then submerged in petroleum ether for 48 hrs and removed. The bones were allowed to air dry overnight in a fume hood then dried at 103°C for 24 hrs in the oven. Dry defatted weight was recorded. Bones were ashed at 600°C in a muffle furnace for 24 hrs (Cheng and Coon 1990). Bones were placed in a desiccator to cool to room temperature. The ashed samples were weighed and percentage of ash was calculated for both defatted tibiae and humeri using following equation.

$$\% \text{ Ash} = \frac{\text{Ash weight (g) x 100}}{\text{fat free dry weight (g)}}$$

4.5.2 Trial 2

This trial extended from the early pullet stage (7 weeks of age) through active egg laying up to 46 weeks of age. Production performance and egg quality were evaluated in hens supplied with higher mineral content in water than trial 1.

4.5.2.1 Experimental design and hen management

a) Pullet stage

Three hundred and twenty, seven-week-old, Lohmann LSL- Lite pullets were housed within 40 experimental cages, 8 birds in each. Four adjacent cages were considered an experimental unit. Therefore, there were 10 experimental units initially with 32 birds per unit. Each treatment had 2 replications from 7-14 weeks of age. Initially it was planned to have 4 replications for the trial. However, with the unavailability of the battery cages at the start of the trial, birds were placed in cages to have 2 replications per treatment. At 15

weeks of age, there were enough battery cages available to split each experimental unit into two thereby increasing to four replications per treatment. Therefore, an experimental unit contained 16 birds from 15 to 18 weeks of age. The experimental design was completely randomised. The pullets were provided with standard grower (7 to 8 weeks), developer (9 to 16 weeks) and pre layer (17 to 18 weeks) diets during the pullet stage (Table 4.3). The diets were formulated to meet the requirements recommended by the breeding company (Lohmann Tierzucht 2013). Feed and water were supplied *ad libitum* throughout the pullet stage. Water was supplied by two nipple drinkers per cage. Hens received 11 hrs of light from week 7 to 16. After that, from 17 to 18 weeks lighting hours gradually increased to 13. Room temperature was kept between 22 to 24°C and checked twice a day. Mortality was recorded as it occurred and all birds that died were necropsied by a veterinary pathologist. All animals were managed in accordance with the Dalhousie university Animal Care and Use Committee guidelines that follow the CCAC (2009) codes of practice.

b) Laying stage

Three hundred pullets were transferred to a layer room facility at 19 weeks of age. The same experimental layout was used for the laying birds as described in mineral trial 1 (section 4.5.1.1) Five birds were placed in a cage and two adjacent cages were considered an experimental unit. There were 30 experimental units with 6 replications per treatment. The experiment was a completely randomised design. Layer phase 1 diet was supplied from 19 to 45 weeks of age (Table 4.4). The diets were formulated based on expected feed intake of the hens. At the start of phase I, the diet was formulated based on a feed intake of 90 g/hen/day. After 3 weeks, the diet was formulated based on feed intake of 100 g/hen/day.

Table 4.3. Formulations and calculated nutrient compositions of grower, developer and pre-layer diets used in the water mineral study (trial 2)

	Phase of diet changes		
	Grower 7-8 wks	Developer 9-16 wks	Pre-layer 17 -18 wks
Ingredients	---------- --- % of Diet (as fed)-------		
Soybean meal	26.92	0.00	24.46
Corn	25.36	43.22	35.56
Barley	30.00	20.00	20.00
Canola meal	0.00	19.84	0.00
Wheat bran	9.98	10.00	10.00
Corn oil	4.00	0.00	0.00
Animal/vegetable fat[1]	0.00	3.46	1.94
Vitamin–mineral premix	0.50[x]	0.50[x]	0.50[y]
Dicalcium phosphate	0.90	0.45	1.59
Iodized salt	0.38	0.31	0.36
Methionine premix[2]	0.17	0.17	0.38
Limestone, ground	1.80	1.65	2.06
Oyster shell	0.00	0.00	1.03
Shell mix[3]	0.00	0.00	1.03
Lysine HCl	0.00	0.39	0.70
Biophytase[4]	0.00	0.01	0.01
Total	100.00	100.00	100.00
Calculated composition			
Metabolizable energy (kcal/kg)	2900	2850	2850
Protein (%)	19	15	18
Methionine+cystine (%)	0.7	0.5	0.6
Available phosphorus (%)	0.5	0.3	0.5
Calcium (%)	1	1	2
Sodium (%)	0.2	0.2	0.2

[1]**Animal vegetable fat** contained free fatty acids 15%, moisture 1%, insoluble matter 0.15%, unsaponifiables 2.5%. S.F. Rendering Ltd. NS, Canada. [2]**Methionine Premix**: 50% Wheat middlings and 50% DL methionine. [3]**Shell mix**: $CaCO_3$ - 97.5%, $MgCO_3$ - 0.3%, Ca - 39%, Mg - 0.1%, Silica (SiO2) - 1.4%, Ferric Oxide (Fe_2O_3) - 0.2%, Alumina (Al_2O_3) 0.2%, total S - 0.01%. Graymont (QC) Inc., QC, Canada. [4]**Biophytase**: 5000 phytase units per g. Canadian Bio-Systems Inc., Calgary, Alberta. [x]**Vitamin mineral premix for grower and developer diets** (Amount per tonne of feed): Vitamin A ($100x10^7$ IU kg^{-1}), 9.75 g; Vitamin D3 premix ($50x10^6$ IU kg^{-1}), 40 g; Vitamin E ($5x10^5$ IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (95%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 12 g; Niacin (99%), 36 g; Folic acid (3%), 33g; Choline chloride (60%), 1335 g; Biotin (0.04%), 750 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (980000 mg kg^{-1}), 2 g; Manganous oxide (60%), 117 g; Zinc oxide (77%), 104 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 148 g; Ethoxyquin (60%), 83 g; Wheat Middlings, 1626.75g; Ground limestone (38%), 500 g. [y]**Layer premix** (Amount per tonne of feed): Vitamin A ($650x10^6$ IU kg^{-1}), 12 g; Vitamin D3 premix ($50x10^6$ IU kg^{-1}), 50 g; Vitamin E ($5x10^5$ IU kg^{-1}), 40 g; Vitamin K (33%), 9 g; Riboflavin (95%), 8 g; DL Ca-pantothenate (45%), 16 g; Vitamin B12 (1000 mg kg^{-1}), 12 g; Niacin (99%), 31 g; Folic acid (3%), 22 g; Choline chloride (60%), 117 g; Biotin (0.04%), 400 g; Pyridoxine (990000 mg kg^{-1}), 4 g; Thiamine (970000 mg kg^{-1}), 220 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 100 g; Copper sulfate (25%), 100 g; Selenium premix (675 mg kg^{-1}), 14.85 g; Ethoxyquin (50%), 100 g; Wheat Middlings, 2189 g; Ground limestone (38%), 500 g.

Table 4.4. Ingredients and calculated nutrient composition of diets used in laying stage (19-46 weeks) of hens in water mineral study (trial 2)

	Diet changes	
	Phase I 90 g/hen/day*	Phase I 100g/hen/day*
Ingredients	-------- --- % of Diet (as fed)----------	
Soybean meal	31.66	27.99
Corn	42.54	36.75
Wheat	10.00	20.00
Animal/vegetable fat[1]	3.25	3.26
Vitamin–mineral premix[2]	0.50	0.50
Dicalcium phosphate	0.96	0.87
Iodized Salt	0.41	0.41
Methionine premix[3]	0.33	0.32
Limestone, ground	5.17	4.94
Oyster shell	2.59	2.47
Shell mix[4]	2.59	2.47
Biophytase[5]	0.01	0.01
Total	100.00	100.00
Calculated composition		
Metabolizable energy (kcal/kg)	2820	2850
Protein (%)	19	19
Methionine+cystine (%)	0.7	0.7
Lysine (%)	1	1
Available phosphorus (%)	0.3	0.3
Calcium (%)	4	4
Sodium (%)	0.2	0.2

*Expected daily feed intake

[1]**Animal vegetable fat** contained free fatty acids 15%, moisture 1%, insoluble matter 0.15%, unsaponifiables 2.5%. S.F. Rendering Ltd., NS, Canada. [2]**Layer premix** (Amount per tonne of feed): Vitamin A ($650x10^6$ IU kg^{-1}), 12 g; Vitamin D3 premix ($50x10^6$ IU kg^{-1}), 50 g; Vitamin E ($5x10^5$ IU kg^{-1}), 40 g; Vitamin K (33%), 9 g; Riboflavin (95%), 8 g; DL Ca-pantothenate (45%), 16 g; Vitamin B12 (1000 mg kg^{-1}), 12 g; Niacin (99%), 31 g; Folic acid (3%), 22 g; Choline chloride (60%), 117 g; Biotin (0.04%), 400 g; Pyridoxine (990000 mg kg^{-1}), 4 g; Thiamine (970000 mg kg^{-1}), 220 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 100 g; Copper sulfate (25%), 100 g; Selenium premix (675 mg kg^{-1}), 14.85 g; Ethoxyquin (50%), 100 g; Wheat Middlings, 2189 g; Ground limestone (38%), 500 g**.** [3]**Methionine Premix:** 50% Wheat middlings and 50% DL methionine.

[4]**Shell mix**: $CaCO_3$ - 97.5%, $MgCO_3$ - 0.3%, Ca - 39%, Mg - 0.1%, Silica (SiO2) - 1.4%, Ferric Oxide (Fe_2O_3) - 0.2%, Alumina (Al_2O_3) - 0.2%, Total S - 0.01%. Graymont (QC)Inc.,QC,Canada. [5]**Biophytase**: 5000 phytase units per g., Canadian Bio-Systems Inc., Calgary, Alberta.

Hens were supplied 13.5 hrs of light per day during the 19th week and thereafter day length was gradually increased to 16 hrs by the 24th week as per the breeder recommendations (Lohmann Tierzucht 2013). Sixteen hrs of light per day was maintained for the remainder of the laying stage. Room temperature was kept between 22 to 24°C and checked twice a day. Mortality was recorded as it occurred and all birds that died were necropsied by a veterinary pathologist. When mortalities occurred, bird weight, feed weigh back and water meter readings were recorded. All animals were managed in accordance with the Dalhousie University Animal Care and Use Committee procedures that followed the CCAC (2009) guidelines.

4.5.2.2 Water treatments and preparation

Four water treatments were prepared by mixing $MgSO_4$ and $CaSO_4$ with the well water normally used in the poultry unit. The water treatment descriptions are provided in Table 4.5. These water treatments were randomly allocated to experimental units at week 7 until hens were 46 weeks of age. The sources of the salts were the same as the mineral trial 1 (section 4.5.1.2). Well water was supplied as the control treatment. Water treatment preparation and distribution was as discussed for trial 1 (section 4.5.1.2). Water samples (200 mL) were analysed for mineral concentration and other water quality parameters including pH, conductivity, hardness, alkalinity in every month. Total dissolved solids of water treatments was determined as described in the trial 1(section 4.5.1.2).

Table 4.5. Description of water treatments, levels of minerals and total dissolved solid contents in water mineral study (trial 2)

Description	Level of test mineral	Total dissolved solids[1] (TDS)
Control	Mg- 9 ppm, Ca- 56 ppm SO_4- 31 ppm	404
Low Mg[2]	2100 ppm $MgSO_4$	2518
High Mg[2]	2600 ppm	3114
Low Ca[2]	2100 ppm $CaSO_4$	2425
High Ca[2]	2600 ppm $CaSO_4$	2957

[1]Analysed by drying at 180°C; units = ppm
[2]The levels added to the control water.

4.5.2.3 Production performance measurements

The feed consumption, water consumption, body weight and hen-day egg production was determined for each 28-day period as described in section 4.5.1.3.

4.5.2.4 Egg quality analysis

Egg quality was analysed for every 28-day period during laying stage from 19 to 45 weeks of age. During the last day of 28-day period, 8 eggs per experimental unit were collected and numbered. Eggs were analysed for specific gravity, egg weight, albumen height, albumen weight, yolk weight, shell weight, shell breaking strength and shell thickness as described in section 4.5.1.4.

4.5.3 Statistical analysis

4.5.3.1 Water mineral trial 1

The first water mineral trial was designed as a completely randomized design with six replications. The production performance data, egg quality data, bone quality data and mineral balance data were subjected to the Proc Mixed procedure of Statistical Analysis Systems (SAS) version 9.3 (SAS Institute Inc., Cary, NC) (Littell et al. 1996) with water treatments as the main effects (Control, 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, 625 ppm

$MgSO_4$ plus 1417 ppm $CaSO_4$ and 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$). If main effects or interaction effects were found to be significant, the differences among the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

4.5.3.2 Water mineral trial 2

The experimental design for the second trial was a completely randomised design. Production performance data were divided into two parts for the pullet phase (7 to 18 weeks) as 7 to 14 weeks and 15 to 18 weeks. This was done due to different replication numbers for the treatments at the start and later in the trial. There were 2 replications from 7 to 14 weeks while 4 replications from 15 to 18 weeks. The production performance data (body weights, feed consumption and water consumption) were subjected to the Proc Mixed procedure of the Statistical Analysis Systems, Inc. with water treatments as the main effects (Control, 2100 ppm $MgSO_4$, 2600 ppm $MgSO_4$, 2100 ppm $CaSO_4$ and 2600 ppm $CaSO_4$). If main effects or interaction effects were found to be significant, the differences among the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

4.5.3.3 General statistical analysis

The following model was used for statistical analysis of data at a given time point for both trials.

$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where Y_{ij} is the variable of interest; μ is the overall mean; α_i is the effect of the i^{th} water treatment (i = 1-5) and ϵ_{ij} is the random effect of error.

For repeated measures analysis, the factor of time and resulting interaction levels were added (production period as the measure of time, k) to the model. Five covariance structures, Compound Symmetry, Heterogeneous Compound Symmetry, Toeplitz,

Heterogeneous Toeplitz and Ante-dependence were compared. For the ANOVA, the covariance structure which gave the smallest corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) numbers was selected. Feed and water consumption, and egg specific gravity were analysed using Ante-dependence covariance structure, while body weight, shell thickness, shell % were analysed using Toeplitz. Egg breaking strength, yolk %, hen day egg production, and egg weight were analysed using Compound Symmetry while albumen height was analysed using Heterogeneous Compound Symmetry. The statistical model for repeated measures was:

$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where, y_{ijk} is the response variable, μ is the overall mean of response variable data (production performance, egg quality data), α_i is the effect of i^{th} level of water treatments, β_j is the effect of j^{th} period, $(\alpha\beta)_{ij}$ is the two-way interaction effect of i^{th} level of water treatment and effect of j^{th} period, ε_{ijk} is the residual error. To compare the water treatment by period means within a specific period, slice option of SAS was used. Correlation coefficient between parameters were determined using Minitab statistical software version 17 (Minitab 17 Statistical Software. 2010. State College, PA: Minitab, Inc.).

4.6 Results and Discussion

The results for diet analysis, water analysis, production performance, egg quality, bone quality and mineral balances were described independently for trial 1 and trial 2.

4.6.1 Trial 1

4.6.1.1 Diet analysis

The composition of diets fed to hens in trial 1 (Table 4.6) indicated the analysed nutrient composition varied depending on the phase. None of the nutrients were below the

calculated values. The crude protein content was similar in phase I and higher in phase II and III to the calculated percentages. The calculated percent values were 17, 15 and 15 for phase I (33 to 45 wks), II (46 to 65 wks) and III (66 to 70 wks), respectively. Calcium content was similar to the calculated percent.

Table 4.6. Analysed composition of the diets fed to laying hens at 3 phases during water mineral study (trial 1)

Nutrient	Diet changes		
	Phase I (33-45 wks)	Phase II (46-65 wks)	Phase III (66-70 wks)
	----------as fed basis----------		
Protein (%)	17±0.2	16±0.2	16±0.1
Fat (%)	3±0.0	3±0.0	4±0.0
Calcium (%)	4±0.0	4±0.1	4±0.1
Potassium (%)	0.7±0.0	0.7±0.0	0.5±0.0
Magnesium (%)	0.2±0.0	0.2±0.0	0.2±0.0
Phosphorus (total) (%)	0.4±0.0	0.4±0.0	0.5±0.0
Sodium (%)	0.1±0.0	0.1±0.0	0.1±0.0
Copper (ppm)	31±5	23±4	24±4
Manganese (ppm)	130±12	71±6	117±11
Zinc (ppm)	95±5	80±3	133±5
Dry matter (%)	89±4	87±2	91±4

4.6.1.2 Water analysis

In the summary of water analysis results for trial 1 (Table 4.7), pH ranged from 7.97 to 8.10 in all treatments. Conductivity changed according to the concentrations of the salts used. Hardness was increased with the increasing Ca and Mg in the water treatments. Alkalinity ranged from 119 to 131 ppm among the treatments. Other mineral concentrations did not deviate significantly among treatments. Mn and K were below detection limits of the equipment. The detection limit for these minerals were 0.1 and 20 ppm, respectively. The TDS concentration of the 625 ppm $MgSO_4$+1417 ppm $CaSO_4$ treatment was lower than calculated concentration of 2342 ppm. That was due to the poor solubility

of $CaSO_4$ in water at higher concentration at room temperature of 22 to 24°C. The TDS of 1250 ppm $MgSO_4$+708 ppm $CaSO_4$ was similar to the calculated concentration of 2200 ppm. $MgSO_4$ was readily dissolved in water at the room temperature.

Table 4.7. The means and standard errors of means of water quality parameters including concentrations of mineral ions of the water treatments used in water mineral study (trial 1)[1]

	Treatment				
	Control	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$+1417 ppm $CaSO_4$	1250 ppm $MgSO_4$+708 ppm $CaSO_4$
Parameter					
pH	7.97±0.06	8.10±0.08	8.00±0.07	7.90±0.04	8.00±0.06
Conductivity	564±42	1375±138	1864±0.3	2406±64	2472±53
Chloride	78±39	60±11	64±11	74±9	70±10
Alkalinity	119±6	125±4	127±3	130±3	131±3
Nitrate/Nitrite-N	3±0	2±0	2±0	3±0	3±0
Hardness	177±25	568±46	1085±80	1711±66	1584±49
Calcium	56±10	39±7	40±7	487±23	284±29
Magnesium	9±0	115±12	234±17	120±6	229±8
Sulphate	31±1	427±46	882±62	1317±51	1309±39
Sodium	41±4	48±2	46±3	48±2	49±3
Manganese	ND	ND	ND	ND	ND
Potassium	ND	ND	ND	ND	ND
Copper	0.12±0.02	0.10±0.01	0.10±0.01	0.10±0.00	0.09±0.01
Iron	0.04±0.01	0.03±0.00	0.02±0.00	0.26±0.22	0.26±0.21
Zinc	0.14±0.08	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.00
TDS[2]	299±67	919±85	1408±96	2130±60	2156±21

[1]Means±SEM of 10 water analysis results. pH measured in pH units. Conductivity was measured in µmhos/cm. Mineral ion concentrations were reported in ppm. Alkalinity and hardness were reported in ppm as $CaCO_3$. ND indicates the concentrations below the detectable limit of the analysis method.
[2]TDS=Total Dissolved Solids in ppm, analysed by drying at 180°C.

4.6.1.3 Effects of water mineral treatments on production performance

4.6.1.3.1 Body weight

There was no interaction between water treatments and age of the birds on body weights of the hens (P>0.05) (Table 4.8). The body weight of the hens was significantly affected by water treatments. The lowest body weight was observed in the high $MgSO_4$ group while highest was observed in the high Mg Ca group. However, when compared to the control water, none of the treatments resulted in a difference in body weights. Production period had significant effect on body weight. Body weight increased as the birds aged from the 33 to 60 weeks of age. However, there was a reduction in body weight during 61 to 69 weeks of age when compared to the other weeks. The feed consumption of the hens reduced during this time period and it could be the reason for the body weight loss. Although there was a reduction in body weight during 61 to 69 weeks of age, average body weight increased from 1692 to 1755 g/bird during the period from 33 to 69 weeks of age. This range was similar to the targeted body weight (1607-1746 g/bird) as proposed by the breeder management guide of Lohmann–Lite white laying hens for this age (Lohmann Tierzucht 2013). Overall there was no negative impact of high mineral drinking water on body weight of the birds. Similar to the findings in the current study, Adams et al. (1975) did not find a significant impact of high $MgSO_4$ content (313 to 5008 ppm) in drinking water on hen body weight from 53 to 57 weeks of age.

Table 4.8. Effects of water mineral treatments and bird age on body weights of the laying hens from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control Water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ +1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ + 708 ppm $CaSO_4$	
	----------g/bird----------					
Period(wks)[1]						
Initial	1687±23	1701±23	1636±23	1705±23	1729±23	1692±10 e
33-36	1750±23	1746±23	1676±23	1751±23	1775±23	1739±10 d
37-40	1751±23	1754±23	1691±23	1756±23	1807±23	1772±10 d
41-44	1768±23	1783±23	1709±23	1775±23	1826±23	1775±10 abc
45-48	1792±23	1774±23	1719±23	1770±23	1821±23	1775±10 ab
49-52	1807±23	1787±23	1725±23	1783±23	1817±23	1784±10 a
53-56	1799±23	1778±23	1728±23	1782±23	1811±23	1780±10 a
57-60	1781±23	1794±23	1721±23	1784±23	1815±23	1779±10 a
61-64	1771±23	1757±23	1686±23	1762±23	1783±23	1752±10 cd
65-69	1775±23	1768±23	1707±23	1776±23	1750±23	1755±10 bcd
Treatment Mean	1768±20 ab	1764±20 ab	1700±20 b	1765±20 ab	1793±20 a	
P value						
Treatment	0.0433					
Period	<.0001					
Treatment × period	0.5937					

[a-e] means±SEM with different letters among treatment and period means are significantly different according to the Tukey-Kramer test (α=0.05)
[1]Period was given in weeks of age of laying hens.

4.6.1.3.2 Feed consumption

There was no interaction between water treatments and age of the birds on feed consumption of the hens (P>0.05) (Table 4.9). Daily feed intake by the hens did not change with changing water mineral levels (P>0.05). Similarly, Adams et al. (1975) did not observe a reduction in feed consumption of hens when fed $MgSO_4$ rich water up to 1252 ppm. However, as the birds aged, feed consumption declined (P<0.0001). From 33 to 40 weeks of age, feed consumption was between 120-123 g/hen/day During 61 to 69 weeks of age, feed consumption of the hens had declined to 100 to 104 g/hen/day. This was accompanied by a reduction in body weights in the last two periods (61 to 69 weeks of age).

4.6.1.3.3 Water consumption

Some water treatments had significant effect on water consumption of hens (P<0.05) (Table 4.10). Water consumption was higher for birds given the control treatment compared to low Mg, high Mg and low Mg Ca treatment groups. However, water consumption was not different for the high Mg Ca group when compared to the control group. The age of the bird had a significant effect on water consumption of hens (P<0.0001). During 57 to 69 weeks of age, water consumption was highest when compared to other periods. Water consumption of a hen ranged from 177 to186 mL per day. Leeson and Summers (2008) reported that a hen in 90% hen day production drinks 180 mL of water per day at 20°C when water is available *ad libitum*. They indicated that water consumption can increase with age of hens. Similar water consumption per bird was found during the current trial where the birds were kept at 22 - 24°C.

Table 4.9. Effects of water mineral treatments and bird age on feed consumption of laying hens from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------g/bird/day----------					
Period(wks)[1]						
33-36	120±1	119±1	119±2	120±1	122±1	120±1b
37-40	122±1	124±1	123±1	125±1	123±1	123±1 a
41-44	109±1	107±1	107±1	109±1	109±1	108±1 e
45-48	117±1	114±1	115±1	115±1	115±1	115±1 c
49-52	120±1	115±1	118±1	119±1	121±1	119±1 b
53-56	112±1	111±1	113±1	115±1	114±1	113±1 cd
57-60	112±1	110±1	110±1	115±2	112±2	112±1 d
61-64	102±1	97±1	99±1	101±2	100±2	100±1 g
65-69	103±1	103±2	105±1	105±1	103±1	104±1 f
Treatment Mean	113±1	111±1	112±1	114±1	113±1	
P value						
Treatment	0.2471					
Period	<.0001					
Treatment × period	0.5813					

[a-g] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).

[1]Period was given in weeks of age of laying hens.

Table 4.10. Effects of water mineral treatments and bird age on water consumption of laying hens from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------mL/bird/day----------					
Period(wks)[1]						
33-36	186±4	177±4	178±5	171±4	186±4	179±2 cde
37-40	179±4	173±4	169±4	174±4	182±3	175±2 def
41-44	179±3	171±3	165±3	167±3	176±3	172±1 f
45-48	181±2	169±2	172±3	171±3	176±2	174±1 ef
49-52	185±2	174±2	172±2	178±2	181±2	179±1 cd
53-56	185±2	176±2	184±2	179±2	185±2	182±1 c
57-60	196±3	183±3	186±3	189±3	191±3	189±1 ab
61-64	190±3	183±3	188±3	190±3	190±3	188±1 b
65-69	193±3	189±3	191±3	193±3	195±3	192±1 a
Treatment Mean	186±2 a	177±2 c	179±2 bc	179±2 bc	185±2 ab	
P value						
Treatment	0.0011					
Period	<.0001					
Treatment × period	0.0877					

[a-f] means±SEM with different letters among period means and treatment means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.1.3.4 Hen day egg production

Hen day egg production results were found to interact with the water treatment and age of hens ($P<0.05$) (Table 4.11). However, the Tukey-Kramer method for separation of the means was not sensitive enough to determine the cause of this relationship as it did not find a significant difference among the means. This could be occurred during the result of the analysis of a large number of data treatment combinations and since the fact that the Tukey-Kramer method is a conservative means separation method. After slicing the data into periods, interaction was found only in periods from 57 to 60 weeks and from 61 to 64 weeks of age. There was no treatment effect on hen day egg production ($P>0.05$). Egg production declined as it normally would with the increasing age of hens ($P<0.05$). It was reduced from about 97 to 90% during the 36 week trial. The expected hen day production % reported by the breeder company was 96 to 90% for this production period (Lohmann-Tierzucht 2013). Lacin et al. (2008) reported that the hen day egg production of Lohmann-Lite white laying hens declined from 89 to 80% during 24 to 68 weeks of age, which is lower than the current study.

4.6.1.3.5 Feed conversion ratio

There was no interaction between water treatments and age of the birds on feed consumption ratio (FCR) of the hens ($P>0.05$) (Table 4.12). FCR was not significantly affected by high water mineral treatments either. The feed consumed (kg) to produce 1 Kg of egg mass ranged from 1.92 to 1.94 in all treatment groups. Bird age had a significant effect on FCR ($P<0.05$). The lowest FCR was observed during 61 to 64 weeks in all water treatment groups. This was expected since the feed consumption of hens during this period was lower than any other period. FCR ranged from 1.71 to 2.09 throughout the trial.

Table 4.11. Effects of water mineral treatments and bird age on hen day egg production of laying hens from 33-69 weeks of age (trial 1)*

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------%----------					
Period(wks)[1]						
33-36	97.1±0.6	97.1±0.7	97.4±0.7	96.4±0.6	97.0±0.6	96.9±0.3
37-40	95.8±0.6	96.6±0.7	96.2±0.7	98.7±0.6	97.9±0.6	97.0±0.3
41-44	96.3±0.6	95.7±0.7	96.6±0.7	96.8±0.6	96.2±0.6	96.3±0.3
45-48	95.9±0.6	95.2±0.7	95.9±0.7	95.5±0.6	96.5±0.6	95.8±0.3
49-52	94.4±0.6	95.1±0.7	95.6±0.7	95.1±0.6	94.3±0.6	94.9±0.3
53-56	95.1±0.7	94.6±0.7	95.2±0.7	96.0±0.7	95.5±0.6	95.3±0.3
57-60	92.4±0.7	92.9±0.7	90.2±0.7	93.5±0.6	92.0±0.6	92.2±0.3
61-64	91.5±0.7	90.3±0.7	91.2±0.7	92.1±0.6	93.3±0.6	91.7±0.3
65-69	90.9±0.7	89.1±0.7	89.9±0.7	91.4±0.6	91.4±0.6	90.5±0.3
Treatment Mean	94.4±0.3	94.1±0.3	94.3±0.3	95.0±0.3	94.9±0.3	
P value						
Treatment	0.1227					
Period	<.0001					
Treatment × period	0.0117[2]					

*Means±SEM.

[1]Period was given in weeks of age of laying hens.

[2]Tukey-Kramer test did not find significant differences among least square means (α=0.05). Only period 7 (57-60 wks) and 8 (61-64 wks) had significant interaction effect ($P<0.05$) according to the slicing results of data into the periods.

Table 4.12. Effects of water mineral treatments and bird age on feed conversion ratio of laying hens from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
Period (wks)[1]						
33-36	2.10±0.03	2.10±0.03	2.05±0.04	2.09±0.03	2.08±0.03	2.09±0.01 a
37-40	2.07±0.03	2.10±0.04	2.01±0.04	2.00±0.03	1.99±0.03	2.03±0.01 ab
41-44	1.81±0.02	1.81±0.02	1.77±0.02	1.81±0.02	1.78±0.02	1.79±0.01 e
45-48	1.95±0.02	1.90±0.02	1.89±0.02	1.90±0.02	1.87±0.02	1.90±0.01 d
49-52	2.00±0.03	2.02±0.03	1.96±0.03	1.94±0.03	1.98±0.03	1.98±0.01 bc
53-56	2.02±0.04	2.19±0.04	2.03±0.04	2.09±0.04	2.04±0.04	2.08±0.02 a
57-60	1.92±0.03	1.96±0.03	1.94±0.04	1.92±0.03	1.93±0.03	1.93±0.01 cd
61-64	1.76±0.03	1.69±0.04	1.71±0.04	1.69±0.04	1.70±0.03	1.71±0.02 f
65-69	2.02±0.04	2.05±0.06	1.97±0.04	2.05±0.04	2.05±0.04	2.03±0.02 ab
Treatment Mean	1.96±0.01	1.98±0.02	1.92±0.01	1.94±0.01	1.94±0.01	
P value						
Treatment	0.0865					
Period	<.0001					
Treatment ×period	0.4534					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Since FCR was calculated using feed consumption, egg production and egg weight, changes of any factor have an influence on FCR. As water mineral levels did not affect feed consumption, egg production or egg weight of the hens in this study, FCR did not change with water treatments.

Lacin et al. (2008) reported that the FCR of Lohmann-Lite white laying hens ranged from 2.04 to 2.42 during 24 to 68 weeks of age. The egg production of these hens declined from 89 to 80% while feed consumption varied from 128 to 130 g/hen/day during the experimental period. In the current trial, the hen-day egg production ranged from 97 to 91% and the feed consumption ranged from 120 to 104 g/hen/day. So that, the lower feed consumption ratio was observed in our study than Lacin et al. (2008).

4.6.1.4 Effects of water mineral treatments on egg quality

4.6.1.4.1 Egg weight

There was no interaction between water treatments and age of the birds on egg weight ($P>0.05$) (Table 4.13). Further, egg weight was not affected significantly by water mineral treatments. The average egg weight was 62 to 63 g in all treatments. When the hen age, egg weight increases ($P<0.05$). Similarly, Roland et al. (1978) found that when hens age, egg production decreased while size of eggs become larger. The egg weight increased from 60.6 to 63.4 g during this trial. According to the Canadian grading of eggs, these eggs fall into the large egg category (56 to 64 g) (Egg Farmers of Canada 2015).

Table 4.13. Effects of water mineral treatments and bird age on egg weight of laying hens from 33-69 week of age (trial 1)*

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------g/egg----------					
Period(wks)[1]						
Initial	59.95±0.75	59.76±0.75	61.27±0.75	60.98±0.75	61.21±0.75	60.63±0.34 d
33-36	60.86±0.75	61.30±0.75	63.00±0.75	62.86±0.75	62.79±0.75	62.16±0.34 c
37-40	62.02±0.75	61.99±0.75	62.89±0.75	63.28±0.75	63.30±0.75	62.69±0.34 bc
41-44	62.40±0.75	62.45±0.75	63.44±0.75	62.37±0.75	63.41±0.75	63.61±0.34 bc
45-48	62.64±0.75	62.62±0.75	63.43±0.75	63.65±0.75	63.77±0.75	63.22±0.34 bc
49-52	63.38±0.75	61.98±0.75	63.61±0.75	63.90±0.75	63.27±0.75	63.23±0.34 bc
53-56	64.21±0.75	61.77±0.75	63.41±0.75	63.06±0.75	63.39±0.75	63.16±0.34 bc
57-60	62.83±0.75	63.27±0.75	63.18±0.75	64.41±0.75	64.32±0.75	63.60±0.34 ab
61-64	64.18±0.75	64.42±0.75	64.28±0.75	65.00±0.75	64.47±0.75	64.47±0.34 a
65-69	62.58±0.75	62.82±0.75	64.94±0.75	63.24±0.75	63.58±0.75	63.43±0.34 ab
Treatment Mean	62.51±0.52	62.24±0.52	63.25±0.52	63.28±0.52	62.35±0.52	
P value						
Treatment	0.4563					
Period	<.0001					
Treatment × period	0.6243					

[a-d] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.1.4.2 Egg specific gravity

There was no interaction between water treatments and age of the birds on egg specific gravity (SG) (P>0.05) (Table 4.14). SG values were similar among treatments (P>0.05) and ranged from 1.0836 to 1.0845 in water treatment groups. SG is an indirect measurement of shell quality, and this range of SG indicates good quality eggshells. SG changed with increasing age of hens (P<0.05). SG was highest during 33 to 36 weeks of age and thereafter decreased with the increasing hen age. SG decreased from 1.0893 during 33 to 36 weeks to 1.0787 during 65 to 69 weeks of age.

The reduction in SG is obvious since hens produce larger eggs with the age (Roland et al. 1978). Therefore, there was less shell density in eggs as the hens aged. Rate of calcium absorption is reduced when the hens get older (Al-Batshan et al. 1994). A decreased activity of 1α, 25-dihydroxyvitamin D-3 [1α, 25(OH)2 D-3] in older hens was reported (Abe et al. 1982), which is important in duodenal calcium absorption and bone calcium mobilization. Since, production of eggshell is not efficient in older hens, this could reduce the amount of shell deposited on an egg. Other than age, genetics of the birds, production rate, nutrition, disease, husbandry, environment temperature are the other major factors that govern eggshell quality (Roberts 2004). In the current study birds were housed in a controlled environment and same management practices were performed throughout the trial. Therefore, these factors would not influence on eggshell quality.

Table 4.14. Effects of water mineral treatments and bird age on egg specific gravity of laying hens from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
Period (wks)						
Initial	1.090±0.001	1.089±0.001	1.089±0.001	1.090±0.001	1.089±0.001	1.089±0.001 a
33-36	1.088±0.001	1.089±0.001	1.087±0.001	1.088±0.001	1.088±0.001	1.088±0.001 b
37-40	1.087±0.001	1.087±0.001	1.086±0.001	1.087±0.001	1.087±0.001	1.087±0.001 c
41-44	1.086±0.001	1.086±0.001	1.085±0.001	1.086±0.001	1.086±0.001	1.086±0.001 c
45-48	1.085±0.001	1.084±0.001	1.084±0.001	1.084±0.001	1.084±0.001	1.084±0.001 d
49-52	1.084±0.001	1.084±0.001	1.083±0.001	1.084±0.001	1.085±0.001	1.084±0.001 d
53-56	1.084±0.001	1.083±0.001	1.083±0.001	1.083±0.001	1.084±0.001	1.083±0.001 d
57-60	1.080±0.001	1.077±0.001	1.077±0.001	1.079±0.001	1.078±0.001	1.078±0.001 f
61-64	1.081±0.001	1.080±0.001	1.083±0.001	1.081±0.001	1.082±0.001	1.082±0.001 e
65-69	1.079±0.001	1.079±0.001	1.079±0.001	1.079±0.001	1.079±0.001	1.079±0.001 f
Treatment Mean	1.085±0.000	1.084±0.000	1.084±0.000	1.084±0.000	1.084±0.000	
P value						
Treatment	0.2352					
Period	<0.0001					
Treatment × period	0.5386					

[a-f]means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.1.4.3 Albumen height

Albumen height (AH) did not differ among treatment groups (P>0.05) (Table 4.15). There was no interaction between water treatments and age of the birds on AH of the eggs (P>0.05). However, it changed with the age of hens (P<0.05). The highest AH of 9.16 was observed in weeks 33 to 36, while lowest of 6.28 was observed during weeks 57 to 60. The lower AH during the initial analysis is unexplainable. Environmental factors and nutrition were not found to significantly affect AH (Williams 1992). Silversides and Scott (2001) also reported decrease in albumen height with the age of hens. During 25 to 59 weeks of age, albumen height dropped from 7.79 to 6.40 mm. The Haugh unit, which is a measure of egg albumen quality was higher in eggs with high albumen height (Haugh 1937).

4.6.1.4.4 Percent eggshell

Water mineral levels did not affect percent shell of eggs (P>0.05) (Table 4.16). Eggshell was about 9.7% in all treatment groups. Kaur et al. (2013) also found that the shell percent of eggs of Lohmann-Lite hens was 9.8%. With increasing bird age, percent shell was reduced significantly (P<0.05). The highest percent eggshell occurred at 33 to 36 weeks of age, thereafter it declined and the lowest value was obtained during weeks 65 to 69. Percent shell decreased from 10.12 to 9.12% during the 36 week trial. The reduction in percent shell could be due to lower amount of shell formation with compared to the increment of egg size (Roland et al. 1978). Zamani et al. (1995) found that percent shell decreased when hens got older. The authors found that the shell percentage of white leghorn hens decreased from 9.27 to 8.37% during the period of 31 to 56 weeks of age. Silversides and Scott (2001) also found reduction in eggshell percent when hens age. The percent was reduced from 10.75 to 9.52% during the period of 25 to 59 weeks of age. During the current trial, we found similar reduction in percent eggshell with the hen age.

Table 4.15. Effects of water mineral treatments and bird age on albumen height of the eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	mm					
Period(wks)[1]						
Initial	6.98±0.23	6.92±0.23	7.27±0.23	7.10±0.23	7.07±0.23	7.07±0.10 efg
33-36	9.43±0.24	9.05±0.24	8.78±0.24	9.10±0.24	9.43±0.24	9.16±0.11 a
37-40	7.13±0.12	7.12±0.12	7.02±0.12	6.95±0.12	7.05±0.12	7.05±0.06 f
41-44	7.85±0.20	7.98±0.20	8.02±0.20	7.85±0.20	8.12±0.20	7.96±0.09 b
45-48	8.00±0.13	7.50±0.13	7.70±0.13	7.93±0.13	7.72±0.13	7.77±0.06 bc
49-52	7.68±0.12	7.42±0.12	7.57±0.12	7.48±0.12	7.73±0.12	7.58±0.05 c
53-56	7.70±0.18	7.18±0.18	7.72±0.18	7.55±0.18	7.47±0.18	7.52±0.08 cd
57-60	6.27±0.12	6.32±0.12	6.40±0.12	6.18±0.12	6.23±0.12	6.28±0.06 h
61-64	7.30±0.15	7.05±0.15	7.35±0.15	7.43±0.15	7.45±0.15	7.32±0.07 de
65-69	6.83±0.13	6.78±0.13	6.77±0.13	6.70±0.13	6.87±0.13	6.79±0.06 g
Treatment Mean	7.52±0.09	7.33±0.09	7.46±0.09	7.52±0.09	7.43±0.09	7.51±0.09
P value						
Treatment	0.5986					
Period	<0.0001					
Treatment × period	0.5627					

[a-h] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Table 4.16. Effects of water mineral treatments and bird age on percent eggshell of the eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$+ 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------%----------					
Period(weeks)[1]						
Initial	10.2±0.1	10.1±0.1	10.0±0.1	10.2±0.1	10.1±0.1	10.1±0.0 a
33-36	10.2±0.1	10.1±0.1	9.8±0.1	10.1±0.1	10.0±0.1	10.0±0.0 ab
37-40	10.0±0.1	10.0±0.1	9.9±0.1	10.0±0.1	9.9±0.1	9.9±0.0 b
41-44	9.7±0.1	9.8±0.1	9.7±0.1	9.8±0.1	9.7±0.1	9.7±0.0 c
45-48	9.8±0.1	9.8±0.1	9.7±0.1	9.8±0.1	9.8±0.1	9.8±0.0 c
49-52	9.6±0.1	9.6±0.1	9.7±0.1	9.8±0.1	9.6±0.1	9.7±0.0 c
53-56	9.6±0.1	9.6±0.1	9.7±0.1	9.6±0.1	9.7±0.1	9.6±0.0 cd
57-60	9.6±0.1	9.4±0.1	9.4±0.1	9.6±0.1	9.4±0.1	9.5±0.0 de
61-64	9.4±0.1	9.3±0.1	9.5±0.1	9.5±0.1	9.5±0.1	9.4±0.0 e
65-69	9.1±0.1	9.3±0.1	9.1±0.1	9.1±0.1	9.1±0.1	9.1±0.0 f
Treatment Mean	9.7±0.0	9.7±0.0	9.7±0.0	9.8±0.0	9.7±0.0	
P value						
Treatment	0.8362					
Period	<.0001					
Treatment × period	0.7633					

[a-f] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.1.4.5 Albumen and yolk percent

Albumen percent (Table 4.17) and yolk percent (Table 4.18) were affected by water treatments ($P<0.05$). However, the Tukey-Kramer test did not find difference among treatment means for percent albumen or percent yolk. This was occurred during analysis of large number of data and since the Tukey-Kramer method is a conservative method. However, the highest albumen percent was occurred in high Mg and high Mg Ca treatments while lowest was from the control treatment. The highest yolk percent was observed in control treatment while lowest was from high Mg Ca treatment. Both albumen and yolk percent were affected by the age of hen ($P<0.05$). Percent albumen did not change consistently throughout the trial and it represented 60 to 62% of egg weight. Percent yolk showed incremental change with the age of hen ($P<0.05$). The yolk percent varied from 28 to 30% throughout the trial. Silversides and Scott (2001) reported that yolk percentage increased from 24 to 28% in white leghorn hens during the weeks 25 to 59. A normal egg could contain 60% and 30 to 33% of albumen and yolk percent respectively (Romanoff and Romanoff 1949).

4.6.1.4.6 Egg breaking strength

Egg breaking strength was not affected by high water mineral content ($P>0.05$) (Table 4.19). The breaking strength was about 5.1 to 5.3 kg force in all treatment groups. Breaking strength decreased with birds age ($P<0.0001$). The force required to break the egg was higher in weeks 33 to 36 when compared to weeks 37 to 69 ($P<0.05$). The lowest force; 4.6 kg force, was obtained at the 65 to 69 week period. Kaur et al. (2013) found that the breaking strength of eggs (61 g of average weight) from Lohmann-Lite hens was 5.16 kg force in a study that compared egg quality characteristics of different breeds of laying hens.

Table 4.17. Effects of water mineral treatments and bird age on percent albumen of the eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm $CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------%----------					
Period(wks)[1]						
Initial	61.5±0.3	61.5±0.3	62.0±0.3	61.8±0.3	62.3±0.3	61.8±0.1 a
33-36	60.8±0.4	59.7±0.4	61.4±0.4	61.2±0.4	61.6±0.4	61.0±0.2 bcde
37-40	60.7±0.3	60.9±0.3	61.0±0.3	61.1±0.3	61.2±0.3	61.0±0.1 cd
41-44	60.9±0.2	61.1±0.2	61.4±0.2	60.7±0.2	61.5±0.2	61.1±0.1 bcd
45-48	61.0±0.3	61.2±0.3	62.1±0.3	61.5±0.3	61.9±0.3	61.5±0.1 ab
49-52	60.6±0.4	61.3±0.4	61.4±0.4	60.8±0.4	61.7±0.4	61.2±0.2 bcd
53-56	60.1±0.3	60.2±0.3	60.9±0.3	60.2±0.3	60.8±0.3	60.4±0.1 e
57-60	60.2±0.2	61.0±0.2	61.3±0.2	60.4±0.2	61.3±0.2	60.8±0.1 de
61-64	60.9±0.3	60.9±0.3	61.6±0.3	61.1±0.3	61.2±0.3	61.1±0.1 bcd
65-69	61.0±0.3	61.7±0.3	61.6±0.3	61.4±0.3	61.5±0.3	61.4±0.1 abc
Treatment Mean	60.8±0.2	61.0±0.2	61.5±0.2	61.0±0.2	61.5±0.2	
P value						
Treatment	0.0312[2]					
Period	<0.0001					
Treatment × period	0.5269					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).

[1]Period was given in weeks of age of laying hens.

[2]Tukey Kramer test did not find significant difference among treatment means.

Table 4.18. Effects of water mineral treatments and bird age on percent yolk of the eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------%----------					
Period(wks)[1]						
Initial	28.2±0.3	28.4±0.3	28.0±0.3	27.9±0.3	27.6±0.3	28.0±0.1 e
33-36	29.0±0.3	30.1±0.3	28.7±0.3	28.7±0.3	28.4±0.3	29.0±0.1 cd
37-40	29.3±0.3	29.1±0.3	29.1±0.3	29.0±0.3	28.8±0.3	29.1±0.1 cd
41-44	29.4±0.3	29.1±0.3	28.9±0.3	29.5±0.3	28.8±0.3	29.1±0.1 bcd
45-48	29.2±0.3	29.0±0.3	28.2±0.3	28.7±0.3	28.3±0.3	28.7±0.1 d
49-52	29.8±0.3	29.1±0.3	28.9±0.3	29.4±0.3	28.6±0.3	29.2±0.1 bcd
53-56	30.3±0.3	30.1±0.3	29.4±0.3	30.2±0.3	29.5±0.3	29.9±0.1 a
57-60	30.2±0.3	29.5±0.3	29.3±0.3	30.0±0.3	29.3±0.3	29.7±0.1 ab
61-64	29.8±0.3	29.7±0.3	28.9±0.3	29.4±0.3	29.3±0.3	29.4±0.1 abc
65-69	29.7±0.3	29.1±0.3	29.3±0.3	29.4±0.3	29.4±0.3	29.4±0.1 abc
Treatment Mean	29.5±0.2	29.3±0.2	28.9±0.2	29.2±0.2	28.8±0.2	
P value						
Treatment	0.0432[2]					
Period	<0.0001					
Treatment × period	0.2362					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.
[2]Tukey Kramer test did not find significant difference among treatment means.

Table 4.19. Effects of water mineral treatments and bird age on breaking strength of eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm$CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------kg force----------					
Period(wks)[1]						
Initial	6.02±0.14	5.68±0.14	5.78±0.14	5.81±0.14	5.85±0.14	5.83±0.06 a
33-36	5.77±0.14	5.91±0.14	5.65±0.14	5.68±0.14	5.87±0.14	5.78±0.06 a
37-40	5.07±0.14	5.47±0.14	5.01±0.14	5.10±0.14	5.35±0.14	5.20±0.06 bcd
41-44	5.08±0.14	5.30±0.14	5.13±0.14	5.25±0.14	5.30±0.14	5.21±0.06 bcd
45-48	5.40±0.14	5.58±0.14	5.27±0.14	5.60±0.14	5.40±0.14	5.45±0.06 b
49-52	5.30±0.14	5.44±0.14	5.10±0.14	5.29±0.14	5.50±0.14	5.32±0.06 bc
53-56	5.06±0.14	5.05±0.14	5.10±0.14	5.09±0.14	4.95±0.14	5.05±0.06 cde
57-60	5.03±0.14	4.95±0.14	5.01±0.14	4.90±0.14	4.99±0.14	4.98±0.06 de
61-64	5.10±0.14	4.95±0.14	4.90±0.14	5.12±0.14	4.53±0.14	4.92±0.06 e
65-69	4.48±0.14	4.73±0.14	4.56±0.14	4.65±0.14	4.62±0.14	4.61±0.06 f
Treatment Mean	5.23±0.06	5.30±0.06	5.15±0.06	5.25±0.06	5.24±0.06	
P value						
Treatment	0.5011					
Period	<.0001					
Treatment × period	0.5129					

[a-f] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Eggshell quality declines as hens get older (Lee 1982; Garlich et al. 1984; Castaldo and Maurice 1988; Al-Batshan et al. 1994). The reduction in breaking strength at the latter part of the trial were related to the reduction in SG when the hen ages. Less efficient Ca absorption and reduced Ca deposition on egg occurred when compared to young laying hens. This could reduce the strength of eggshell. Hamilton (1982), reported that for eggshell strength, both type and association of mineral-organic materials in eggshell is important. Moreover, structural factors such as egg size, shape, distribution of shell and shell thickness are important factors that can affect shell strength (Hamilton 1982).

4.6.1.4.7 Eggshell thickness

Eggshell thickness was not affected by water treatments (P>0.05) (Table 4.20). With increasing bird age, shell thickness changed (P<0.05), but the change was not consistent. When compared to the week 33 to 36 period, thickness was lower in the periods including 45 to 48, 53 to 56 and 65 to 69. In other periods, thickness was not changed compared to first four weeks on test. Kaur et al. (2013) also found similar eggshell thickness (0.43 mm) for the Lohmann-Lite laying hens and reduced shell quality with age during the study which compared different breeds of hens. Roland et al. (1978) reported that shell materials did not produce at similar rate in which egg size increased with increasing bird age. Therefore, the amount of shell material that cover a unit area of an egg gets reduced. That could reduce the shell thickness of an egg with the age. This effect was reflected in lower specific gravity values at the end of the current trial. Hamilton (1982) reported that, there was a strong positive correlation between eggshell thickness and specific gravity based on the published data in the literature (r = 0.56 to 0.88), but the correlation for these two parameters for the current trial was r = 0.13 (P= 0.537), which indicate a low insignificant correlation between the two parameters.

Table 4.20. Effects of water mineral treatments and bird age on shell thickness of the eggs from 33-69 weeks of age (trial 1)

	Treatment					Period mean
	Control water	625 ppm $MgSO_4$	1250 ppm $MgSO_4$	625 ppm $MgSO_4$ + 1417 ppm $CaSO_4$	1250 ppm $MgSO_4$ +708 ppm $CaSO_4$	
	----------mm----------					
Period (wks)[1]						
Initial	0.477±0.006	0.439±0.006	0.449±0.006	0.449±0.006	0.442±0.006	0.445±0.002 abc
33-36	0.452±0.006	0.447±0.006	0.457±0.006	0.458±0.006	0.455±0.006	0.454±0.002 a
37-40	0.441±0.006	0.445±0.006	0.446±0.006	0.453±0.006	0.450±0.006	0.447±0.002 abc
41-44	0.446±0.006	0.450±0.006	0.453±0.006	0.455±0.006	0.459±0.006	0.453±0.002 ab
45-48	0.435±0.006	0.444±0.006	0.443±0.006	0.442±0.006	0.441±0.006	0.441±0.002 cd
49-52	0.442±0.006	0.449±0.006	0.456±0.006	0.453±0.006	0.448±0.006	0.449±0.002 abc
53-56	0.456±0.006	0.435±0.006	0.442±0.006	0.439±0.006	0.439±0.006	0.442±0.002 bc
57-60	0.419±0.006	0.425±0.006	0.428±0.006	0.436±0.006	0.443±0.006	0.430±0.002 de
61-64	0.442±0.006	0.436±0.006	0.444±0.006	0.433±0.006	0.434±0.006	0.438±0.002 cd
65-69	0.426±0.006	0.423±0.006	0.430±0.006	0.431±0.006	0.421±0.006	0.426±0.002 e
Treatment Mean	0.441±0.003	0.439±0.003	0.445±0.003	0.445±0.003	0.443±0.003	
P value						
Treatment	0.7142					
Period	<.0001					
Treatment × period	0.8027					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.1.5 Effects of water mineral treatments on bone quality

Humerus and tibia bones collected from 70 week old hens (group A) were subjected to QCT analysis followed by breaking strength analysis. Humerus and tibia bones from other hens (group B) were used to measure ash percentage.

4.6.1.5.1 QCT analysis

4.6.1.5.1.1 Humerus

Length, width or length of humerus bones did not change with water treatments (P>0.05) (Table 4.21). The weight ranged from 5.8 to 7 g while length varied from 7.2 to 7.6 cm in all groups. The force required to break the bone at the midpoint did not differ among treatment groups (P>0.05). Fleming et al.(1998) reported that humerus breaking strength in laying hens at 70 weeks of age was about 12.1 kg force. Those birds were given a standard diet with 3.5% Ca, compared to the breaking force which was from 12.2 to 14.8 kg force when hens were provided with 4% Ca in the diet plus the Ca in the water in the current study. Area, density and mineral content of total bone, cortical and trabecular bones were evaluated using QCT (Saunders-Blades et al. 2009). Total, cortical or trabecular area measurements did not differ among treatment groups (P>0.05) (Table 4.22). Trabecular area was about two times greater at the end of the humerus bone compared to the middle. Cortical area was more or less similar at the middle and end. The total area of the humeri bones was high at the 30% position. The total, cortical and trabecular bones areas at the midpoint of humerus of hens at 70 weeks of age ranged from 31 to 33, 9 to 11, and 21 to 24 mm^2, respectively, which were in similar to the findings by Jendral et al. (2008). The authors found that the total, cortical and trabecular area at the midpoint of the humerus of white leghorn laying hens at 65 weeks of age were 39.91, 9.30 and 28.80 mm^2, respectively.

Table 4.21. The effect of water mineral treatments on morphology and breaking strength of humerus bones in laying hens at 70 weeks of age used for density analysis by QCT[1] (trial 1)

	Bone morphological measurement[2]			Bone breaking measurement[2]		
	Weight (g)	Length (cm)	Width (mm)	Breaking strength (kg force)	Gradient (kg/sec)	Area (kg.sec)
Treatment						
Control	6.3±0.3[2]	7.5±0.1	5.8±0.1	14.8±0.8	2.7±0.2	37.1±3.3
625 ppm $MgSO_4$	6.5±0.3	7.4±0.1	5.8±0.1	12.9±0.8	2.9±0.2	26.7±3.3
1250 ppm $MgSO_4$	6.2±0.3	7.4±0.1	5.7±0.1	14.2±0.8	2.8±0.2	31.3±3.3
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	5.8±0.3	7.2±0.1	5.7±0.1	13.7±0.8	2.4±0.2	31.3±3.3
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	7.0±0.3	7.6±0.1	5.9±0.1	12.2±0.8	2.4±0.2	31.1±3.0
ANOVA P value	0.1164	0.1915	0.4669	0.1825	0.3928	0.3087

[1]QCT=Quantitative Computed Tomography.
[2]Means±SEM for 6 observations.

Table 4.22. The effect of water mineral treatments on bone area of humerus bones in laying hens at 70 weeks of age measured by QCT[1] (trial 1)

	Total area (mm^2)		Cortical area (mm^2)		Trabecular area (mm^2)	
Scan position[2]	30%	50%	30%	50%	30%	50%
Treatment						
Control	53.60±1.73[3]	31.69±0.91	12.44±0.49	10.37±0.40	40.62±1.63	21.65±0.83
625 ppm $MgSO_4$	56.75±1.73	33.29±0.91	11.73±0.49	9.77±0.40	43.51±1.63	23.73±0.83
1250 ppm $MgSO_4$	54.11±1.73	32.07±0.91	11.70±0.49	10.06±0.40	41.88±1.63	22.30±0.83
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	55.33±1.73	32.80±0.91	13.04±0.49	10.69±0.40	41.88±1.63	22.27±0.83
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	55.58±1.73	32.95±0.91	11.37±0.49	9.65±0.40	43.47±1.63	23.66±0.83
ANOVA P value	0.7241	0.7160	0.1260	0.3594	0.6924	0.3223

[1]QCT=Quantitative Computed Tomography.
[2]Scan positions; 50% =at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end.
[3]Means±SEM for 6 observations per treatment.

Cortical and trabecular bones were separated using the density values of 500 and 400 mg/cm^3, respectively (Korver et al. 2004). Medullary bone content cannot be differentiated using this technique. Since medullary bone is associated with trabecular space of bones, it was speculated that any changes in trabecular bone measurements reflected medullary bone changes (Riczu et al. 2004). Humerus, a hollow bone generally does not contain medullary bone. In some instances, some birds can have medullary bone in their humerus (Korver et al. 2004). There was only one bone that had trabecular density at the 30% scanning position out of 30 humerus bones analysed. However, cross sectional analysis at the midpoint (50% scanning) found in 3 bones which had trabecular densities. These data were not statistically analysed. Therefore, for mineral density of humerus bones, only total and cortical density measurements are discussed (Table. 4.23).

There were no differences for total or cortical densities of humeri bones among any water treatments (P>0.05). However, the total mineral density at the 30% scanning position had marginal effect of water treatment, where highest total mineral density was observed in control water treatment while lowest was from low Mg treatment. Mineral density of total and cortical bones were higher at the midpoint cross section than at the 30% scanning position taken, closer to the proximal end of bone in all treatments. Therefore, minerals were more densely distributed at the middle of the humeri. However, total and cortical mineral contents were not different (P>0.05) (Table 4.24). The total and cortical mineral content close to proximal end and at the midpoint were similar. Differences in the mineral content closer to proximal end were approaching significant differences (P=0.052) in some of the water treatments. The highest mineral content was observed from control water treatment while the lowest from low Mg treatment.

Table 4.23. The effect of water mineral treatments on total and cortical density of humerus bones in laying hens at 70 weeks of age measured by QCT[1] (trial 1)

	Total density (mg/cm^3)		Cortical density (mg/cm^3)	
Scan position[2]	30%	50%	30%	50%
Control	181.03±18.86[3]	309.13±28.17	1000.68±16.14	1157.80±13.79
625 ppm $MgSO_4$	120.73±18.86	231.02±28.17	994.00±16.14	1136.77±13.79
1250 ppm $MgSO_4$	129.73±18.86	285.22±28.17	1012.98±16.14	1155.12±13.79
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	174.27±18.86	281.20±28.17	999.08±16.14	1144.78±13.79
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	124.88±18.86	235.27±28.17	1008.82±16.14	1133.87±13.79
ANOVA P value	0.0796	0.2474	0.9215	0.6665

[1]QCT=Quantitative Computed Tomography.
[2]Scan positions; 50% = at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end.
[3]Means±SEM for 6 observations per treatment.

Table 4.24. The effect of water mineral treatments on total and cortical bone mineral content of humerus in laying hens at 70 weeks of age calculated using QCT measurements (trial 1)

	Total mineral content[1] (mg/mm)		Cortical mineral content[2] (mg/mm)	
Scan position[2]	30%	50%	30%	50%
Treatment				
Control	9.63±0.92[3]	9.32±1.11	12.47±0.57	12.01±0.46
625 ppm $MgSO_4$	6.71±0.92	7.65±1.11	11.67±0.57	11.12±0.46
1250 ppm $MgSO_4$	6.92±0.92	8.79±1.11	11.87±0.57	12.16±0.51
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	9.55±0.92	9.07±1.11	13.03±0.57	12.23±0.46
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	6.84±0.92	7.57±1.11	11.47±0.57	10.95±0.46
ANOVA P value	0.0516	0.4769	0.3119	0.1858

[1]Bone mineral content was calculated by multiplying the area and density measurements for each bone type measured at 30 % or 50 % of the total length of bone by QCT.
[2]Scan positions; 50% = at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end.
[3]Means±SEM for 6 observations per treatment.

4.6.1.5.1.2 Tibia

The area measurement of total, cortical or trabecular bones did not differ among treatments (P>0.05) (Table 4.25). Saunders-Blades et al. (2009) reported that total, cortical and trabecular area of tibia bones of DeKalb laying hens at 74 weeks of age was 35, 18 and 15, respectively. There was no much difference between 30% and 50% area measurements of tibia bones unlike humerus. Trabecular bone area was reasonably similar at the proximal end and the middle.

The mineral density of total, cortical or trabecular bones at both 30 and 50% scanning positions were not different among treatment groups (P>0.05) (Table 4.26). These values were consistent with what others have found for similar birds of similar age. Saunders-Blades et al. (2009) found that total, cortical and trabecular densities were about 626, 1034 and 160 mg/cm^3 respectively at the midpoint of tibia of laying hens at 74 weeks of age. In our study, we observed relatively higher densities than Saunders-Blades et al. (2009), which could be due to the difference of commercial lines used in the two studies.

Mineral content of total, cortical or trabecular bones were not affected by water treatments (P>0.05) (Table 4.27). At the 30% scanning position, the bone mineral content was slightly higher than at the midpoint of the tibia. That could be due to the slightly higher bone area measurements obtained at the 30% length cross section analysis. According to the Saunders-Blades et al. (2009), these measurements were about 23, 20 and 2 mg/mm respectively at the midpoint of tibia bones. Mineral content of a 1 mm longitudinal slice of total, cortical and trabecular bone was calculated by multiplying density and area measurement of each bone type (Saunders-Blades et al. 2009).

Table 4.25. The effect of water mineral treatments on total, cortical and trabecular area of tibia bones in laying hens at 70 weeks of age measured by QCT[1] (trial 1)

	Total area (mm^2)		Cortical area (mm^2)		Trabecular area (mm^2)	
Scan position[2]	30%	50%	30%	50%	30%	50%
Control	41.43±0.99[3]	32.54±1.16	18.10±0.65	14.43±0.64	22.91±0.85	17.95±1.36
625 ppm $MgSO_4$	39.40±0.99	33.24±1.16	17.44±0.65	14.05±0.64	21.30±0.85	16.73±1.49
1250 ppm $MgSO_4$	38.37±0.99	32.56±1.16	17.78±0.59	14.56±0.59	20.50±0.78	18.02±1.34
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	40.18±0.99	34.07±1.16	17.78±0.65	14.54±0.64	22.32±0.85	18.50±1.49
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	41.73±0.99	33.49±1.16	18.52±0.59	15.24±0.64	22.50±0.78	16.70±1.36
ANOVA P value	0.1226	0.8690	0.7823	0.2247	0.2379	0.8565

[1]QCT=Quantitative Computed Tomography.
[2]Scan positions; 50% = at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end.
[3]Means±SEM for 6 or 5 observations per treatment.

Table 4.26. The effect of water mineral treatments on total, cortical and trabecular mineral density of tibia bones in laying hens at 70 weeks of age measured by QCT[1] (trial 1)

	Total density (mg/cm^3)		Cortical density (mg/cm^3)		Trabecular density (mg/cm^3)	
Scan position[3]	30%	50%	30%	50%	30%	50%
Treatment						
Control	606.82±26.25[2]	669.43±38.93	1063.58±16.83	1211.38±18.55	200.90±14.28	249.40±16.96
625 ppm $MgSO_4$	633.43±26.25	689.50±38.93	1092.67±16.83	1166.43±18.55	226.13±14.28	221.42±16.96
1250 ppm $MgSO_4$	638.93±26.25	667.15±38.93	1121.60±16.83	1217.60±18.55	229.60±14.28	222.15±16.96
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	630.62±26.25	710.63±38.93	1089.48±16.83	1187.85±18.55	201.57±14.28	196.73±16.96
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	595.78±26.25	675.87±38.93	1103.57±16.83	1214.96±20.32	200.73±14.28	208.23±16.96
ANOVA P value	0.7288	0.9288	0.2082	0.4451	0.3949	0.2818

[1]QCT=Quantitative Computed Tomography.
[2]Means±SEM for 6 or 5 observations per treatment.
[3]Scan positions; 50% =at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end.

Table 4.27. The effect of water mineral treatments on total, cortical and trabecular mineral content of tibia bones in laying hens at 70 weeks of age calculated using QCT measurements (trial 1)

	Total mineral content[2] (mg/mm)		Cortical mineral content[2] (mg/mm)		Trabecular mineral content[2] (mg/mm)	
			Scan position[3]			
	30%	50%	30%	50%	30%	50%
Control	25.14±1.11[4]	21.75±0.81	19.68±0.81	17.36±1.14	4.28±0.42	4.44±0.35
625 ppm $MgSO_4$	24.99±1.11	19.77±0.99	19.14±0.81	19.36±1.14	4.41±0.42	3.18±0.35
1250 ppm $MgSO_4$	24.50±1.11	21.63±0.81	19.94±0.74	17.99±1.14	4.70±0.42	3.93±0.35
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	23.95±1.22	22.47±0.88	19.58±0.81	18.71±1.25	4.03±0.42	3.78±0.38
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	24.93±1.11	21.56±0.88	20.42±0.74	17.69±1.25	4.52±0.42	3.56±0.35
ANOVA P value	0.9938	0.7604	0.8249	0.7439	0.8376	0.1630

[1]QCT=Quantitative computed tomography.
[2] Bone mineral content was calculated by multiplying the area and density measurements for each bone type measured at 30 % or 50 % of the total length of tibia bone by QCT.
[3]Scan positions; 50% =at the midpoint of the bone; 30% = at the 30% of the total length of bone from the proximal end. The mineral contents at 30 % and 50% positions were calculated using density and area measurements at these locations of the bones measured by QCT.
[4]Means±SEM for 6 or 5 observations per treatment.

Tibia bones are long bones which act as calcium stores for hens. Medullary bone, which is located in the trabecular space of long bones is a readily available calcium source of laying hens (Korver et al. 2004). Therefore, any changes in trabecular space would be considered as changes in medullary bones. Since there were not significant differences in trabecular area, density or mineral content of tibia, water mineral levels did not affect tibia medullary bone measures in laying hens.

Supporting the QCT analysis results, morphological measurements and breaking strength of tibia bones were not different among treatment groups (P>0.05) (Table 4.28). Since there were no differences among cortical bone mineral density, it would suggest that Ca requirement for eggshell formation was maintained only at the expense of medullary bones in all treatments. Therefore, the high Mg, Ca or SO_4 did not negatively affect bone mineral homeostasis. Tibia length, width or weight were similar among groups. The force required to break the bones at their midpoint was not different among the groups (P>0.05). This was also expected since cortical or trabecular bone densities were not different among treatments. Therefore, high Ca, Mg and SO_4 in water did not have a negative effect on bone quality measurements. Fleming et al. (1998) found that tibia breaking strength of laying hens at 70 weeks of age was 23.6 kg force which was similar to the current findings. The unaffected mineral retention of essential minerals including Ca and P at 70 weeks of age would support these results. The balance study conducted at the end of the mineral trial 1 at 70 weeks of age before the bone collection. If negative Ca balance occurred, bone Ca reserves would mobilize and compensate the acute deficiency (Whitehead 2004) and bone mineral density would get reduced. Therefore, unaffected bone mineral density and mineral content in bones fit with the mineral balance study findings at 70 weeks of age of the hens.

Table 4.28. The effect of water mineral treatments on morphology and breaking strength of tibia bones in laying hens at 70 weeks of age used for density analysis by QCT[1] (trial 1)

	Bone morphological measurement			Bone breaking measurement		
	Weight (g)	Length (cm)	Width (mm)	Breaking force (kg)	Gradient (kg/sec)	Area (kg.sec)
Treatment						
Control	12.8±0.6[2]	11.2±0.1	6.1±0.1	23.1±2.0	6.9±0.4	41.1±5.9
625 ppm $MgSO_4$	12.8±0.6	11.2±0.1	6.0±0.1	24.1±2.0	6.0±0.4	50.9±5.9
1250 ppm $MgSO_4$	12.4±0.6	11.2±0.1	5.9±0.1	24.9±2.0	6.9±0.4	46.5±5.9
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	13.1±0.6	11.3±0.1	6.1±0.1	25.7±2.0	6.5±0.4	54.5±5.9
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	13.7±0.6	11.3±0.1	6.2±0.1	26.3±2.2	6.7±0.4	50.9±6.4
ANOVA P value	0.6806	0.7229	0.1252	0.8365	0.4497	0.5610

[1]QCT=Quantitative Computed Tomography.
[2]Means±SEM for 6 or 5 observations per treatment.

4.6.1.5.2 Percent ash determination of tibia and humerus bones

Tibia and humerus bones from group B collected from hens at 70 weeks of age were used for the ash determination. The morphological measurements including length, weight and width and breaking strength were measured prior to ash determination.

Similar to the bones used for density analysis by QCT, the width, length or weight of the tibia and humerus bones did not differ among the treatments for these bones ($P>0.05$) (Table 4.29). Bone breaking strength showed the same lack of effects as previously demonstrated for humerus and tibia bones. Breaking strength was similar in all treatments ($P>0.05$). However, when compared to previously measured humeri in density analysis group, this group of humeri had higher breaking strength values. The same effect was found for tibia bones. The reason could be related to the freshness of the bones used for performing the breaking test. The breaking strength analysis for this group bones was performed on fresh bones once they were cleaned of adhering tissues and morphological measures were taken in same day. However, for previously analysed bones (density analysis group), breaking strength was performed after the density analysis by QCT. Bones were sent to University of Alberta for density analysis and returned to Nova Scotia for breaking strength analysis. During that period bones can lose moisture. And that could affect breaking strength of bones. Kim et al. (2004) found that the breaking strength of dry bones were significantly lower when compared to fresh bones.

Ash percentage of humerus (Table 4.29) or tibia (Table 4.30) bones was not significantly different among treatment groups ($P>0.05$). The ash percentage of fat free dry humerus was 60 to 61% compared to 52 to 54 % in fat free tibia. Similarly, Hess and Britton (1997) found that percent ash in dry defatted tibia of white leghorn laying hens at 65 weeks was 54%.

Table 4.29. The effect of water mineral treatments on ash percentage and other bone quality measurements of humerus bones from laying hens at 70 weeks of age (trial 1)

	Bone quality measurement[1]							
	Wet weight[2] (g)	Length (cm)	Width (mm)	Breaking Strength (kg force)	Gradient (Kg/sec)	Area (kg.sec)	Defatted weight[3] (g)	Ash [4] (%)
Treatment								
Control	3.2±0.2	7.2±0.1	5.8±0.1	16.3±1.2	2.8±0.4	42.4±2.6	2.1±0.1	60.9±0.8
625 ppm $MgSO_4$	3.1±0.2	7.1±0.1	5.7±0.1	15.2±1.3	3.0±0.4	43.5±2.9	2.0±0.1	60.3±0.9
1250 ppm $MgSO_4$	2.9±0.2	7.1±0.1	5.8±0.1	15.9±1.3	3.9±0.4	40.5±2.9	1.9±0.1	61.2±0.8
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	3.0±0.2	7.1±0.1	5.9±0.1	15.3±1.2	2.6±0.4	45.5±2.9	1.9±0.1	61.5±0.8
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	3.3±0.2	7.3±0.1	5.9±0.1	16.8±1.2	3.9±0.4	44.4±2.6	2.1±0.1	61.3±0.8
ANOVA P value	0.5610	0.5774	0.7136	0.8758	0.0985	0.7669	0.1871	0.8872

[1]Means±SEM for 6 or 5 observations per treatment.
[2] Fresh weight of the bone after removing adhering tissues.
[3]Weight after defatting and drying.
[4] percent ash in defatted dried bone.

Table 4.30. The effect of water mineral treatments on bone quality measures of tibia bones used in ash determination of laying hens at 70 weeks of age (trial 1)

Treatment	Bone quality measurement[1]							
	Wet weight[2] (g)	Length (cm)	Width (mm)	Breaking Strength (kg force)	Gradient (Kg/sec)	Area (kg.sec)	Defatted weight[3] (g)	Ash[4] (%)
Control	9.1±0.3	11.4±0.2	6.2±0.1	30.5±2.1	6.2±1.2	6.0±5.7	5.2±0.3	54.3±1.0
625 ppm $MgSO_4$	8.7±0.3	11.4±0.2	6.2±0.1	26.8±2.1	6.0±1.2	6.1±5.7	4.9±0.3	53.6±1.0
1250 ppm $MgSO_4$	8.5±0.3	11.4±0.2	6.0±0.1	28.5±2.1	5.3±1.2	6.2±5.7	4.8±0.3	54.0±1.0
625 ppm $MgSO_4$ plus 1416 ppm $CaSO_4$	8.7±0.3	11.5±0.2	5.9±0.1	27.0±2.1	4.6±1.2	5.5±5.7	5.1±0.3	52.4±1.0
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	9.1±0.3	11.6±0.2	6.0±0.1	33.6±2.1	7.1±1.2	7.0±5.7	5.5±0.3	52.4±1.0
ANOVA P value	0.6910	0.9225	0.1325	0.1458	0.6746	0.4424	0.3289	0.4973

[1]Means±SEM for 6 observations per treatment.
[2] Fresh weight of the bone after removing adhering tissues.
[3]Weight after defatting and drying.
[4]percent ash in defatted dried bone.

4.6.2 Trial 2

This trial evaluated the effect of high Ca, Mg and SO_4 in drinking water of laying hens provided from early pullet stage (7 weeks of age) to 46 weeks of age representing the post peak production.

4.6.2.1 Diet analysis

The changes in diets from pullet stage to laying phase (Table 4.31) indicated analysed protein levels were equal or exceed the calculated values. Calcium percentages were similar to the calculated percentages in grower and developer diets. In pre-layer and layer diets analysed percent Ca exceeded the calculated concentration. The analysed contents of all nutrients met or exceeded the recommendations by the breeder company (Lohmann Tierzucht 2013) except Mn in developer diet. The recommendation was 100 ppm while analysed value was 89 ppm. However, this concentration surpassed the NRC (1994) recommendation of Mn for developer bird (30 ppm).

Pre-layer diet was given at 17 weeks of age when the body weight of 1200 g per bird was achieved according to the breeder recommendations (Lohmann Tierzucht 2013). Pre layer diet was fed 2 weeks then from 19 weeks onwards layer phase I diet was fed. Developer diet was formulated to be low in Ca and protein contents when compared to pre-layer diet. A pre-layer diet should contain 2 to 2.5% calcium (Lohmann Tierzucht 2013). The supply of pre-layer diet is important to improve flock uniformity and development of medullary bones, which is important in egg production during the egg laying stage. Layer phase I diet was formulated to have high Ca content to fulfill the requirement during egg production. The analyzed contents of the all nutrients exceeded the NRC (1994) recommendations for birds at any production stage.

Table 4.31. Analysed nutrient composition of diets fed in different phases of laying hen production cycle from 7-46 weeks of age in water mineral trial 2[1]

Nutrient	Phase of feeding			
	Grower (7- 8 wks)	Developer (9-16 wks)	Pre layer (17-18wks)	Layer Phase I (19-46 wks)
Protein (%)	19±0.1	18±0.1	20±0.1	22±0.1
Fat (%)	4±0.1	7±0.1	5±0.1	9±0.1
Calcium (%)	0.9±0.1	0.8±0.1	2.4±0.1	4.9±0.1
Potassium (%)	0.9±0.1	0.7±0.0	0.8±0.0	0.9±0.1
Magnesium (%)	0.2±0.0	0.3±0.1	0.2±0.0	0.3±0.1
Phosphorus (total) (%)	0.6±0.0	0.7±0.0	0.8±0.0	0.6±0.0
Sodium (%)	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Copper (ppm)	43±3	29±2	32±2	29±3
Manganese (ppm)	105±6	89±4	121±3	122±5
Zinc (ppm)	133±5	107±3	115±2	106±3

[1]Diets were formulated to meet or exceed the breeder company recommendation (Lohmann Tierzucht, 2013). n=2.

4.6.2.2 Water analysis

Water composition during pullet (Table 4.32) and laying stages (Table 4.33) were averaged within the phases. The pH of the water measured between 7.8 to 8.0. The conductivity of water samples increased with increasing mineral addition. The expected concentrations for two $CaSO_4$ treatments was not obtained because of poor solubility of $CaSO_4$ at higher concentration at room temperature for the both pullet and laying stages. The analyzed TDS was lower than the calculated TDS content in $CaSO_4$ treatments for the both production stages. The calculate TDS were 2525 and 3025 ppm, respectively, for low Ca and high Ca treatments for both the pullet stage. The reduction of analyzed TDS were 0.6% and 9% in low Ca and high Ca treatments, respectively, compared to calculated TDS for the pullet stage while 0.9% and 7 % reduction occurred in low and high Ca treatments for laying stage. However, similar analyzed TDS content to the calculated TDS content for $MgSO_4$ treatments were obtained. $MgSO_4$ was readily soluble in water at the room temperature.

The calculated TDS content for low Mg (2100 ppm) and high Mg (2600 ppm) were 2525 ppm and 3025 ppm, respectively. The concentrations of other minerals in water treatments were similar among treatments.

Table 4.32. The average composition of pH, mineral ions and other quality parameters of the water treatments used in pullet phase (7-18 weeks), mineral study (trial 2)[1]

	Water Treatment				
	Control	Low Mg	High Mg	Low Ca	High Ca
Parameter[2]					
pH	7.91±0.10	7.87±0.11	7.91±0.10	7.99±0.00	7.88±0.10
Conductivity	594±132	3022±286	3504±368	2618±42	2679±95
Chloride	83±39	89±39	88±28	77±21	85±24
Alkalinity	126±8	139±5	140±6	137±5	134±5
Nitrate/Nitrite-N	3±1	3±1	3±1	3±0	3±0
Hardness	190±82	2039±235	2389±374	1938±240	1991±189
Calcium	59±31	50±19	57±19	713±98	786±78
Magnesium	10±1	465±53	562±64	8±2	8±1
Sulphate	34±3	1681±223	1988±270	1435±121	1500±140
Sodium	44±10	49±7	47±6	55±9	70±42
Potassium	ND	ND	ND	ND	ND
Copper	0.09±0.01	0.10±0.05	0.10±0.01	0.07±0.03	0.06±0.02
Iron	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Zinc	0.04±0.01	0.02±0.00	0.02±0.01	0.01±0.00	0.02±0.01
Manganese	ND	ND	ND	ND	ND
TDS[3]	425±90	2592±82	3132±178	2506±57	2764±59

[1]Means±SEM of 4 water analysis results during pullet stage.
[2]pH measured in pH units. Conductivity was measured in µmhos/cm. Mineral ion concentrations were reported in ppm. Alkalinity and hardness were reported in ppm as $CaCO_3$.
ND indicates the concentrations below the detectable limit of the analysis method.
[3]TDS=Total Dissolved Solids (ppm), analysed by drying at 180°C.

Table 4.33. The average composition of pH, mineral ions and other quality parameters of the water treatments used in laying stage (19-46 weeks), mineral study (trial 2)[1]

	Treatments				
	Control	Low Mg	High Mg	Low Ca	High Ca
Parameter[2]					
pH	7.91±0.14	7.90±0.11	7.92±0.1	7.94±0.09	7.93±0.08
Conductivity	662±129	2910±324	3293±374	2551±65	2629±25
Chloride	103±39	70±24	90±23	66±22	71±23
Alkalinity	125±6	141±7	141±7	137±3	138±6
Nitrate/Nitrite	3±1	3±1	3±0	3±1	3±0
Hardness	224±69	1933±272	2237±325	1807±226	1862±189
Calcium	72±26	43±16	57±16	710±91	732±77
Magnesium	11±1	443±58	508±35	8±1	8±1
Sulphate	35±2	1606±222	1818±121	1386±291	1416±102
Sodium	42±8	51±6	47±7	55±6	55±8
Copper	0.19±0.01	0.09±0.01	0.09±0.02	0.05±0.02	0.06±0.03
Iron	0.05±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Manganese	0.01±0.00	ND	ND	ND	ND
Potassium	ND	ND	ND	ND	ND
Zinc	0.04±0.01	0.02±0.00	0.02±0.00	0.01±0.00	0.02±0.00
TDS[3]	343±45	2558±65	3106±138	2421±44	2731±56

[1]Means±SEM of 7 water analysis results during laying stage.
[2]pH measured in pH units. Conductivity was measured in µmhos/cm. Mineral ion concentrations were reported in ppm. Alkalinity and hardness were reported in ppm as $CaCO_3$.
ND indicates the concentrations below the detectable limit of the analysis method.
[3]TDS=Total Dissolved Solids (ppm), analysed by drying at 180°C.

4.6.2.3 Effects of water mineral treatments on production performance

4.6.2.3.1 Pullet grower phase (7 to 14 weeks of age)

4.6.2.3.1.1 Feed consumption

During 7 to 14 weeks of age pullet growers did not have differences in feed consumption among water treatments (Table 4.34). Average feed per bird was 62±1 to 65±1 g per day. Feed consumption increased by 22% in the second period concurrent with the growth of

pullets. There was no interaction between water mineral treatment and bird age on feed consumption from 7 to 14 weeks of age of the birds.

Table 4.34. Feed consumption of pullets from 7-14 weeks of age fed different waters with different mineral content (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------g/bird/day----------					
Period(wks)[1]						
7-10	56±1	57±1	58±1	58±1	56±1	57±0 b
11-14	69±1	69±1	72±1	71±1	70±1	70±0 a
Treatment mean	62±1	63±1	65±1	64±1	63±1	
P value						
Treatment		0.2630				
Period		<0.0001				
Treatment × period		0.1522				

[a-b]means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.3.1.2 Water consumption

There was significant effect of water treatment on water consumption of pullet growers during the period from 7 to 14 weeks (P<0.05) (Table 4.35). Water consumption of high Mg treatment was lower compared to the low Mg and low Ca treatments. Adams et al. (1975) found similar effect of high $MgSO_4$ in water on water consumption of laying hens. Water consumption declined significantly when $MgSO_4$ level increased up to 1000 ppm in water during their study. The reason for low intake of water could be due to the high TDS content in high Mg treatment. The TDS was more than 3000 ppm in this treatment, which is the suggested desirable limit for poultry drinking water. The TDS levels of low Mg and

low Ca were lower than 3000 ppm. There was no difference in water consumption between the high Ca treatment, which contained 2764 ppm TDS, and high Mg treatment.

Birds drank 21% more water during the 11 to 14 week period compared to the 7 to 10 week period. The incremental increase in water consumption was similar to the increase in feed consumption during 11 to 14 weeks compared to 7 to 10 weeks of age. Feed: water ratio during these production periods was 1:1.3 for pullets.

Table 4.35. The effect of water mineral treatments on water consumption of pullet growers from 7-14 weeks of age (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------mL/bird/day----------					
Period[1]						
7-10	79±2	76±2	71±2	77±2	77±2	76±1 b
11-14	88±2	94±2	89±2	93±2	91±2	91±1 a
Treatment mean	83±1 ab	85±1 a	80±1 b	85±1 a	84±1 ab	
P value						
Treatment		0.0209				
Period		<0.0001				
Treatment × period		0.2146				

[a-b] means±SEM with different letters among period means and treatment means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.3.1.3 Body weight

Body weights of the birds at 7 to 14 weeks age, did not differ among water treatments (P>0.05) (Table 4.36). With increasing age, birds weights increased (P<0.05). Body weights were almost double at the 14th week compared to 7th week. There was no treatment

by bird age interaction on body weights. Adams et al. (1975) found that high $MgSO_4$ in water up to 4000 ppm did not affect body weights of laying hens.

Table 4.36. Body weights of pullets from 7 to 14 weeks of age fed waters with different mineral contents (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------g/bird----------					
Period[1]						
Initial	504±18	491±18	527±18	479±18	495±18	499±8 d
7-8	749±18	723±18	716±18	749±18	761±18	739±8 c
9-10	911±18	922±18	944±18	907±18	952±18	927±8 b
11-14	1023±18	1041±18	1046±18	1045±18	1030±18	1037±8 a
Treatment mean	797±7	794±7	808±7	795±7	809±7	
P value						
Treatment		0.4734				
Period		<0.0001				
Treatment × period		0.5448				

[a-d] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.3.2 Pullet developer phase (15 to 18 weeks of age)

4.6.2.3.2.1 Feed consumption

Feed consumption was reduced in high Mg treatment during the period of 15 to 18 weeks of age (P<0.05) (Table 4.37). The feed consumption of other groups were not different (P>0.05). Therefore, high Mg content (562 ppm) in water negatively affect feed intake of birds at developer phase. However, Adams et al. (1975) did not find a reduction in feed consumption in hens at 53 weeks of age given 1000 ppm $MgSO_4$ in their drinking water, but at 4000 ppm $MgSO_4$. In this study, pullet developer birds received 2600 ppm $MgSO_4$

in the water. Young birds may more sensitive to high mineral content in their drinking water than older birds (NRC 1974).

4.6.2.3.2.2 Water consumption

Water consumption did not differ among treatments (P>0.05) (Table 4.37). Average daily consumption per bird ranged from 83 to 96 mL/day. Feed: water ratio was about 1: 1.1 during this period. Although importance of water is discussed for laying hens in the literature, information on daily water consumption of birds is limited. Leeson and Summers (2008) only compared water intake of laying hens at high and low temperature. Generally it is assumed that a bird consumes as much as twice the water as feed on a weight basis at higher temperatures (35ºC vs 22ºC) (Leeson and Summers 2008).

Table 4.37. The effect of water mineral treatments on feed and water consumption of pullet birds from 15-18 weeks age (trial 2)

	Feed consumption	Water consumption
	g/bird/day	mL/bird/day
Treatment		
Control	79±2 a	89±3
Low Mg	82±2 a	88±3
High Mg	72±2 b	89±3
Low Ca	80±2 a	84±3
High Ca	80±2 a	96±3
P value		
Treatment	0.0137	0.1257

[a-b]means±SEM with different letters among feed consumption means are significantly different according to the Tukey-Kramer test (α=0.05).

4.6.2.3.2.3 Body weight

There was no interaction between water treatment and period on body weight of the birds from 15-18 weeks of age (P>0.05) (Table 4.38). Water treatments alone did not affect the

body weights either (P>0.05). Even though feed consumption was low in high Mg group during this period, body weight was not affected. Body weights increased with age of pullets (P<0.05). At the end of the pullet stage, average weight per bird was 1219 g in all treatment groups, which was similar to expected body weight recommended indicated by the breeder company (Lohmann Tierzucht 2013).

Table 4.38. The effect of water mineral treatments on body weights of pullet growers from 15-18 weeks age (trial 2)

Item	Treatment					Period Mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------g/bird----------					
Period[1]						
Initial	1023±12	1041±12	1046±12	1045±12	1030±12	1037±6 c
15-16	1165±12	1157±12	1161±12	1116±12	1152±12	1150±6 b
17-18	1216±12	1220±12	1221±12	1209±12	1229±12	1219±6 a
Treatment mean	1135±9	1139±9	1142±9	1123±9	1137±9	
P value						
Treatment		0.6014				
Period		<0.0001				
Treatment × period		0.1644				

[a-c]means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.3.3 Laying phase (19 to 46 weeks of age).

4.6.2.3.3.1 Feed consumption

Feed consumption by hens did not differ among water mineral treatments (P>0.05) (Table 4.39). There was no interaction between water mineral level and bird age on feed consumption of laying hens (P>0.05).

Table 4.39. Effects of water mineral treatments and bird age on feed consumption of laying hens from 19-46 weeks of age (trial 2

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------g/bird/day----------					
Period (wks)[1]						
19-22	74±1	75±1	76±1	74±1	77±1	75± 1 e
23-26	105±1	107±1	106±1	104±1	107±1	106±1 d
27-30	114±1	115±1	113±1	115±1	114±1	114±1 c
31-34	117±1	118±1	117±1	119±1	117±1	118±1 b
35-38	119±1	120±1	120±1	119±1	120±1	120±1 a
39-42	119±1	121±1	120±1	120±1	119±1	120±1 a
43-46	119±1	121±1	122±1	120±1	120±1	120±1 a
Treatment Mean	110±1	111±1	110±1	110±1	111±1	
P value						
Treatment	0.7783					
Period	<0.0001					
Treatment × period	0.9871					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

The average daily feed consumption per bird was 110 to 111 g in all groups over the whole period (19 to 46 weeks). However, with time, feed consumption increased parallel to the increase in body weights during the 19 to 46 week period (P<0.05).

4.6.2.3.3.2 Water consumption

Same as feed intake, water intake (Table 4.40) was not affected by the high mineral content in water (P>0.05). The average daily water consumption among treatments ranged from 175 to 188 mL/hen. The feed: water ratio ranged from 1:1.59 to 1: 1.69 among the treatment groups. The ratio was low in the pullet stage (1:1.1 during 15 to 18 weeks of age). Water consumption increased (P<0.05) after the first period (19 to 22 weeks of age) according to the increase in egg production. Francesch et al. (1995) found the feed: water ratio ranged from 1:1.8 to 1: 2.1 from 26 to 37 weeks of age in laying hens in a study that evaluate enzyme supplementation on laying hen performance.

Table 4.40. Effects of water mineral treatments and bird age on water consumption of laying hens from 19-46 weeks of age (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------mL/bird/day----------					
Period (wks)[1]						
19-22	135±6	144±6	143±6	133±7	139±6	139±3 c
23-26	196±6	200±6	200±6	189±6	197±6	197±3 a
27-30	184±7	191±7	197±7	192±7	189±7	191±3 ab
31-34	186±6	193±6	193±6	183±7	191±6	189±3 ab
35-38	194±6	195±6	196±6	166±6	196±6	189±3 ab
39-42	190±6	197±6	197±6	188±6	196±6	193±3 a
43-46	183±6	191±6	190±6	171±6	190±6	185±3 b
Treatment Mean	181±4	188±4	188±4	175±4	185±4	
P value						
Treatment	0.1327					
Period	<.0001					
Treatment × period	0.3363					

[a-c] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.3.3.3 Body weight

Body weights of hens were not affected by high mineral content in water ($P>0.05$) (Table 4.41). There was no water mineral treatment by bird age interaction on body weight. Hens become heavier with age ($P<0.05$). The average body weight of the hens increased from 1220 to 1884g during weeks of 19 to 46 age. A rapid increase in body weight occurred during weeks of 19 to 22 of age. Thereafter, the increment in four weeks was not as large.

4.6.2.3.3.4 Hen day egg production

There was no interaction between water mineral treatments and bird age on hen day egg production ($P>0.05$) (Table 4.42). Water mineral treatments alone did not affect hen day egg production ($P>0.05$). Bird age had a significant effect on egg production. During the 19 to 22 weeks of age egg production was as low as 30 to 32% which is corresponded with the early stage of egg production (Lohmann Tierzucht 2013). Thereafter, egg production increased with age of birds. At 46 weeks hen day production was 96%, where the birds were still at their peak egg production.

According to these results, Ca, Mg or SO_4 up to 765, 520 and 2080 ppm, respectively, in water did not affect body weight, feed intake, and water intake or egg production.

Table 4.41. Effects of water mineral treatments and bird age on body weight of laying hens from 19-46 weeks of age (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	g/bird					
Period (wks)[1]						
Initial	1235±12	1216±12	1208±12	1217±12	1225±12	1220±5 h
19-22	1493±19	1511±19	1495±19	1518±19	1515±19	1506±8 g
23-26	1657±28	1668±28	1572±31	1518±31	1515±28	1634±13 f
27-30	1687±21	1693±21	1699±21	1689±21	1707±21	1695±9 e
31-34	1729±21	1733±21	1733±21	1727±21	1744±21	1733±9 d
35-38	1803±24	1813±24	1811±24	1797±21	1813±24	1807±11 c
39-42	1845±26	1851±26	1855±26	1835±25	1860±26	1849±12 b
43-46	1861±27	1891±27	1891±27	1882±27	1899±27	1884±12 a
Treatment Mean	1664±15	1672±15	1658±15	1664±15	1674±15	
P value						
Treatment	0.9434					
Period	<0.0001					
Treatment × period	0.6144					

[a-h] means±SEM for period means with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Table 4.42. Effects of water mineral treatments and bird age on hen day egg production of laying hen from 19-46 weeks of age (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------%----------					
Period (wks)[1]						
19-22	30.3±1.61	30.8±1.61	29.2±1.61	32.6±1.61	31.5±1.61	30.9±0.72 e
23-26	91.6±1.61	93.4±1.61	91.6±1.61	90.7±1.61	92.6±1.61	92.0±0.72 d
27-30	94.8±1.61	95.7±1.61	95.4±1.61	95.2±1.61	95.4±1.61	95.3±0.72 bc
31-34	94.5±1.61	94.3±1.61	93.5±1.61	93.0±1.61	93.1±1.61	93.7±0.72 cd
35-38	97.1±1.61	95.5±1.61	95.4±1.61	97.1±1.61	95.4±1.61	96.0±0.72 abc
39-42	99.3±1.61	97.6±1.61	96.5±1.61	98.5±1.61	98.1±1.61	98.0±0.72 a
43-46	97.4±1.61	97.0±1.61	95.2±1.61	98.1±1.61	94.3±1.61	96.4±0.72 ab
Treatment Mean	86.4±0.97	86.3±0.97	85.3±0.97	86.4±0.97	85.7±0.97	
P value						
Treatment	0.8771					
Period	<0.0001					
Treatment × period	0.9721					

[a-e] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.4 Effects of water mineral treatments on egg quality

4.6.2.4.1 Egg weight

Egg weight changed with water treatments for certain bird ages (P<0.05) (Table 4.43). After slicing the data according to periods, only 23 to 26 weeks period and 27 to 30 weeks period had significant interaction with water treatments. However, the Tukey-Kramer procedure did not identify significant differences among least square means. The highest egg weights were observed in high Ca water treatment during the both periods. Egg weight increased when hens aged. The egg weight increased from 51 to 63 g from 19 to 43 weeks of age in the current study. Silversides and Scott (2001) reported increased egg weight in white leghorn laying hens with age. In their study, egg weight increased 53 to 63 g from 25 to 59 weeks of age compared to 56 to 63 g from 23 to 46 weeks of age in our study.

Table 4.43. Effects of water mineral treatments and bird age on egg weight from 19-46 weeks of age in laying hens (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------g/egg----------					
Period (wks)[1]						
19-22	51.3±0.5	51.2±0.5	49.9±0.5	51.0±0.5	51.0±0.5	50.9±0.2
23-26	56.0±0.5	56.0±0.5	55.4±0.5	55.7±0.5	57.5±0.5	56.1±0.2
27-30	58.0±0.5	58.5±0.5	58.5±0.5	58.5±0.5	60.2±0.5	58.7±0.2
31-34	60.5±0.5	61.2±0.5	61.8±0.5	60.6±0.5	61.0±0.5	61.0±0.2
35-38	61.3±0.5	62.9±0.5	62.2±0.5	62.0±0.5	62.4±0.5	62.1±0.2
39-42	62.1±0.5	63.0±0.5	62.0±0.5	62.2±0.5	63.5±0.5	62.6±0.2
43-46	62.4±0.5	63.3±0.5	63.5±0.5	62.7±0.5	63.0±0.5	63.1±0.2
Treatment mean	58.8±0.3	59.5±0.3	59.0±0.3	59.0±0.3	59.8±0.3	
P value						
Treatment	0.1612					
Period	<.0001					
Treatment × period	0.0378*					

*Tukey-Kramer procedure did not identify significant differences among least square means (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.4.2 Specific gravity

There was no interaction between water treatment and bird age on SG (P>0.05). Moreover, water treatments did not affect SG of eggs (P>0.05) (Table 4.44). However, SG decreased with bird age (P<0.05). As described in trial 1 results, this is due to the increment of egg size without proportionate increase in production of eggshell materials with increasing the age of hen (Roland et al. 1978).

4.6.2.4.3 Albumen height

As observed in the first trial, albumen height was not affected by water treatments even with higher levels up to 2600 ppm $MgSO_4$ and $CaSO_4$ (P>0.05) (Table 4.45). However, AH significantly changed as birds grew older (P<0.05). During the 19 to 22 weeks, AH was greater than during any other periods. The lowest AH was occurred during the last period, 43 to 46 weeks of age. Similarly, Silversides and Scott (2001) reported a decrease in albumen height with the age.

4.6.2.4.4 Percent yolk

Percent yolk was similar among treatments (P>0.05). There was no significant treatment by bird age interaction on percent yolk (Table 4.46). However, percent yolk significantly increased with birds age (P<0.05). Percent yolk was lowest (22%) during 19 to 22 weeks when the egg weight was also lowest. The first period (19 to 22 weeks of age) was the start of lay, so hens produced smaller and fewer eggs. With the increase in egg weight, percentage of yolk increased with age. A similar effect was reported by Silversides and Scott (2001) where percent yolk increased from 23 to 28% during 25 to 59 weeks of age. Similarly, in our study, percent yolk increased from 22 to 29% during 19 to 46 weeks of age in laying hens.

Table 4.44. Effects of water mineral treatments and bird age on specific gravity of eggs from 19-46 weeks of age in laying hens (trial 2)

	Treatment					Period Mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
Period (wks)[1]						
19-22	1.096±0.001	1.096±0.001	1.096±0.001	1.096±0.001	1.097±0.001	1.096±0.001 bc
23-26	1.097±0.001	1.097±0.001	1.097±0.001	1.095±0.001	1.097±0.001	1.097±0.001 a
27-30	1.095±0.001	1.094±0.001	1.094±0.001	1.094±0.001	1.094±0.001	1.094±0.001 b
31-34	1.092±0.001	1.092±0.001	1.092±0.001	1.093±0.001	1.093±0.001	1.093±0.001 bc
35-38	1.091±0.001	1.090±0.001	1.089±0.001	1.090±0.001	1.090±0.001	1.090±0.001 c
39-42	1.088±0.001	1.086±0.001	1.087±0.001	1.087±0.001	1.087±0.001	1.087±0.001 d
43-46	1.087±0.001	1.087±0.001	1.086±0.001	1.086±0.001	1.086±0.001	1.086±0.001 e
Treatment mean	1.092±0.001	1.092±0.001	1.091±0.001	1.092±0.001	1.092±0.001	
P value						
Treatment	0.3375					
Period	<0.0001					
Treatment × period	0.7854					

[a-c]means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Table 4.45. Effects of water mineral treatments and bird age on albumen height from 19-46 weeks of age in laying hens (trial 2)

	Treatment					Period Mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------mm----------					
Period (wks)[1]						
19-22	8.85±0.17	8.87±0.17	8.86±0.17	8.71±0.17	8.79±0.17	8.82± 0.07 a
23-26	7.62±0.17	7.64±0.17	7.62±0.17	7.61±0.17	7.59±0.17	7.62± 0.07 de
27-30	7.85±0.17	8.14±0.17	7.99±0.17	8.09±0.17	8.15±0.17	8.04± 0.07 bc
31-34	8.01±0.17	8.19±0.17	7.84±0.17	8.11±0.17	8.06±0.17	8.04± 0.07 bc
35-38	7.97±0.17	8.23±0.17	8.07±0.17	8.10±0.17	8.20±0.17	8.11± 0.07 b
39-42	7.79±0.17	7.93±0.17	7.67±0.17	7.81±0.17	8.03±0.17	7.84± 0.07 cd
43-46	7.59±0.17	7.54±0.17	7.60±0.17	7.57±0.17	7.66±0.17	7.59± 0.07 e
Treatment mean	7.95±0.12	8.07±0.12	7.95±0.12	7.80±0.12	8.07±0.12	
P value						
Treatment	0.9135					
Period	<.0001					
Treatment × period	0.9839					

[a-e] Means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.

Table 4.46. Effects of water mineral treatments and bird age on % yolk from 19-46 weeks of age in laying hens (trial 2)

	Treatment					Period mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------%----------					
Period (wks)[1]						
19-22	22.0±0.3	22.3±0.3	21.5±0.3	22.0±0.3	22.2±0.3	22.0±0.1 f
23-26	25.0±0.3	24.4±0.3	24.5±0.3	24.5±0.3	24.3±0.3	24.5±0.1 e
27-30	25.9±0.3	25.7±0.3	25.9±0.3	26.3±0.3	25.7±0.3	25.9±0.1 d
31-34	26.7±0.3	26.8±0.3	26.6±0.3	27.1±0.3	26.9±0.3	26.8±0.1 c
35-38	27.8±0.3	27.6±0.3	27.4±0.3	27.6±0.3	27.7±0.3	27.6±0.1 b
39-42	27.8±0.3	27.4±0.3	28.2±0.3	27.1±0.3	27.4±0.3	27.6±0.1 b
43-46	27.7±0.3	28.7±0.3	28.8±0.3	28.1±0.3	29.0±0.3	28.7±0.1 a
Treatment mean	26.3±0.2	26.1±0.2	26.1±0.2	26.1±0.2	26.2±0.2	
P value						
Treatment	0.9581					
Period	<0.0001					
Treatment × period	0.0516					

[a-f] means±SEM with different letters among period means are significantly different (α=0.05).
[1]Period was given in weeks of age of laying hens.

4.6.2.4.5 Percent eggshell

Percent eggshell (% eggshell) was significantly affected by treatment by period interaction (P<0.05) (Table 4.47). However, there was no significant difference among any treatment means according to the Tukey-Kramer test. After slicing of data into the periods, only 31 to 34 weeks, 39 to 42 and 43 to 46 weeks periods had significant effect of interaction. The percent shell was lowest in high Mg treatment for both periods from 31 to 34 weeks of age and from 39 to 42 weeks of age, but not for period from 43 to 46 weeks of age. The high Mg treatment had lowest percent eggshell among the water treatments regardless of the interaction between treatments and bird age. Eggshell percentage tend to increase with the water Ca increase (10.2% vs 10.3% in low Ca and high Ca treatments, respectively), while eggshell percentage tend to decrease with the water Mg increase in water (10.2% vs 10.1% in low Mg and high Mg treatments, respectively).

Table 4.47. Effects of water mineral treatments and bird age on % eggshell from 19-46 weeks of age in laying hens (trial 2)[1]

	Treatment					Period Mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	----------%----------					
Period (wks)[2]						
19-22	10.8±0.1	10.9±0.1	10.7±0.1	11.0±0.1	10.9±0.1	10.9±0.0
23-26	10.9±0.1	11.0±0.1	10.9±0.1	10.8±0.1	11.1±0.1	11.0±0.0
27-30	10.4±0.1	10.2±0.1	10.2±0.1	10.3±0.1	10.3±0.1	10.3±0.0
31-34	10.2±0.1	10.1±0.1	9.8±0.1	10.2±0.1	10.2±0.1	10.1±0.0
35-38	10.1±0.1	9.8±0.1	9.7±0.1	9.9±0.1	9.9±0.1	9.9±0.0
39-42	10.0±0.1	9.8±0.1	9.7±0.1	9.9±0.1	10.0±0.1	9.9±0.0
43-46	9.8±0.1	9.7±0.1	9.6±0.1	9.5±0.1	9.7±0.1	9.7±0.0
Treatment mean	10.4±0.0	10.2±0.0	10.1±0.0	10.2±0.0	10.3±0.0	
P value						
Treatment	0.0456					
Period	<0.0001					
Treatment ×period	0.0331[3]					

[1]Means±SEM.
[2]Period was given in weeks of age of laying hens.
[3]Tukey-Kramer procedure did not identify significant differences among least square means.

These 0.1% eggshell percent changes demonstrate biological response of hens to the excess Ca and Mg contents in water. In the current study, % eggshell decreased from 10.9 to 9.7 during 19 to 46 weeks of age of hens as normally occurred with the hen age. Silversides and Scott (2001) reported decrease in % eggshell when hens ages. The % eggshell decreased from 10.75 to 9.52 during the period of 25 to 59 weeks of age in their study.

4.6.2.4.6 Egg breaking strength

Similar to the result observed during the first trial, egg breaking strength was not affected by water treatments or treatments by bird age interaction ($P>0.05$) even at higher levels of Ca, Mg and SO_4 in water than the first trial. The force required to break the egg became significantly lower during 35 to 38, 39 to 42 and 43 to 46 weeks of age of birds ($P<0.05$) (Table 4.48).

Table 4.48. Effects of water mineral treatments and bird age on egg breaking strength from 19-38 weeks of age in laying hens (trial 2)

	Treatments					Period mean
	Control	2100 ppm $MgSO_4$	2600ppm $MgSO_4$	2100 ppm $CaSO_4$	2600 ppm $CaSO_4$	
	----------kg force----------					
Period (wks)[1]						
19-22	6.38±0.14	6.05±0.14	6.17±0.14	6.07±0.14	6.00±0.14	6.13±0.87 a
23-26	6.13±0.14	6.05±0.14	6.09±0.14	6.00±0.14	6.44±0.14	6.16±0.87 a
27-30	5.52±0.14	5.27±0.14	5.39±0.14	5.42±0.14	5.72±0.14	5.45±0.87 b
31-34	5.69±0.14	5.27±0.14	5.31±0.14	5.43±0.14	5.72±0.14	5.49±0.87 b
35-38	5.41±0.14	5.32±0.14	5.31±0.14	5.24±0.14	5.30±0.14	5.32±0.87 b
Treatment mean	5.83±0.64	5.59±0.64	5.68±0.64	5.63±0.64	5.82±0.64	
P value						
Treatment	0.2281					
Period	<.0001					
Treatment × period	0.5647					

[a-b]means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Period was given in weeks of age of laying hens.
Note: Data for the last 2 periods (39-46 weeks) were not analyzed due to error occurred in texture analyzer.

The breaking strength was not statistically analyzed for the two periods from 39 to 46 weeks in the current trial due to an error occurred in texture analyzer machine.

Reduction in shell thickness during these periods could have resulted in lower breaking force needed. Hamilton (1982) reported that the compression force was highly correlated with eggshell thickness (r = 0.60 to 0.83) based on the data in the literature. In our study the correlation between the breaking force and eggshell thickness was r = 0.26 (*P*=0.172), which indicate non-significant low correlation between the two parameters.

4.6.2.4.7 Eggshell thickness

Eggshell thickness did not differ among water treatments (*P*>0.05) (Table 4.49). There was no significant interaction between water mineral treatments and bird age on shell thickness. However, with bird age, shell thickness was significantly changed but without a consistent pattern. There was a decrease of shell thickness when hens were older when compared to the early production periods. As described in trial 1 results, although egg size become larger with age, shell materials are not produced at similar rate (Roland et al. 1978). Therefore, the amount of shell material that covers a unit area of an egg is reduced, resulting in reduced shell thickness of an egg with later periods. This effect was reflected in lower specific gravity values at the end of the trial. Hamilton (1982) reported that there was a strong positive correlation between eggshell thickness and specific gravity (r = 0.56 to 0.88). The correlation between shell thickness and specific gravity in the current study was r =0.50 (*P* = 0.005) which indicate a significant medium correlation between the two parameters.

Table 4.49. Effects of water mineral treatments and bird age on eggshell thickness from 19-46 weeks of age in laying hens (trial 2)

	Treatment					Period Mean
	Control	Low Mg	High Mg	Low Ca	High Ca	
	mm					
Period (wks)[1]						
19-22	0.436±0.005	0.434±0.005	0.429±0.005	0.442±0.005	0.441±0.005	0.437±0.002 b
23-26	0.485±0.005	0.477±0.005	0.481±0.005	0.468±0.005	0.485±0.005	0.479±0.002 a
27-30	0.432±0.006	0.432±0.005	0.427±0.005	0.432±0.005	0.436±0.005	0.432±0.002 bc
31-34	0.424±0.005	0.426±0.005	0.423±0.005	0.425±0.005	0.432±0.005	0.426±0.002 cd
35-38	0.422±0.005	0.420±0.005	0.410±0.005	0.412±0.005	0.412±0.005	0.415±0.002 e
39-42	0.404±0.005	0.408±0.005	0.395±0.005	0.419±0.005	0.404±0.005	0.406±0.002 f
43-46	0.426±0.005	0.423±0.005	0.420±0.005	0.420±0.005	0.418±0.005	0.421±0.002 de
Treatment mean	0.433±0.002	0.431±0.002	0.426±0.002	0.431±0.002	0.433±0.002	
P value						
Treatment	0.2831					
Period	<.0001					
Treatment × period	0.1429					

[a-f] means±SEM with different letters among period means are significantly different according to the Tukey-Kramer test α=0.05.
[1]Period was given in weeks of age of laying hens.

4.6.3 Integration of trial 1 and trial 2 results

Two mineral trials were conducted to evaluate high levels of Ca, Mg and SO_4 in water on laying performance throughout their production cycle. During the first trial, effects of high Ca, Mg and SO_4 up to 487, 234 and 1317 ppm, respectively, were evaluated on laying hens from peak production to the end of production cycle (33 to 69 weeks of age). The second trial was conducted to study the impacts during the pullet stage (7 to 18 weeks) through the laying stage (19 to 46 weeks of age) with higher mineral contents than used in the first trial.

Ca, Mg and SO_4 up to 487 ppm, 234 ppm and 1317 ppm, respectively, in water did not affect feed consumption, water consumption, body weight, egg production and egg quality of laying hens during their peak to late egg production phases under the given experimental conditions in water mineral trial 1. Bone quality of these hens at the end of production cycle (70 weeks of age) was not affected by the mineral levels in water. Pullet grower and developer birds (7 to 18 weeks) tolerated Ca, Mg and SO_4 in water up to 786, 562 and 1988 ppm, respectively, without having negative impacts on water consumption and body weights during the second mineral trial. Feed consumption was affected only during 15 to 18 weeks of age of birds, but body weight was not reduced. During early to peak egg production of these birds, no impacts were found on feed consumption, water consumption, body weight, egg production and egg quality up to 732 ppm, 508 ppm and 1818 ppm, Ca, Mg and SO_4, respectively in drinking water.

4.7 Conclusions

The mineral content of water containing Ca, Mg and SO_4 up to 786 ppm, 562 ppm and 1988 ppm, respectively, did not negatively affect pullet grower and developer production performance. The Ca, Mg and SO_4 up to 732 ppm, 508 ppm and 1818 ppm, respectively, in drinking water, did not negatively affect laying hen production performance and egg quality. Bone quality of the laying hens at the end of production cycle was not affected by Ca, Mg and SO_4 up to 487 ppm, 234 ppm and 1317 ppm, respectively, in water. Hens at all stages of production tolerated higher concentrations of these minerals than were recommended for poultry by Carter and Sneed (1996); Weltzien (2002) and Fairchild and Ritz (2012) under given experimental conditions.

CHAPTER 5 THE EFFECT OF WATER pH ON PRODUCTION PERFORMANCE AND EGG QUALITY OF LAYING HENS AT THE END OF PRODUCTION

5.1 Abstract

The pH of drinking water, currently being used for commercial laying hens across Canada, ranged from pH 5.97 to 9.2. The effects of extreme water pH levels on laying hens performance have not been well documented. This pilot study assessed the water pH effects from pH 6 to 8.2 on laying hen production performance and egg quality. The effects of water pH 6, 6.5, 7.9 (control) and 8.2 were evaluated in a completely randomized experiment with 320 Lohmann–Lite laying hens from 66 to 69 weeks of age. Birds were given a standard diet and water *ad libitum* throughout the trial. Water treatments were prepared daily into 10 L containers, which was connected to nipple drinkers in each experimental unit. Increasing pH to 8.2 with sodium bicarbonate significantly reduced feed consumption, water consumption, body weight, egg production and egg weight. This treatment caused high mortality (5%) and was terminated after 5 days and thereafter birds were given pH 7.9 water for the remainder of the trial. Reducing pH to 6 or 6.5 with citric acid did not affect body weight, feed consumption or water consumption ($P>0.05$). Specific gravity, egg weight, albumen height, percentage of yolk, shell or albumen, and breaking strength were not affected by pH 6 or 6.5 ($P>0.05$). Shell thickness was reduced by pH 6 (0.307 mm) compared to pH 7.9 (0.345 mm) during Week 2. Percent soft-shelled and broken eggs were not different among treatments. Production performance and egg quality were not different in the pH 8.2/7.9 group, compared to other treatments. Therefore, providing drinking water with a pH ranging from 6 to 7.9 did not negatively affect production performance or egg quality of the laying hens late in production. The pH 8.2 was undesirable for laying hen performance when water pH was adjusted with sodium bicarbonate. A full production cycle should be conducted to determine the effects of pH at different stages of egg production of hens.

Key words: drinking water, egg quality, laying hens, performance, pH

5.2 Introduction

Water pH is a major indicator of water quality. The pH of water can be changed by many natural factors, including acid rain, dissolved minerals in water, such as iron sulphide (FeS), dissolved atmospheric carbon dioxide in shallow wells or surface water. As well, agricultural chemicals, such as fertilizers and pesticides (Sullivan et al. 2005). According to the results of the water quality survey described in Chapter 03, the pH of water offered to commercial laying hens varies from 5.97 to 9.20 across Canada.

Information on the effects of different levels of drinking water pH on laying hen performance is limited. More commonly there are reports of broiler studies carried out to evaluate the antibacterial effects of acidified water (Chaveerach et al. 2004; Watkins et al. 2004; Açıkgöz et al. 2011). Watkins et al. (2004) found that pH treatments ranging from 3 to 8 did not affect body weight, feed conversion efficiency or water intake per gram of body weight for broilers. However, Açıkgöz et al. (2011) found a reduction in body weight when broilers were supplied with water of pH 4.5 compared to control water with a pH of 7.4. But feed intake, feed conversion ratio or mortality were not affected by the pH 4.5 treatment. In both studies, gizzard content pH did not change with the pH levels in water. Chaveerach et al. (2004) did not find body weight change in broilers supplied with water treatments with a pH range from 3.9 to 6.9. Neither feed intake nor water intake were measured. These findings on broiler performance with changes to water pH varied from study to study and did not always make the same measurements. Body weight change is the commonly evaluated parameter in these studies while mortality, feed conversion efficiency and water intake of the birds were the other measurements that assessed in those studies. Additionally, those findings did not agree with what has been suggested for poultry

drinking water by other authors. According to Carter and Sneed 1996; Weltzein 2002; Fairchild and Ritz 2012, water pH below 6 or 6.5 could negatively affect poultry performance. The original research data were not discussed in these popular press articles and could not be found in peer reviewed literature. However, the above broiler studies showed that broilers can tolerate a wide range of pH in water, as low as 3 and as high as 8. No studies were found that evaluated water pH above 8 for commercial poultry and none were conducted to determine the impact of drinking water pH on laying hen production performance and egg quality. Therefore, evaluation of different pH levels in water on production performance and egg quality would fill a gap of knowledge on water pH effect on laying hens. Since guidelines suggest pH 6 to 8.5 (Weltzein 2002) test pH levels were selected within this range.

5.3 Objectives

a) To determine the effect of adjusting the pH of well water pH 7.9 (control) to 6, 6.5, or 8.2 on production performance of laying hens measured as body weight change, feed consumption, water consumption and hen-day egg production at the end of production (66 to 69 weeks of age).
b) To determine the effect of adjusting the pH of well water pH 7.9 (control) to 6, 6.5, or 8.2 on the egg quality parameters measured as egg weight, specific gravity, albumen height, breaking strength, shell thickness, percent shell, yolk and albumen of eggs.

5.4 Hypothesis

a) Adjusting the pH of drinking water to 6, 6.5, and 8.2 will affect production performance of laying hens including: body weight change, feed consumption,

water consumption and hen-day egg production at end of production when compared to control water pH 7.9.

b) Adjusting the pH of drinking water to 6, 6.5, and 8.2 will affect egg quality parameters including: egg weight, specific gravity, albumen height, breaking strength, shell thickness, percent shell, yolk and albumen of eggs when compared to control water pH 7.9.

5.5 Materials and Methods

5.5.1 Experimental design and hen management

The trial was a 4-week production study using 320, 66-week old Lohmann LSL-Lite laying hens. The hens were housed in 64 experimental battery cages, 5 birds in each cage. Adjacent four cages in a row of a battery cage unit (50 cm × 60 cm × 44 cm; length × width × height) were considered an experimental unit. There were 16 experimental units and each treatment had 4 replications. The hens were provided with a standard phase III layer diet (Table 5.1). The diet was formulated to meet or exceed the breeder company recommendations (Lohmann Tierzucht 2012). Two feed samples were analyzed for crude protein, crude fat and mineral content at the Department of Agriculture Feed Analysis laboratory in Truro, Nova Scotia. Water and feed were supplied *ad libitum* throughout the study. Hens were supplied with 16 hrs light and 8 hrs dark per day as per the breeder recommendation. Room temperature was kept between 22-24℃ and checked twice a day. Data was analysed using analysis of variance in a completely randomized design with drinking water pH treatments (7.9 (control), 6.0, 6.5 and 8.2) as the main effect. Mortality was recorded as it occurred and all birds that died were necropsied by a veterinary pathologist. All birds were managed in accordance with the local Animal Care and Use

Committee guidelines that follow the Canadian Council on Animal Care Codes of Practice (2009).

Table 5.1. The ingredients and calculated nutrient composition of the diet supplied to laying hens receiving waters with different pH from 66-69 weeks of age

Ingredients	As fed basis %
Soybean meal	19.41
Corn	59.49
Wheat	10.00
Animal/vegetable fat [1]	0.32
Vitamin –mineral premix[2]	0.50
Oyster shells	2.46
Salt	0.30
Methionine premix[3]	0.14
Shell mix[4]	2.46
Limestone	4.91
Biophytase[5]	0.01
Total	100.00
Calculated composition	
Metabolizable energy(Kcal/kg)	2820
Protein (%)	15
Fat (%)	3
Calcium (%)	4
Phosphorus (available) (%)	0.3
Sodium (%)	0.1
Potassium (%)	0.7
Magnesium (%)	0.1
Manganese (ppm)	84
Copper(ppm)	31
Zinc(ppm)	110
Fe (ppm)	68

* Expected feed intake115 g/hen/day. [1]**Animal vegetable fat** contained free fatty acids 15%, moisture 1%, insoluble matter 0.15%, unsaponifiables 2.5%. S.F. Rendering Ltd., NS, Canada. [2]**Layer premix** (Amount per tonne of feed): vitamin A ($650x10^6$ IU kg^{-1}), 12 g; vitamin D3 premix ($50x10^6$ IU kg^{-1}), 50 g; vitamin E ($5x10^5$ IU kg^{-1}), 40 g; vitamin K (33%), 9 g; riboflavin (95%), 8 g; DL Ca-pantothenate (45%), 16 g; Vitamin B12 (1000 mg kg^{-1}), 12 g; Niacin (99%), 31 g; Folic acid (3%), 22 g; Choline chloride (60%), 117 g; Biotin (0.04%), 400 g; Pyridoxine (990000 mg kg^{-1}), 4 g; Thiamine (970000 mg kg^{-1}), 220 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 100 g; Copper sulfate (25%), 100 g; Selenium premix (675 mg kg^{-1}), 14.85 g; Ethoxyquin (50%), 100 g; Wheat middlings, 2189 g; Ground limestone (38%), 500 g**.** [3]**Methionine Premix:**50% Wheat middlings and 50% DL methionine. [4]**Shell mix**:$CaCO_3$ 97.5%,$MgCO_3$ 0.3%, Ca 39%,Mg 0.1%, Silica(SiO2) 1.4%, Ferric Oxide (Fe_2O_3) 0.2%,Alumina (Al_2O_3) 0.2% Total S 0.01%.Graymont (QC)Inc., QC, Canada. [5]**Biophytase**: 5000 phytase units per g. Canadian Bio-Systems Inc., Calgary, Alberta.

5.5.2 Water treatment preparation

Water treatments with a pH of 6.0 and 6.5 were prepared using citric acid (0.56 g and 1.0 g per 10 L well water respectively) while pH 8.2 was prepared by adding 120 g sodium bicarbonate into 10 L of well water. Atlantic Poultry Research Centre (APRC) well water was used to prepare water treatments. The water treatments were prepared daily and pH was measured daily using a portable pH meter (pH Tester 20, Fisher Scientific Company, Ottawa, Ontario, Canada). Prepared waters were placed in 10 L containers, one per experimental unit, with the water supplied from the containers to nipple drinkers for in each cage within the experimental unit. The water consumption was measured weekly by weighing the water remaining.

5.5.3 Production performance measurements

At 66 weeks of age and thereafter at 7-day intervals, body weights, feed consumption and water consumption were measured. Birds were weighed in groups at 1300 hr on the last day of each 7-day interval. Egg production was recorded daily including marketable eggs and unmarketable eggs (cracked, without shells, and soft-shelled).

5.5.4 Egg quality measurements

Eight eggs were collected from each experimental unit during the last day of each 7-day interval for measurement of egg specific gravity, egg weight, eggshell breaking strength, albumen height, yolk weight, and eggshell thickness. The egg quality measurements were assessed using the same methods described in chapter 4 section 4.5.1.4.

5.5.5 Statistical analysis

In this completely randomized experiment the production performance and egg quality data were subjected to ANOVA using Proc Mixed procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) (Littell et al. 1996) with water treatment as the main effect. The statistical model used for measurements for single time data was:

$Y_{i} = \mu + \tau_i + \varepsilon_{ij}$

Where Y_i is the variable of interest; μ is the overall mean; τ_i is the effect of i^{th} water treatment (i =1-3); ε_{ij} is the random effect of error with j representing replicates (j=1-4).

If significant effects were found, the Tukey-Kramer procedure (Gbur et al. 2012) was used to compare differences among the least square means. The α-level of significance was 0.05.

For repeated measures analysis, the factor of time and resulting interaction levels were added (weeks of bird age as the measure of time, k) to the model. Five covariance structures, Compound Symmetry, Heterogeneous Compound Symmetry, Toeplitz, Heterogeneous Toeplitz and Ante-dependence were compared. For the ANOVA, the covariance structure which gave the smallest corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) numbers was selected. Body weight, egg specific gravity and eggshell thickness data were analysed using Ante-dependence covariance structure while feed consumption, egg breaking strength, albumen height, % shell, % yolk and egg weight were analysed using Compound Symmetry. Water consumption analysed using Heterogeneous Compound Symmetry. The statistical model used for repeated measures analysis was:

$Y_{i} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$

Where Y_i is the variable of interest; μ is the overall mean; τ_i is the effect of i^{th} water treatment (i =1-3); β_j is the effect of time (j =1-4); $(\tau\beta)_{ij}$ is the effect of the interaction between water treatments and time; and ε_{ijk} is the random effect of error with k representing replicate measurements (k =1-4).

5.6 Results and Discussion

5.6.1 Diet and water analysis

The analyzed composition of most nutrients was similar or exceeded the calculated values in the diet (Table 5.2). The analyzed contents of protein, fat, Ca, P and Na were similar to the calculated contents. K was lower than calculated content while Mg content was higher. The Mn concentration was higher than the calculated composition while the Cu and Zn concentrations were lower. However, these analyzed contents of nutrients met or exceeded the NRC (1994) recommendations for laying hens. The pH levels of water treatments were measured daily once the water treatments were prepared (Table 5.3). The average pH of water treatments during 4 weeks study was calculated.

Table 5.2. Analyzed composition of the diet supplied to the hens from 66-69 weeks of age in pH study*

Nutrient	As fed basis
Dry matter (%)	90±0.1
Protein (%)	15±0.1
Fat (%)	3±0.1
Calcium (%)	4±0.1
Phosphorus (available) (%)	0.3±0.0
Sodium (%)	0.1±0.0
Potassium (%)	0.6±0.0
Magnesium (%)	0.2±0.0
Manganese (ppm)	117±12
Copper (ppm)	30±3
Zinc (ppm)	97±3

*Diets were analyzed in duplicates, n=2.

Table 5.3. Water pH level in water treatments in each week of the pH study

Water treatment	pH of water
pH 6.0	6.02±0.01
pH 6.5	6.51±0.00
pH 7.9	7.91±0.09
pH 8.2/7.9	7.93±0.07*

*pH of water after switching the birds to control water. Before that birds received pH 8.28± 0.05 water for 5 days.

5.6.2 Effects of water pH on production performance of laying hens

Four different pH levels for drinking water were evaluated on laying hen production performance and egg quality. Two of the pH (6 and 6.5) levels were acidic and the other 2 were alkaline at pH 7.9 and 8.2. These pH levels can be found in natural water and were considered as safe for poultry species (Weltzien 2002). The water from the natural source was the pH 7.9 treatment.

During the first 5 days of the study, two hens in two experimental units provided pH 8.2 water died (Table 5.4). The body weight of hens supplied with pH 8.2 water were reduced by 4% and 10% compared to the pH 7.9 group. Watery feces was observed in all pH 8.2 experimental units during this period. The hens were removed from the treatment and supplied with well water after 5 days. The effects of pH 6, 6.5 and 7.9 were evaluated during the rest of trial. The pH 8.2 treatment was indicated as pH 8.2/7.9 in the tables.

Negative impacts of $NaHCO_3$ were reported in some broiler and laying hen studies when supplied with either water or feed at the similar level that we used to increase water pH in this study. Hayat et al. (1999) found high mortality rate among broilers when fed 10 g/L of $NaHCO_3$ in water. Junqueira et al. (1984) reported high mortality in laying hens when 1.6% (16 g/kg) $NaHCO_3$ supplied in the diet. Reduced egg production, feed intake and egg

weight were occurred in the latter study. Similar effects were found in the current study when 12g/L of $NaHCO_3$ was used to increase water pH. In above mentioned studies, blood acid-base balance was affected and metabolic alkalosis was occurred where pH, HCO_3, CO_2 contents in blood of hens were increased, at these levels. Davison and Wideman (1992) also reported that 30g/kg sodium bicarbonate caused high mortality, reduce egg production and diarrhea in a commercial laying flock, but this level is higher than the level used in our study.

Table 5.4. The mortality and body weight change of laying hens received pH 8.2 and pH 7.9 water treatments during 5 days

Unit	Treatment pH	Initial weight (g)	Weight after 5 days (g)	% body weight change	No. of Mortality	Egg production%
3	7.9	1680	1701	1.3%	0	85
4	8.2	1672	1605	- 4%	1	61
13	8.2	1740	1563	-10%	1	54
14	7.9	1770	1805	1.9 %	0	94

5.6.2.1 Body weight

There was no interaction between pH treatments and age of the birds on body weights of the hens (P>0.05) (Table 5.5). Body weight of the birds did not differ among different pH groups (P>0.05). There was reduction in body weight of pH 8.2 group at the first week. However, after introduction of control water, body weight increased and no change was observed at the end of the trial when compared to the other pH groups. During the four week period, body weights remained the same for all water treatments. The average hen body weight ranged from 1726±20 to 1769±20 g.

Table 5.5. The effect of adjusting water pH on body weights of laying hens from 66-69 weeks of age[1]

	Body weight (g/bird)					Treatment Mean
	Age (weeks)					
Treatment	**Initial**	**66**	**67**	**68**	**69**	
pH 6.0	1709±24	1745±24	1740±22	1721±23	1713±21	1726±20
pH 6.5	1755±24	1798±24	1784±22	1770±23	1735±21	1769±20
pH 7.9	1729±24	1788±24	1765±22	1768±23	1717±21	1753±20
pH 8.2/7.9[2]	1749±24	1727±24	1764±22	1734±23	1731±21	1741±20
Age mean	1736±24	1741±24	1740±24	1725±24	1702±24	
ANOVA P value						
Treatment	0.7805					
Age	0.1049					
Treatment × Age	0.2776					

[1]Means±SEM

[2]pH 8.2 group was transferred to pH 7.9 water treatment on day 5 due to high mortality and loss of body weights by hens.

5.6.2.2 Feed consumption

There was an interaction between the water pH treatments and age of the birds for feed consumption (P<0.05) (Table 5.6). The feed consumption was lowest for the pH 8.2 group during the first week of the study (66 weeks of age). This was reflected by the body weight reduction of the hens of pH 8.2 group during 66 weeks of age. Birds on the pH 8.2 water were provided with pH 7.9 water for the remainder of the experiment and had similar feed intake to birds on the other treatments. Similarly, Açıkgöz et al. (2011) did not find reduction in feed consumption when broilers were supplied with water of pH 4.5 compared to control water with a pH of 7.4. However, we evaluated low pH only up to pH 6 in water on laying hens performance.

Table 5.6. The effect of adjusting water pH on feed consumption of laying hens from 66-69 weeks of age

	Feed consumption (g/bird/ day)				Treatment Mean
	Age (weeks)				
Treatment	**66**	**67**	**68**	**69**	
pH 6.0	124±2 a	120±2 a	122±2 a	124±2 a	122±2
pH 6.5	124±3 a	125±3 a	120±3 a	121±3 a	123±2
pH 7.9	127±2 a	124±2 a	124±2 a	124±2 a	125±2
pH 8.2/7.9	104±3 b	128±3 a	120±3 a	123±2 a	119±2
Age Mean	120±1	124±1	121±1	122±1	
ANOVA P value					
Treatment	0.3919				
Age	0.0122				
Treatment × Age	<0.0001				

[a-b] means±SEM with different letters among interaction means are significantly different according to the Tukey-Kramer test (α=0.05).

5.6.2.3 Water consumption

There was interaction between pH treatments and age of the birds on water consumption of the hens (P<0.05) (Table 5.7).

Table 5.7. The effect of adjusting water pH on water consumption of laying hens from 66-69 weeks of age

	Water consumption (mL/bird/day)				Treatment Mean
	Age (weeks)				
Treatment	**66**	**67**	**68**	**69**	
pH 6.0	2 15±5 a	205±5 abc	203±5 abc	209±5 ab	208±3
pH 6.5	210±5 ab	206±5 ab	195±5 abc	196±5 abc	202±3
pH 7.9	220±5 a	209±5 ab	213±5 ab	206±5 ab	212±3
pH 8.2/7.9	176±6 c	213±5 a	196±5 abc	185±5 bc	192±3
Age mean	205±3	208±3	201±3	198±3	
ANOVA P value					
Treatment	0.1305				
Age	<0.0001				
Treatment ×Age	0.0432				

[a-c] means±SEM with different letters among interaction means are significantly different according to the Tukey-Kramer test (α=0.05).

Water consumption of pH 8.2 group decreased significantly when compared to the other treatments during the first week of the study (66 weeks of age). There was no difference of water intake among the other groups of pH 6, 6.5 and pH 7.9 during that week. At the 2nd week of the trial (67 weeks of age), water intake of all groups were similar. pH 8.2 group was supplied with pH 7.9 water treatment for the remainder of the trial. With the introduction of pH 7.9 water, intake of water increased. During the 68 and 69 weeks of age, water consumption was similar among the treatments. There was no difference in water intake of hens given pH 6, 6.5 or 7.9 water throughout the trial. These results would indicate that palatability of water with low pH was not changed compared to the pH 7.9. The feed: water ratio in pH 6, 6.5 and 7.9 groups were 1:1.7, 1:1.6 and1:1.7, respectively in laying hens during 66-69 weeks. The ratio for pH 8.2 at the first week of the trial was 1:1.5 then 1:1.6 for the remainder of the trial.

5.6.2.4 Hen day egg production

There was interaction between pH treatments and age of the birds on hen day egg production (P<0.05) (Table 5.8). Egg production of pH 8.2 treatment group was low when compared to the other water treatments during 66 and 67 weeks of age. Even though pH 7.9 water treatment supplied to the hens that initially received pH 8.2 during 66 weeks of age, low egg production continued during 67 weeks of age. During 68 and 69 weeks of age egg production was not different among treatments.

Table 5.8. The effect of adjusting water pH on hen day egg production of laying hens from 66-69 weeks of age

	Hen day egg production (%)				Treatment Mean
	Age (weeks)				
Treatment	**66**	**67**	**68**	**69**	
pH 6.0	85.3±2.0a-d	85.5±2.0a-d	78.2±2.0 cd	76.2±2.0 de	81.3±1.3
pH 6.5	90.3±2.0a	88.7±2.0abc	87.3±2.0abc	79.5±2.0 bcd	86.4±1.3
pH 7.9	89.8±2.0ab	87.1±2.0abc	78.9±2.0cd	80.6±2.0 a-d	84.1±1.3
pH 8.2/7.9	59.2±2.0f	67.4±2.0ef	82.5±2.0a-d	81.8±2.0 a-d	72.8±1.3
Age Mean	81.1±1.0	82.2±1.0	81.7±1.0	79.5±1.0	
ANOVA P value					
Treatment	<0.0001				
Age	0.1958				
Treatment × Age	<0.0001				

[a-f] means±SEM with different letters among interaction means are significantly different according to the Tukey-Kramer test (α=0.05).

5.6.3. Effects of water pH on egg quality

5.6.3.1 Egg weight

There was interaction between pH treatments and age of the birds on the egg weight (P<0.05) (Table 5.9). There was a significant effect of water pH at certain ages on the weight of the eggs produced. Initially there was no difference in egg weight among treatment groups. The egg weight for the pH 8.2 group was reduced after the first week of the study compared to the control and pH 6 groups, but no difference was observed between pH 6.5 and 8.2 treatments. After introduction of control water to the pH 8.2 hens at 67 weeks of age, egg weight increased and was similar to the other treatments. There were no difference among treatments during 68 and 69 weeks of age. Egg weight of more than 63 are graded as extra-large eggs by the Canadian egg industry (Egg Farmers of Canada 2015).

5.6.3.2 Specific gravity

There was no interaction between pH treatments and age of the birds on SG of eggs (P>0.05) (Table 5.9). Egg SG was not different among treatment groups (P>0.05). However, bird age had a significant effect (P<0.05) with a low SG at the start and 66 weeks of age compared to 67 weeks of age. During 69 weeks of age, SG decreased compared to the 67 and 68 weeks of age. No significant difference occurred from the first week to the last week of the trial. The SG ranged from 1.081-1.082 for treatment groups. SG should be at least 1.080 to be considered as good quality (Bell and Weaver 2002). SG below 1.070 would indicate thin shells, while 1.090 or more would indicate thick shells (Bell and Weaver 2002). Since the SG of the eggs of this study was above 1.080, the egg shell quality was satisfactory.

Table 5.9. The effect of adjusting water pH on specific gravity and egg weight of laying hens at 66-69 weeks of age

	Age (weeks)					
	Initial	66	67	68	69	Treatment Mean
Specific gravity						
Treatment						
pH 6.0	1.077±0.001	1.080±0.001	1.084±0.001	1.083±0.001	1.081±0.001	1.081±0.007
pH 6.5	1.079±0.001	1.082±0.001	1.083±0.001	1.083±0.001	1.082±0.001	1.082±0.007
pH 7.9	1.079±0.001	1.081±0.001	1.082±0.001	1.084±0.001	1.081±0.001	1.081±0.007
pH 8.2/7.9	1.079±0.001	1.084±0.001	1.085±0.001	1.083±0.001	1.082±0.001	1.083±0.007
Age mean	1.079±0.001 d	1.082±0.001 bc	1.084±0.001 a	1.083±0.001 ab	1.081±0.001 c	
ANOVA P value						
Treatment	0.3747					
Age	<.0001					
Treatment × Age	0.1729					
Egg weight (g)						
Treatment						
pH 6.0	63.24±0.87 bc	66.73±0.60 ab	67.83±0.94 ab	67.53±0.94 ab	66.90±1.10 abc	66.45±0.57
pH 6.5	64.70±0.87 abc	65.98±0.60 abc	67.14±0.94 abc	67.40±0.94 ab	65.97±1.07 abc	66.23±0.57
pH 7.9	65.20±0.86 abc	67.07±0.60 ab	66.67±0.97 abc	66.64±0.94 abc	67.47±1.07 abc	66.61±0.57
pH 8.2/7.9	65.42±0.86 abc	63.17±0.60 c	65.90±0.94 bc	69.40±0.94 a	67.65±1.10 ab	66.31±0.57
Age mean	64.65±0.50	65.73±0.30	66.89±0.46	67.75±0.47	67.00±0.51	
ANOVA P value						
Treatment	0.9654					
Age	0.0001					
Treatment × Age	0.0002					

[a-d] means±SEM with different letters among interaction means and week means are significantly different according to the Tukey-Kramer test (α=0.05).

5.6.3.3 Albumen height

Albumen height (AH) of the eggs (Table 5.10) were not affected by interaction between water treatments and bird age (P>0.05). AH had a significant treatment and age effect. AH reduced during 66, 67 and 69 weeks of age compared to initial and 68 weeks of age. During 66 to 69 weeks of age, AH ranged from 6.63 to 7.33 mm. Silversides and Scott (2001) reported AH decreased with increasing age of the hen. Even within this four weeks study, albumen quality fluctuated with age. AH was higher in pH 7.9/8.2 treatment compared to pH 6 treatment. There was no difference of AH among pH 6.5, 7.9 and 7.9/8.2 treatments. AH is an indicator of freshness of eggs and it is measured as the height of thick albumen portion of an egg. It is mostly important to the consumer's satisfaction, since thick white is more preferred than thinner white. Based on our results, drinking water pH of laying hen did not affect AH.

5.6.3.4 Yolk percentage

There was no interaction between pH treatments and age of the birds on yolk percentage of eggs (P>0.05) (Table 5.10). Akbar et al. (1983) found that egg yolk % increased from 26 to 30% during 24 to 63 weeks of age of laying hens. During our 4 week study, yolk % was ranged from 28 to 29%. Yolk % is not a commonly used egg quality measurement in the industry (Akbar et al. 1983). However, it is important to know how egg components including yolk, change with water pH changes.

Table 5.10. The effect of adjusting water pH on albumen height and percent yolk of eggs of laying hens from 66-69 weeks of age

	Age (weeks)					
	Initial	66	67	68	69	Treatment Mean
Albumen height (mm)						
Treatment						
pH 6.0	7.02±0.15	7.05±0.16	6.72±0.15	7.05±0.14	6.46±0.18	6.86±0.10 b
pH 6.5	7.31±0.15	6.91±0.16	7.02±0.15	7.42±0.14	6.55±0.16	7.05±0.09 ab
pH 7.9	7.33±0.15	6.87±0.16	6.75±0.15	7.25±0.14	6.55±0.16	6.95±0.09 ab
pH8.2/7.9	7.65±0.15	6.97±0.16	7.20±0.15	7.47±0.14	6.97±0.16	7.25±0.09 a
Age mean	7.33±0.07 a	6.96±0.08 b	6.92±0.07 b	7.30±0.07 a	6.63±0.08 b	
ANOVA P value						
Treatment	0.0490					
Age	<.0001					
Treatment × Age	0.1365					
Yolk percentage (%)						
Treatment						
pH 6.0	27.2±0.5	28.1±0.5	28.9±0.5	28.6±0.5	28.9±0.5	28.4±0.3
pH 6.5	27.9±0.5	28.2±0.5	28.6±0.5	28.6±0.5	28.6±0.5	28.4±0.3
pH 7.9	28.4 ±0.5	27.5±0.5	27.9±0.5	28.7±0.5	28.7±0.5	28.2±0.3
pH 8.2/7.9	27.5±0.5	28.0±0.5	28.2±0.5	27.9±0.5	28.4±0.5	28.0±0.3
Age mean	27.7±0.2	27.9±0.2	28.4±0.2	28.5±0.2	28.7±0.3	
ANOVA P value						
Treatment	0.7667					
Age	0.1319					
Treatment × Age	0.7303					

[a-b] means±SEM with different letters among week means and treatment means are significantly different according to the Tukey-Kramer test (α=0.05).

5.6.3.5 Eggshell percentage

There was interaction between pH treatments and age of the birds on eggshell % of eggs ($P<0.05$) (Table 5.11). Eggshell % was higher during 66 and 67 weeks compared to 68 week in pH 8.2/7.9 treatment. This would suggest increase in eggshell deposition when the birds in this group were at pH 8.2 compared to pH 7.9. To increase eggshell %, more shell materials should be deposited on eggs and therefore, more Ca and CO_3 need to be supplied into the shell gland. It cannot be expected that high pH in water improve the mineral absorption in the digestive tract of hens since low pH in the gut favor the mineral solubility (Guenter and Sell 1973). However, Frank and Burger (1965) found that elevated HCO_3 ion concentration in the blood increased eggshell deposition when $NaHCO_3$ supplied in drinking water. Therefore, increase in eggshell % possibly could be due to the supply of HCO_3 ions through added $NaHCO_3$ into water to adjust pH rather than due to the pH effect. No other significant interactions were observed among the treatments during 66 to 69 weeks of age of the hens. Eggshell % is an important eggshell quality measure and lower shell % values at same egg weight would cause increase in eggshell damages.

Table 5.11. The effect of adjusting water pH on eggshell percentage and albumen percentage of eggs of laying hens from 66-69 weeks of age

	Age (weeks)					
	Initial	66	67	68	69	Treatment Mean
Eggshell percentage (%)						
Treatment						
pH 6.0	9.1±0.1 bc	9.1±0.1 abc	9.4±0.1 abc	9.1±0.2 abc	9.3±0.1 abc	9.2±0.1
pH 6.5	9.4±0.1 abc	9.5±0.1 abc	9.3±0.1 abc	9.2±0.2 abc	9.3±±0.1 abc	9.3±0.1
pH 7.9	9.2±0.1 abc	9.2±0.1 abc	9.0±0.1 abc	9.0±0.2 abc	9.1±0.1 abc	9.1±0.1
pH 8.2/7.9	9.3±0.1 abc	9.7±0.1 a	9.5±0.1 ab	9.0±0.2 c	9.3±0.1 abc	9.4±0.1
Age mean	9.2±0.1	9.4±0.1	9.3±0.1	9.1±0.1	9.3±0.1	
ANOVA P value						
Treatment	0.2522					
Age	0.0017					
Treatment × Age	0.0079					
Albumen percentage (%)						
Treatment						
pH 6.0	63.7±0.5	62.8±0.6	61.7±0.5	60.9±0.3	61.0±0.7	62.0±0.3
pH 6.5	62.7±0.5	62.3±0.6	62.1±0.5	60.4±0.3	61.5±0.7	61.8±0.3
pH 7.9	62.4±0.5	63.2±0.6	63.1±0.5	60.4±0.3	61.6±0.7	62.1±0.3
pH8.2/7.9	63.0±0.5	62.2±0.6	62.3±0.5	61.3±0.3	61.2±0.7	62.0±0.3
Age mean	63.0±0.3 a	62.6±0.3 a	62.3±0.2 ab	60.8±0.2 c	61.4±0.3 bc	
ANOVA P value						
Treatment	0.8798					
Age	<0.0001					
Treatment × Age	0.0763					

[a-c] means±SEM with different letters among interaction means and week means are significantly different according to the Tukey-Kramer test (α=0.05).

5.6.3.6 Albumen percentage

There was no interaction between pH treatments and age of the birds on albumen % of eggs (P>0.05) (Table 5.11). The low water pH alone did not affect albumen % either (P>0.05). As bird become older, the albumen % changed (P<0.05). When compared to initial and week 66 measurements, 68 and 69 weeks albumen % were significantly lower. Akbar et al. (1983) reported that increment in yolk % and decline in eggshell and albumen % occurred with age in a long term study from 25 to 95 weeks of age of hens. In this short study, only the albumen % declined with bird age.

5.6.3.7 Eggshell breaking strength

Eggshell breaking strength, an important indicator of eggshell quality did not differ among treatments (P>0.05) (Table 5.12). Further, there was no effect of bird age or an interaction between water treatment and bird age on the shell strength (P>0.05). The average force required to break the egg shell was about 4.2 to 4.8 kg force in all treatment groups throughout the trial.

The breaking strength of the egg of Lohmann white hen eggs was about 4.3 kg force (Ketta and Tůmová 2014). According to the breeder recommendations, Lohmann white laying hen eggs should have breaking strength of at least 40 Newton, which was equal to 4.08 kg force (Lohmann Tierzucht 2013). Quality of the eggshell is important to the egg industry since, poor quality can result in losses and reduce profits to the farmer (Dhawale 2008).

Table 5.12. The effect of adjusting water pH on eggshell breaking strength and eggshell thickness of laying hens at 66-69 weeks of age

	Age (weeks)					
	Initial	66	67	68	69	Treatment Mean
Breaking strength (kg force)						
Treatment						
pH 6.0	4.52±0.14	4.19±0.23	4.59±0.16	4.73±0.20	4.40±0.14	4.49±0.09
pH 6.5	4.25±0.14	4.28±0.23	4.49±0.16	4.67±0.20	4.51±0.14	4.44±0.09
pH 7.9	4.47±0.14	4.54±0.23	4.07±0.16	4.19±0.20	4.12±0.14	4.28±0.09
pH 8.2/7.9	4.83±0.14	4.67±0.23	4.47±0.16	4.44±0.20	4.11±0.14	4.50±0.09
Age mean	4.51±0.07	4.42±0.11	4.41±0.07	4.51±0.10	4.29±0.07	
ANOVA P value						
Treatment	0.2825					
Age	0.2499					
Treatment × Age	0.1206					
Eggshell thickness (mm)						
Treatment						
pH 6.0	0.560±0.01 cd	0.610±0.01 ab	0.345±0.01 e	0.340±0.01 ef	0.317±0.01 ef	0.435±0.01
pH 6.5	0.525±0.01 d	0.595±0.01 abc	0.327±0.01 ef	0.337±0.01 ef	0.330±0.01 ef	0.423±0.01
pH 7.9	0.545±0.01 d	0.610±0.01ab	0.307±0.01 f	0.305±0.01 ef	0.305±0.01 f	0.414±0.01
pH 8.2/7.9	0.565±0.01bd	0.605±0.01 ac	0.330±0.01ef	0.325±0.01ef	0.310±0.01 ef	0.427±0.01
Age mean	0.549±0.01	0.605±0.01	0.327±0.01	0.327±0.01	0.317±0.01	
ANOVA P value						
Treatment	0.0038					
Age	<.0001					
Treatment ×Age	0.0294					

[a-f] means±SEM with different letters among interaction means are significantly different according to the Tukey-Kramer test (α=0.05).

6.3.8 Eggshell thickness

There was interaction between pH treatments and age of the birds on eggshell thickness ($P<0.05$) (Table 5.12). However, the difference was observed only between control and pH 6 group during 67 weeks of age where shell thickness was lower in pH 7.9 group than pH 6 group. Shell thickness decreased after 66 weeks of age in all groups. A reduction of shell thickness with the age was reported by Hamilton (1982). At the start of the trial shell thickness was about 0.5 mm and at the end of first week it was about 0.6 mm in all groups. But, at the end of 67 weeks of age it was reduced up to 0.327 and did not change much during 68 and 69 weeks of age.

The reported eggshell thickness ranged from 0.3 to 0.4 in the literature (Um and Paik 1999; Zamani et al. 2005; Kaur et al. 2013; Ketta and Tůmová 2014). In our study, the eggshell thickness during the initial and the first weeks were higher (0.5 to 0.6 mm) than those reported range, but after 66 weeks of age, eggshell thickness was about 0.3 mm among all treatments during the remainder of the trial. An error occurs during height calibration of the texture analyzer could lead to a higher eggshell thickness measurement.

Percent soft-shelled (Table 5.13) was not affected by water pH ($P>0.05$). The values were about 1% or below in all treatment groups. Percent broken eggs was affected by age of birds (Table 5.14). During 68 and 69 weeks broken egg % were higher compared to 66 and 67 weeks of age. However, eggshell breaking strength was not affected by age of the birds.

Table 5.13. The effect of adjusting water pH on soft shelled eggs percentage of laying hens at 66-69 weeks of age

	Broken eggs (%)				
	Age (weeks)				
Treatment	**66**	**67**	**68**	**69**	**Mean**
pH 6.0	0.4±0.4	0.0±0.4	1.4±0.4	0.7±0.4	0.6±0.2
pH 6.5	0.4±0.4	0.0±0.4	0.9±0.4	0.9±0.4	0.5±0.2
pH 7.9	0.2±0.4	0.2±0.4	0.7±0.4	1.2±0.4	0.6±0.2
pH 8.2/7.9	0.0±0.4	0.0±0.4	0.5±0.4	0.3±0.4	0.2±0.2
Age mean	0.3±0.2 b	0.0±0.4 b	0.8±0.4 a	0.8±0.4 a	
	ANOVA				
Treatment	0.3301				
Age	0.0409				
Treatment × Age	0.9527				

Table 5.14. The effect of adjusting water pH on broken eggs percentage of laying hens at 66-69 weeks of age

	Soft shelled eggs (%)				
	Age (weeks)				
Treatment	**66**	**67**	**68**	**69**	**Mean**
pH 6.0	0.6±0.5	1.1±0.5	0.9±0.5	0.7±0.5	0.8±0.3
pH 6.5	0.2±0.5	0.6±0.5	0.4±0.5	0.5±0.5	0.4±0.3
pH 7.9	0.8±0.5	1.5±0.5	2.1±0.5	0.9±0.5	1.3±0.3
pH 8.2/7.9	1.9±0.5	1.1±0.5	0.5±0.5	0.5±0.5	1.0±0.3
Age mean	0.9±0.3	1.1±0.3	1.0±0.3	0.6±0.3	
	ANOVA				
Treatment	0.3767				
Age	0.6384				
Treatment × Age	0.3940				

5.7 General discussion

The trial was conducted as an initial step to plan a study to evaluate the effect of water pH on laying hen performance. pH 6, 6.5, 7.9 and 8.2 were tested with Lohmann-Lite white laying hens at 66 weeks of age until 69 weeks of age in a completely randomized experiment.

A pH of 8.2 significantly reduced feed consumption, water consumption, body weight, egg production and egg weight. Since this pH treatment caused high mortality (5%), it was terminated on day 5. Hens were transferred to the pH 7.9 group and monitored for the remainder of the trial. Based on the survey results (Chapter 03 section 3.6), pH of water from some commercial egg farms were more than 8.2. Hayat et al. (1999) and Junqueira et al. (1984) found high mortality rate when fed 10 g/L of $NaHCO_3$ in water to broilers and 16 g/kg $NaHCO_3$ in diet to laying hens and found alkalosis in birds in the both studies. These levels are similar to the level (12 g/L) of $NaHCO_3$ that we used to increase water pH to 8.2. Therefore the negative effects occurred during our study may be due to the alkalogenic effect of $NaHCO_3$ on birds. Further studies need to be conducted to determine the pH 8.2 effects on laying hen performance with alternative sources to adjust pH.

Low pH of 6 and 6.5 did not affect body weight, feed consumption or water consumption, when compared to the pH 7.9 water. Specific gravity, egg weight, albumen height, percentage of yolk, eggshell or albumen, and breaking strength were not affected by pH 6, 6.5 and 7.9. Shell thickness was reduced by pH 6 compared to pH 7.9 only during 77 weeks of age. There was no difference among pH 8.2 and other treatments on production performance and egg quality after hens were transferred to pH 7.9 treatment.

The water currently being used by commercial egg production units across Canada contained pH from 5.97 to 9.20 (Chapter 3 section 3.6). Based on the results of this study, it can be recommended that from pH 6 to 7.9 is safe for laying hens when water pH lowered using citric acid. However, different effects could occur under different conditions when water pH is lowered by means of other sources such as minerals. Future studies need to be conducted to verify the impacts of water pH 8.2 since the negative effects occurred during our study was observed when water pH increased using $NaHCO_3$. Furthermore, this was a short study conducted at the end of the production cycle, therefore, a full production cycle should be conducted to determine the long term effects of water pH during different stages of egg production.

5.8 Conclusions

Adjusting water pH with citric acid at levels from 6 to 7.9 did not show negative effects on production performance or egg quality of laying hens during late production. Adjusting pH 8.2 with sodium bicarbonate decreased laying hen performance.

CHAPTER 6 EFFECT OF WATER pH AND HIGH CONTENT OF CALCIUM, MAGNESIUM AND SULPHATE IN DRINKING WATER ON MINERAL RETENTION AND PERCENT RETENTION OF LAYING HENS AT DIFFERENT PHASES OF PRODUCTION

6.1 Abstract

Impacts of extremes of pH and dissolved minerals in drinking water on laying hen mineral metabolism have not been well examined. Therefore, effects of high and low water pH or high content of Ca, Mg and SO_4 in water on apparent mineral retention and percent mineral retention of total intake in laying hens were evaluated in 4 balance studies. The effect of water pH 6, 6.5 and 7.9 on mineral retention and percent mineral retention was evaluated at the end of the pH production study (69 weeks of age) with Lohmann-Lite laying hens in a completely randomised experiment with 4 replications. Excreta and eggs were collected each experimental unit for 4 days. Mineral content of excreta, eggshell, egg content and feed samples were determined using Inductively Coupled Argon Plasma - Optical Emission Spectrometry (ICP-OES). Mineral intake from feed and output through excreta and eggshell plus egg content were calculated. Daily mineral retention of Ca, Mg, P, K, Na, SO_4, Fe, Cu, Mn and Zn were determined by total intake minus total output of a mineral. Percent retention of a mineral was calculated as the percent of mineral retained of total intake. There was no effect of water pH on mineral retention in the hens except for Fe. Fe retention decreased in pH 6 treatment when compared to the both pH 6.5 and 7.9 treatments ($P<0.05$). The birds were in positive mineral retention at the late production. To evaluate the effects of Ca, Mg and SO_4 in water, 3 balance studies were conducted during the water mineral study, trial 1, at 42, 55 and 70 weeks of age of hens. Five water treatments: well water (control), 625 ppm $MgSO_4$, 1250 ppm $MgSO_4$, 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$ and 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$, were tested using Lohmann-Lite hens in a completely randomised experiment with 6 replications. Excreta and eggs were collected for 4 days and samples were analysed for the same minerals as described in pH mineral balance study using ICP-OES. At 42 weeks, only the SO_4 retention and percent retention was affected ($P<0.05$). The birds were in positive SO_4 balance among all the treatments. SO_4 retention and percent retention increased in groups given 1500 ppm SO_4 compared to the control. At 55 weeks, Mg and SO_4 retention and percent retention were significantly affected by water treatments ($P<0.05$). Mg retention and percent retention increased in groups given the highest Mg concentration in water (250 ppm). SO_4 retention and percent retention increased in the groups given high SO_4 containing water (1000-1500 ppm). At 70 weeks, similar to what occurred at 55 weeks, Mg retention and percent retention increased in the hens given 250 ppm Mg water treatment than the control. Hens in the high Mg and high Mg Ca groups retained more SO_4 than the control birds. Therefore, based on these results, high Mg and SO_4 in water significantly increased the retention and retention percent of Mg and SO_4. However, retention or retention percent of other minerals were not affected by the high Ca, Mg and SO_4 content in the drinking water at any stage and birds were in positive balance. Apparent retention of Na, P, Fe and SO_4 increased with bird age while K and Mg retention decreased with the age.

Key words: laying hens, mineral retention, minerals in water, water pH

6.2 Introduction

Minerals, are associated with the skeletal system, extracellular and intracellular fluids of animals (Georgievskiĭ et al. 1981). Minerals, such as Mg and Zn are important for many enzyme activities. Mg and Zn are involved with carbohydrate metabolism and egg shell formation, respectively (Georgievskiĭ et al. 1981). The requirements for minerals depend on the physiological state of the bird (NRC 1994). High production rates in modern commercial laying hens lead to high demands for minerals, such as calcium (Leeson and Summers 2008). Minerals can be ingested through feed and water into the body. Once absorbed and transferred into plasma, minerals can be either used for metabolism or stored. Bones store Ca in laying hens and can release Ca for eggshell formation when plasma Ca level is lowered (Hurwitz and Bar 1966). It is important for poultry to maintain adequate amounts of minerals in body fluids for normal metabolism. Mineral levels are maintained by regulating absorption in the digestive tract or excretion through feces and urine (Hurwitz 1970). Bones are important in maintaining mineral homeostasis of calcium in laying hens (Hurwitz and Bar 1966).

The balance study technique can be used to evaluate mineral metabolism by determining mineral balance or retention in the bodies of laying hens (Świątkiewicz et al. 2010). Retention of minerals is calculated by measuring intake and output contents. Minerals in feces and urine can originate either from diet or endogenous sources, such as gastric juices and pancreatic secretions (Guenter and Sell 1973). Minerals can be deposited in products such as eggs and are lost from the body. Apparent balance or retention of minerals can be calculated without estimating endogenous loss of minerals into the digestive tract (Liu et al. 2012). A positive balance would indicate retention of mineral in the body or adequate

intake, while a negative balance would suggest utilization of body mineral reserves when intake is inadequate.

High levels of Ca and Mg were known to reduce absorption of other minerals, such as Fe, I, Mg, Mn, P and Zn (McDowell 1992). High Mg or Ca with SO_4 in the water may cause diarrhoea in poultry and this may lead to poor nutrient absorption (Weltzien 2002). Concentrations of mineral ions in plasma and body fluids are important in regulating the acid-base balance (Mongin 1981). Cations, such as Ca and Mg, are known as alkali producing ions, while anions such as sulphate and phosphate are acidogenic. An appropriate range of acid-base balance should be maintained for normal metabolic functions. Excess intake of minerals may affect the balance. SO_4 is an acidic ion which reduced pH in the blood of chickens (Ruíz-lópez and Austic 1993). The changes in acid-base balance in the blood can affect eggshell calcification in laying hens (Kesharvaz and Austic 1990).

In natural water, Ca, Mg and SO_4 can be found in high amounts (USEPA 2012) and pH can vary from 5.97 to 9.21 (data from the current cross-Canada survey of this project). The effects of high concentrations of Ca, Mg and SO_4 and different pH levels in drinking water, on mineral balance and retention in laying hens, are not well known. Therefore, the present study evaluated the effects of Ca, Mg and SO_4 minerals on the balance and retention of Ca, Mg, K, P, Na, SO_4, Cu, Fe, Mn and Zn in early, mid and late production phases. These minerals are considered to be essential for laying hen performance (NRC 1994). Water pH 6, 6.5 and 7.9 were evaluated at the end of production of the laying hen production cycle.

6.3 Objectives

1. To determine the effect of three different pH levels: 6, 6.5 and 7.9 in drinking water on the apparent retention and retention percent of macro and micro minerals including; Ca, Mg, P, K, Na, SO_4, Cu, Fe, Mn and Zn at the late phase of production.
2. To determine the effect of high Ca, Mg and SO_4 ions in drinking water on apparent retention and retention percent of macro and micro minerals including: Ca, Mg, P, K, Na, SO_4, Cu, Fe, Mn and Zn at early, mid and late phases of production.

6.4 Hypothesis

1. pH levels of 6, 6.5 and 7.9 in drinking water of laying hens will change the apparent retention and retention percent of macro and micro minerals including Ca, Mg, P, K, Na, SO_4, Cu, Fe, Mn and Zn during the late stage of egg production.
2. High levels of Ca, Mg and SO_4 ions in drinking water of laying hens will change apparent retention and retention percent of macro and micro minerals including Ca, Mg, P, K, Na, SO_4, Cu, Fe, Mn and Zn at any stage of the production.

6.5 Materials and Methods

6.5.1 Experimental design, treatments and hen management

To determine the effect of Ca, Mg and SO_4 ions on mineral retention and percent retention in laying hens at different stages of production, 3 mineral balance studies were conducted at 42, 55 and 70 weeks of age during the first water mineral trial that evaluated high Mg, Ca and SO_4 effect in drinking water on production performance and egg quality of laying hens from 33 to 69 weeks (Chapter 4). Water treatments and mineral levels were as described in Chapter 4 section 4.5.1.2.

For the evaluation of pH effect on mineral balance at late stage of production of laying hens, a mineral balance study was conducted at the end of the pH study that evaluated the water pH effect on production performance and egg quality (Chapter 5). Only pH 6, 6.5 and 7.9 were evaluated for mineral balance since pH 8.2 was removed from the trial. The treatment preparation was as described in chapter 5 section 5.5.2.

6.5.2 Sample collection and preparation

Total excreta and total eggs produced were collected from under each cage unit over a period of 4 days. On the first day of the balance study week, pre-weighed metal trays were placed on the manure belt, underneath each cage unit at 0800 h. Excreta were scraped from the metal trays below the cage, thoroughly mixed, weighed and a representative sample collected in plastic containers. The samples were then frozen at -20°C until mineral analysis was conducted. Feed and water were weighed back in the morning at 0830 h and 0930 h respectively. Weighed amounts of feed were added daily for 4 days. Birds were weighed at 1300 h in the afternoon.

All the eggs were collected and numbered daily from each cage unit after excreta collection. Eggs from each experimental units were weighed, egg shells removed and egg content was collected into a labelled freezer bag. Egg shell weight was recorded and shells collected into labelled freezer bags. The samples were pooled over the four days collection and frozen at -20°C until analysis.

On the last day, feed and water were weighed back in the morning in order to determine feed and water consumptions. Birds were weighed in the afternoon in order to determine body weight changes. Feed samples collected for mineral analysis were kept in freezer at -20°C until analysis.

Excreta samples were thawed at room temperature, weighed and refrozen at -20°C for 12 hours before being placed on a freeze dryer. The samples were then freeze dried for 24 to 48 hrs, weighed, ground and pooled. Egg contents were thawed at room temperature, homogenised in a blender. Then a representative sample was weighed into a pan and frozen at -20°C for 12 to 24 hrs. Frozen samples were then dried for 24 to 48 hrs in the freeze dryer, weighed and ground. Eggshells were thawed at room temperature and dried at 65°C for 24 hrs. Dried egg shells were weighed, and ground using a coffee grinder.

6.5.3 Mineral analysis of excreta, eggshells and egg content

Mineral content of excreta, eggshell, egg content and feed was determined by the AOAC method 968.08) (AOAC 2005). In duplicate, 1g of excreta, 1g of egg content and 0.35g of eggshell samples were ashed overnight in a muffle furnace at 550°C. Ash samples were moistened with distilled water and transferred into a 200 mL beaker. 1:3 diluted hydrochloric acid (HCl) was prepared by diluting 500 mL of concentrated HCl (Trace metal grade concentrated HCl, Fisher Scientific Company, Ottawa, Ontario) in to 1500 mL distilled water. Forty mL of diluted HCl and a few drops of concentrated nitric acid (Trace metal grade concentrated HNO_3, Fisher Scientific Compay, Ottawa, Ontario) were added. The mixture was placed on a hot plate covered with a watch glass and boiled for 10 minutes, to solubilize the minerals in the sample. The mixture was cooled then filtered (using Whatman #42 ashless filter paper) into 250 mL flasks and made up to volume 250 mL with distilled water. Beakers, watch glasses and filter papers were rinsed several times with distilled water to avoid losses during the filtering process. Then a sample was filled into scintillation vials and sent to Nova Scotia Department of Agricultural lab for mineral analysis. Inductively Coupled Argon Plasma analyzer (ICAP) (Varian Vista Pro Axial ICP-

OES, Agilent Technologies Inc., USA) was used for the analysis. The same method was used to analyse minerals in feed samples for each stage. For the analysis of mineral content of water, ICP-OES method was used as described in chapter 3.

Individual Mineral balance (A) and % Individual Mineral Retention (B) were determined by the following equations (Saunders-Blades 2002). Total mineral intake was calculated as intake from feed and water. Total output includes minerals in excreta and eggs.

(A) Mineral retention (g) = total mineral intake (g) − total mineral output (g)

(B) % Mineral retention $= \frac{\text{total mineral intake} - \text{excreta plus egg output}}{\text{total mineral intake}} \text{ X } 100$

6.5.4 Statistical analysis

The experimental design was a completely randomised design with 6 replications. The mineral retention and percent retention data were subjected to ANOVA using Proc Mixed procedure of the SAS version 9.3 (SAS Institute Inc., Cary, NC) (Littell et al. 1996) with water mineral treatment as the main effect. The statistical model used for measurements was:

$Y_i = \mu + \tau_i + \varepsilon_{ij}$, where Y_i is the variable of interest; μ is the overall mean; τ_i is the effect of i^{th} water mineral treatment; ε_{ij} is the random effect of error with j representing replicate measurements. If the significant main effects were found, the Tukey-Kramer procedure was used to compare differences among the least square means. The α-level of significance was 0.05 (Gbur et al. 2012).

For repeated measures analysis, the factor of time and resulting interaction levels were added (bird age as the measure of time, k) to the model. Five covariance structures, Compound Symmetry, Heterogeneous Compound Symmetry, Toeplitz, Heterogeneous

Toeplitz and Ante-dependence were compared. For the ANOVA, the covariance structure which gave the smallest corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) numbers was selected. The mineral retention data for the three balance studies at early, mid and late phases in first water mineral trial were analysed using repeated measures analysis. The statistical model used for repeated measures analysis as follows.

$Y_i = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$, where Y_i is the variable of interest; μ is the overall mean; τ_i is the effect of i^{th} water treatment (i =1-3); β_j is the effect of time (j =1-4); $(\tau\beta)_{ij}$ is the effect of the interaction between water treatments and time; and ε_{ijk} is the random effect of error with k representing replicate measurements (k =1-4).

The retentions of Ca, Mg, SO_4, Na, P and K were analyse using Compound Symmetry covariance structure while Cu, Fe, Mn and Zn retentions were analysed using Heterogeneous Compound Symmetry.

6.6 Results and Discussion

6.6.1 Effect of water pH on mineral retention and percent retention

The apparent retention data for macro minerals were presented in g/hen/day on a dry matter basis while for micro minerals in mg/hen/day on a dry matter basis. The feed, excreta and egg samples were analyzed using ICP-OES (AOAC 2005). By using this method, mineral concentrations can be measured in ppm. There were trace minerals in egg samples, which the reference method could not detect due to concentrations below the minimum detectable limits. These were identified as ND in the results tables. The mineral loss through panting were considered negligible, since hens were housed in a thermo neutral temperature at the poultry facility (22 to 24°C). Losses via feathers were not considered since there was no

significant feather loss observed during the trial. Endogenous losses of minerals were not considered for the calculations. Therefore, apparent mineral retention and percent retention were presented. The major routes of mineral excretions were through feces, urine and eggs. Since urinary and fecal mineral excretions of the hens could not be separated under normal conditions, excreta accounted for the losses through both urine and feces.

The mineral composition was same as the pH production trial, since the same diet was used for the balance study (Table 6.1). The intake of mineral was mainly from the common diet fed to all birds. Since the same diet was given to the all treatment groups, mineral levels in the diet for the treatment groups were the same within each phase. The actual intake of a mineral varied with feed and water consumption of the cage of birds. The output varied with amount of excreta produced and egg production of the hens within each phase. The feed consumption, water consumption, hen day egg production, egg weight and shell weight for treatments during the balance week were reported (Table 6.2).

6.1. Analysed mineral concentrations of diet used in balance study at 69 weeks of age of laying hens in pH trial[1]

Nutrient	ppm
Calcium	43000
Phosphorus (available)	3350
Sodium	1675
Potassium	5500
Magnesium	1851
Sulphate	1800
Manganese	145
Copper	53
Zinc	109
Iron	205

[1]Diets were analysed in duplicate using ICP-OES.

Table 6.2. The feed intake, water intake, egg weight, egg production and shell weight during balance study at 69 weeks of age of hens[1]

Treatment	Feed consumption (g/bird/day)	Water consumption (mL/bird/day)	Egg production (%)	Egg weight (g)	Shell weight (g)
pH 6	124±2	208±4	76.2±2.0	67.42±1.1	6.30±0.1
pH 6.5	121±2	195±4	79.5±2.0	65.97±1.1	6.13±0.1
pH 7.9	124±2	206±4	80.6±2.0	67.43±1.1	6.16±0.1
ANOVA P value					
Treatment	0.5863	0.1118	0.3220	0.5768	0.6818

[1]Means ±SEM.

6.6.1.1 Calcium

Water pH treatments did not affect Ca intake of the hens (P>0.05) (Table 6.3). The Ca intake ranged from 5.10 to 5.23 g/hen/day in all treatments. Similar feed consumption by the hens in different groups (Table 6.2) resulted in similar Ca intake among hens.

Ca excretion via feces plus urine was not affected by low water pH 6 and 6.5 compared to pH 7.9 (P>0.05). Hens excreted 2.27 to 2.30 g of Ca daily which represents 44 to 45% of the Ca intake. Similarly, Neijat et al. (2011) found that the Ca excretion of the caged laying hens from 20 to 63 weeks was 2.29 g/hen/day.

Eggshell Ca deposition was not affected by water pH level (P>0.05). In the eggshell, 2.13 to 2.29 g of Ca was deposited by the hen daily. Hurwitz (1970) found about 2 g of Ca was deposited in an eggshell. Neijat et al. (2011) also found that hens deposited 2.2 g of Ca on eggshells. Both the literature eggshell Ca and reported for the current study were similar.

Table 6.3. The effect of water pH on Ca intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	----------g/bird/day----------						(%)
PH 6	5.23±0.06	2.39±0.11	2.13±0.07	0.030±0.00	4.55±0.12	0.69±0.11	13.1±2.1
pH 6.5	5.10±0.06	2.27±0.11	2.29±0.07	0.034±0.00	4.59±0.13	0.51±0.12	10.1±2.4
pH 7.9	5.23±0.06	2.30±0.11	2.24±0.07	0.029±0.00	4.57±0.12	0.66±0.11	12.5±2.1
ANOVA P Value							
Treatment	0.3866	0.7425	0.2232	0.2885	0.9764	0.5829	0.6328

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

The amount of Ca deposited in egg yolk and white did not differ among treatments (P>0.05). The Ca in the yolk and white varied from 29 to 34 mg/egg. The analyzed Ca content in eggs of the current study was similar to the values reported by Stadelman (1995) and Neijat et al. (2011). Neijat et al. (2011) found that the Ca amount that was deposited in yolk and white were about 34 and 4 mg respectively. According to Stadelman (1995), most of the Ca was found in egg yolk (about 25 mg) with little in egg white (about 3.8 mg). In the current study, 2.1 to 2.3 g of Ca was removed daily from the hen via egg production. From 41 to 45% of feed Ca was deposited in the egg. Part of the Ca for the shell calcification comes from the bone reserves, since most of the shell is deposited during the night, when feed Ca is limited for the hens (Hurwitz 1970). Therefore, part of the feed Ca ingested daily, must be used to replenish the bone Ca, that was used for the shell formation during the previous day (Hurwitz 1970).

Ca Output was not different among treatments (P>0.05). That was expected since the excreta Ca and egg Ca contents were not changed. Hens at 69 weeks of age, excreted 4.6 g of Ca within a day through the excreta and eggs.

Based on total intake and output of Ca, the hens were in positive Ca balance in all treatments. There was no effect of water pH on Ca retention of laying hens at 69 weeks of age (P>0.05). Hens retained 0.51 to 0.69 g of calcium in their body daily. Retention percentage of total Ca intake was not affected by the water pH changes (P>0.05). The percentage of retention was 10 to 13% among all water pH treatments. Keshavarz (1986) demonstrated that Ca retention by hens was 2.01 g, when given 4.5% Ca in the diet. The retention was calculated only using excreta Ca output of the hens. The retention percentage was 45% of total intake. Um and Paik (1999) also found hens retained 2.5 to 3.5 g of Ca,

without considering Ca loss via eggs, in a study that evaluated effects of phytase enzyme on mineral retention in laying hens. In the current study, the retention of Ca calculated without considering egg loss ranged from 2.83 to 2.94 g.

Positive Ca balance is important in laying hens to have good productivity (NRC 1994). Positive retention indicates hens had enough Ca to produce sound egg shell and to replenish bone Ca, which had been used during eggshell formation during the night, when feed Ca was not consumed. If a hen is in calcium deficiency, bone Ca is extensively utilized for egg production (Hurwitz and Bar 1966) and if not replenished may lead to bone weaknesses. Therefore, the intestinal absorption of the Ca was not affected, when low pH drinking water at 6 and 6.5 was provided compared to well water at pH 7.9.

6.6.1.2 Magnesium

Mg retention or retention percentage was not affected by water pH level (P>0.05) while birds were in positive Mg retention among all treatments (Table 6.4). The hens retained about 0.01 to 0.02 g of Mg per day regardless of the water pH levels. The percentage of retention ranged from 3.7% to 10% among the hens in all treatment groups. Therefore, water pH levels did not affect Mg retention or retention in hens at 69 weeks of age.

Mg intake was not affected by water pH levels (P>0.05). The intake of Mg through the diet was 0.20 g in all groups. All hens received the same diet and feed intake was similar among treatment groups (106 to 108 g/bird/day) (Table 6.1). The total output was not different (P>0.05) in the three treatment groups since Mg content in excreta, eggshell and egg content were almost same (P>0.05). The output varied from 0.18 to 0.19 g/hen/day.

Table 6.4. The effect of water pH on Mg intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	----------g/bird/day----------						(%)
PH 6	0.20±0.01	0.17±0.01	0.02±0.00	0.005±0.00	0.19±0.01	0.01±0.01	3.7±2.9
pH 6.5	0.20±0.01	0.16±0.01	0.02±0.00	0.006±0.00	0.18±0.01	0.01±0.01	10.1±2.9
pH 7.9	0.20±0.01	0.16±0.01	0.02±0.00	0.005±0.00	0.18±0.01	0.02±0.01	8.1±2.9
ANOVA P Value							
Treatment	0.5459	0.5087	0.2143	0.1576	0.6679	0.7980	0.3600

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake

The daily Mg excretion per hen was 0.16 to 0.17 g. Um and Paik (1999) found that a hen at 40 weeks of age excreted 0.27 g of Mg in a day. These hens were supplied a similar diet to that used in the current study. The retention of Mg in the body and eggs was 0.25 g/hen/day. The Mg content in eggs were not reported in their study. According to the results in the current study, the retention of Mg ranged from 0.1 to 0.2 g/hen/day without considering egg Mg output.

Mg content in eggshell or content was not affected by the water pH levels (P>0.05). Through each egg, 0.025 to 0.026 g was removed daily from the hen. Eggshell Mg was about 0.02 g while 0.005 to 0.006 g was in egg contents. Similar to the Mg content obtained in egg content in our study, Stibilj et al. (2002) found that Mg in egg yolk and white was about 0.003 mg.

6.6.1.3 Phosphorus

Total P intake and output were not affected by water pH treatments (P>0.05) (Table 6.5). Meanwhile, excreta, eggshell or egg content P contents were not different among the groups (P>0.05). All treatment groups had positive P retention without differences among the treatments (P>0.05). P retention ranged from 0.03 to 0.04 g/bird/day. The retention percentage for P was not affected by water pH and the percentage retention at pH 6, 6.5 and pH 7.9 were 9.8%, 11.7% and 11.4%, respectively. Lim et al. (2003) reported that hens at 30 weeks of age had daily P retention of 0.23 g while excreta P was 0.34 g/hen/day, when P% in the diet was 0.25%, which is lower compared to our diet P% (0.3%). They determined P retention based on the excreta P output only. In the current study, we found less daily P retention (0.13 to 0.15 g/hen/day), without considering P output in eggs compared to Lim et al. (2003).

Table 6.5. The effect of water pH on P intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------						(%)
PH 6	0.36±0.01	0.23±0.01	0.005±0.0	0.09±0.01	0.33±0.01	0.03±0.01	9.8±2.8
pH 6.5	0.35±0.01	0.20±0.01	0.006±0.0	0.11±0.01	0.31±0.01	0.04±0.01	11.7±2.8
pH 7.9	0.36±0.01	0.23±0.01	0.005±0.00	0.09±0.01	0.32±0.01	0.04±0.01	11.4±2.8
ANOVA P Value							
Treatment	0.5459	0.3114	0.2482	0.0731	0.7234	0.9023	0.8888

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

6.6.1.4 Sodium

Na retention and percent retention were not affected by water pH treatment groups (P>0.05) (Table 6.6). The birds were in positive Na retention. Excreta, eggshell or egg Na contents were similar among the treatments (P>0.05). Daily Na excretion via faeces and urine ranged from 0.07 to 0.09 g per hen. Eggshell contained 0.01g of Na in all the groups. Egg content Na ranged from 0.06 to 0.08 g. Na is important for acid base balance in body fluids of birds and number of studies have been conducted to evaluate NaCl in water on laying hen performance. Those studies found that high content of Na in water affect shell quality by influencing carbonic anhydrase activity, through changes in acid base balance of the shell gland of the hens (Yoselewitz and Balnave 1989a). However, no information was found in the literature on water pH effect on Na balance or retention of laying hens.

6.6.1.5 Potassium

There was no pH effect on K retention or retention percentage in the laying hens (P>0.05) (Table 6.7) and birds were in positive K retention. The retention for K ranged from 0.03 to 0.05 g/hen/day while percent retention was from 5.2% to 8.5% among the treatments. The K content in excreta, eggshell or egg content were not affected by water pH. Therefore, low pH of 6 and 6.5 would not affect K metabolism in the laying hens. K is important electrolyte in acid base balance of the body fluids. K are known is known as alkalogenic ion (Mongin 1981). Changes in acid base balance can reduce the eggshell quality (Gezen et al. 2005).

Table 6.6. The effect of water pH on Na intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------						(%)
PH 6	0.18±0.00	0.09±0.00	0.01±0.00	0.06±0.00	0.16±0.01	0.02±0.01	12.6±4.7
pH 6.5	0.18±0.00	0.07±0.00	0.01±0.00	0.08±0.00	0.16±0.01	0.02±0.01	10.9±4.7
pH 7.9	0.18±0.00	0.08±0.00	0.01±0.00	0.06±0.00	0.15±0.01	0.03±0.01	14.2±4.7
ANOVA P Value							
Treatment	0.5459	0.1618	0.7491	0.1941	0.9745	0.8918	0.8983

[1]Means±SEM
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content
[4]Retention = (intake - output)
[5]Retention % = retention in the body compared to the intake.

Table 6.7. The effect of water pH on K intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	----------g/bird/day----------						(%)
PH 6	0.59±0.01	0.49±0.01	ND	0.07±0.01	0.56±0.01	0.03±0.01	5.2±1.9
pH 6.5	0.59±0.01	0.49±0.01	ND	0.07±0.01	0.56±0.01	0.04±0.01	7.3±1.9
pH 7.9	0.60±0.01	0.48±0.01	ND	0.06±0.01	0.55±0.01	0.05±0.01	8.5±1.9
ANOVA P Value							
Treatment	0.5486	0.8498		0.6695	0.7520	0.4242	0.4400

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.
ND = not detected (concentration was below the minimum detectable limit of analysis method).

6.6.1.6 Copper

Cu retention and percent retention were not affected by the water pH (P>0.05) (Table 6.8). The Cu content in excreta, eggshell and egg content were not affected by water pH. Cu is important in laying hen nutrition and egg quality. Cu deficiency cause anaemia and poor eggshell and eggshell membrane quality in laying hens (Baumgartner et al. 1978). There was no recommendation for daily Cu requirement for laying hens in NRC (1994). Lohmann-Lite laying hen management guide recommended 5mg of Cu supplementation per kg of diet. However, the hens in our study had positive balance of 2 to 3 mg/hen/day.

6.6.1.7 Iron

Fe retention or percent retention was affected by water treatments (P<0.05) (Table 6.9). The Fe retention and percent retention in the pH 6 group were negative and lower than the pH 7.9 group. There was no significant difference in Fe retention and percent retention between pH 6 and 6.5 or between pH 6.5 and 7.9 treatments.

The eggshell Fe content was significantly affected by the water pH (P<0.05). The Fe content in eggshells of pH 6 treatment was higher compared to pH 6.5 and 7.9. Further, there was a trend of increasing Fe content in eggshells with low water pH. Low dietary pH cause improved mineral absorption of birds (Dibner and Buttin 2002). Therefore it could be speculate low pH 6 in water may have effect on solubility of Fe in the digestive tract which enhance the absorption of Fe. However, egg content Fe concentration did not increase. Egg yolk is rich source of Fe and Fe is deposited in egg yolk by binding to phosvitin protein for the use of embryo (Vieira 2007).

Table 6.8. The effect of water pH on Cu intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	----------mg/bird/day----------						(%)
PH 6	5.78±0.01	3.41±0.01	ND	ND	3.41±0.01	2.27±0.01	39.3±4.0
pH 6.5	5.66±0.01	2.89±0.01	ND	ND	2.89±0.01	2.93±0.01	51.7±4.0
pH 7.9	5.80±0.01	3.43±0.01	ND	ND	3.43±0.01	2.37±0.01	41.0±4.0
ANOVA P value							
Treatment	0.5459	0.3336			0.3336	0.2277	0.1561

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - Output).
[5]Retention % = retention in the body compared to the intake.
ND = not detected (concentration was below the minimum detectable limit of analysis method).

Table 6.9. The effect of water pH on Fe intake, output, retention and percent retention of the laying hens at 69 weeks of age

Treatment	Intake[1]	Excreta	Eggshell	Egg content	Output[2]	Retention[3]	Percent retention[4]
	----------mg/bird/day----------						(%)
PH 6	22.2±0.3	20.2±0.8	0.35±0.05 a	1.8±0.5	22.4±1.0	-0.2±0.1b	-0.9±4.5 b
pH 6.5	21.7±0.3	16.8±0.9	0.14±0.05 ab	1.9±0.5	18.6±1.0	3.1±0.1ab	14.3±4.5 ab
pH 7.9	22.2±0.3	17.5±0.8	0.03±0.05 b	1.1±0.5	18.6±1.0	3.6±0.1a	16.2±4.5 a
ANOVA P Value							
Treatment	0.5459	0.0533	0.0308	0.4199	0.0524	0.0354	0.0346

[a-b] means±SEM with different letters in same column are significantly different (α=0.05).
[1]Intake = from feed.
[2]output = total output from excreta, eggshell and egg content.
[3]Retention = (intake - output).
[4]Retention % = retention in the body compared to the intake.
ND = not detected (concentration was below the minimum detectable limit of analysis method).

The reported Fe content of chicken eggshell highly varied from study to study. Dobrzanski et al. (2008) found that eggshell Fe content of Lohmann-brown laying hens ranged from 2.3 to 3 ppm on a dry matter basis. Schaafsma et al. (2000) noted that Fe content of dried eggshell of hens reared under different housing and feeding practices ranged from 22 to 23 ppm. Abduljaleel et al. (2011) found that Fe concentration of chicken eggshell was 1422 ppm in dry matter basis. In the current study eggshell Fe ranged from 0.03 to 0.35 mg (5 ppm to 58 ppm). Eggshell Fe content could be changed with the dietary concentration of Fe (Skrivan et al. 2005). However, no literature was found to compare the Fe content of brown and white eggshells.

Egg content Fe was not affected by water pH treatments (P>0.05). Naber (1979), in his review, compared reported Fe contents of eggs from three studies. The average value of Fe ranged from 2.1 to 2.3 mg per 100g of liquid egg. Based on these results, Fe content in a 60 g egg was 1.2 mg in dry matter basis. Georgievskiĭ et al. (1981) also reported that Fe content in an egg was 1.1 to 2 mg. In current study, total Fe content in an egg ranged from 1.1 to 2.1 mg which is similar to the range of Fe content in eggs reported in the literature.

The excreta Fe was slightly affected by the water pH (P = 0.0533). pH 6 group had relatively high excreta Fe content. The intake of Fe in three groups were not different (P>0.05). Therefore, the negative retention could be due to the higher Fe in eggshells of the pH 6 group and slightly higher excreta Fe, when compared to the pH 7.9 and pH 6.5 groups. However, a negative retention would indicate the use of body reserves of Fe for the homeostasis.

6.6.1.8 Managanese

Manganese (Mn) retention or percent retention were not affected by water pH levels (P>0.05) (Table 6.10). The birds were in positive Mn balance. The intake or output of Mn were not different among treatment groups. The Mn content in the egg was very low. The retention ranged from 0.3 to 1.9 mg/hen/day while percent retention varied from 1.7 to 12.0% among treatments. No information could be found regarding Mn retention in literature to compare the results of our study. Mn is important for eggshell quality by involving formation of proteoglycans in eggshell matrix (Zamani et al. 2005). Therefore, changes in Mn retention would affect eggshell quality. However, low water pH did not have negative effect on Mn retention.

6.6.1.9 Zinc

There was no significant effect of water pH on Zn retention or percent retention (Table 6.11). Zn balance was negative in all the groups. The total output was higher than total intake of the hens in all treatment groups. When compared to Zn intake and excreta Zn, hens excreted more Zn than they consumed. However, the hen production performance or egg quality was not affected during the week 69. The NRC recommendation for Zn was 2.9 mg/hen/day (NRC 1994) for laying hens at 120 g of feed intake per day. Hens received adequate Zn from their diet in this study based on NRC recommendation for Zn. Since there was no literature on Zn balance with different water pH, the results could not be compared. The high content of excreta Zn could be due to sample contamination during collection since the cages and metal trays used were galvanized with Zn. Klevay et al. (1971) reported that galvanised cages are a source of Zn intake for animals. A controlled environment is critical for trace metal studies since trace metal contamination can occur through air, equipment and utensils used in animal facility (Klevay et al. 1971).

Table 6.10. The effect of water pH on Mn intake, output, retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	----------mg/bird/day----------						(%)
PH 6	15.7±0.2	15.4±0.5	ND	ND	15.4±0.5	0.3±0.5	1.7±0.5
pH 6.5	15.8±0.2	13.9±0.5	ND	ND	13.9±0.5	1.9±0.5	12.0±0.5
pH 7.9	15.7±0.2	14.4±0.5	ND	ND	14.4±0.5	1.3±0.5	8.3±0.5
ANOVA P Value							
Treatment 0.5459		0.2738			0.2738	0.1327	0.1285

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output)
[5]Retention % = retention in the body compared to the intake.
ND = not detected (concentration was below the minimum detectable limit of analysis method).

Table 6.11. The effect of water pH on Zn retention and percent retention of the laying hens at 69 weeks of age[1]

Treatment	Intake[2]	Excreta	Eggshell	Egg content	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------						(%)
PH 6	11.0±0.10	13.5±0.50	0.3±0.0	0.09±0.0	13.9±0.5	-2.90±0.5	-26.36±4.86
pH 6.5	10.8±0.10	11.8±0.50	0.4±0.0	0.10±0.0	12.6±0.5	-1.50±0.5	-13.88±4.86
pH 7.9	11.0±0.10	11.7±0.50	0.3±0.0	0.09±0.0	12.9±0.5	-1.90±0.5	-17.27±4.86
ANOVA P Value							
Treatment	0.5459	0.2638	0.5150	0.9303	0.3575	0.2467	0.2649

[1]Means±SEM.
[2]Intake = from feed.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

Further, Georgievskiĭ et al. (1981) reported that a chicken carcass contain about 30 mg of Zn in a one kg defatted tissue. A layer chicken carcass has 6% fat of body weight (Robinson et al. 2001), therefore Zn content could be calculated in 1.7 kg hen at 69 weeks of age as 48 mg/hen. The negative balance occurred at 69 weeks of age was -1.5 to - 2.9 mg/hen/day. If this negative balance continued, bird can only survive up to 16 to 30 days based on the estimated body content of Zn in the hens. However, the production trial was not continued after 69 weeks to assess production performance since the birds were culled at the end of production cycle.

6.6.1.10 Summary of the water pH balance study

There was no effect of water pH 6, 6.5 and 7.9 on Ca, Mg, P, Na, K, Cu, Mn and Zn retention and retention percent of the hens at 69 weeks of age. Fe retention in the hen body and percent retention were significantly decreased by water pH 6 compared to pH 6.5 and 7.9 while increasing eggshell Fe content. Therefore, a negative Fe balance occurred in pH 6 treatment. The negative Zn balance occurred among all treatments could possibly be due to the Zn contamination of excreta samples.

6.6.2 The effect of high content of Ca, Mg and SO_4 in the drinking water on mineral retention and percent retention at early, mid and late phases of egg production

Mineral balance studies were conducted at 42, 55 and 70 weeks of age to evaluate the effect of high Ca, Mg and SO_4 on retention and retention percentage in different phases of production in laying hens. The three time points represented the different production phases, when the phase 1, 2 and 3 diets were given. The mineral concentration in each diet was analysed in duplicate (Table 6.12). The sodium concentration in the diet given at 55 weeks of age was higher than expected and it should be similar to the diets given at 42 and 70 weeks of age. Further, Ca concentration of this diet given at 55 weeks of age was unacceptably low, analysed Ca % (4.2%) during the production trial was used for the calculations. The water treatments supplied to hens were control (well water), low Mg (625 ppm $MgSO_4$), high Mg (1250 ppm $MgSO_4$), low Mg Ca (625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$) and high Mg Ca (1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$). The composition of the water treatments were as same as the water mineral trial 1 (chapter 4 section 4.6.1.2). The same water treatments were fed to birds at different age.

6.12. Analysed mineral concentrations of diets used in balance studies at 42, 55 and 70 weeks of age of laying hens, water mineral trial 1*

Mineral	Concentration (ppm)		
	Balance study I 42 weeks	Balance study II 55 weeks	Balance study III 70 weeks
Calcium	41000	42000	42000
Phosphorus (total)	4235	4065	5395
Sodium	2030	1615	1530
Potassium	8295	7675	5840
Magnesium	2255	1965	2580
Sulphate	2325	2100	2600
Manganese	140	122	138
Copper	45	41	32
Zinc	127	126	109
Iron	164	225	210

[1]Diets were analysed in duplicate using ICP-OES.

The total intake of a mineral represent the sum of mineral ingested through feed and water by the hen. For some minerals, the concentrations were below the equipment detection limits. These were indicated as ND in the tables. The total output was calculated as the sum of mineral that eliminated through excreta, eggshell and egg content.

6.6.2.1 The mineral retention and percent retention at 42 weeks age of laying hens

At 42 weeks of age, production performance or egg quality were not different among treatments. Effects on feed intake, water intake, egg weight or shell weights were reported (Table 6.13). The hen day egg production was 96% and birds were at 4 weeks after their peak production.

Table 6.13. The feed intake, water intake, egg weight, and shell weight during balance study at 42 weeks of age of laying hens

Treatment	Feed intake (g/bird/day)	Water intake (mL/bird/day)	Egg weight (g)	Shell weight (g)
Control	107±1	175±6	61.80±0.58	8.41±0.88
625 ppm $MgSO_4$	107±1	177±6	61.07±0.58	8.32±0.88
1250 ppm $MgSO_4$	105±1	168±6	61.71±0.58	8.32±0.88
625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$	108±1	168±6	62.46±0.58	8.51±0.88
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	107±1	184±6	62.64±0.58	8.44±0.88
ANOVA P Value				
Treatment	0.3890	0.3788	0.6785	0.4355

6.6.2.1.1 Calcium

Intake of Ca was not affected by the mineral levels in the drinking water (P>0.05). (Table 6.14). Since the same diet was given to all treatment groups and feed consumption was similar, equal intake of Ca occurred. Ca intake though drinking water was different among the treatment groups (P<0.05).

Table 6.14. The effect of high mineral content in drinking water on Ca intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Rtention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	4.46± 0.06	0.004±0.0c	2.06±0.13	2.20±0.03	0.036±0.01	4.46±0.04	4.30±0.11	0.16±0.11	3.7±2.5
Low Mg	4.40± 0.06	0.004±0.0c	1.87±0.13	2.20±0.03	0.036±0.01	4.40±0.01	4.11±0.11	0.30±0.11	6.7±2.5
High Mg	4.34± 0.06	0.004±0.0c	2.08±0.13	2.22±0.03	0.033±0.01	4.34±0.01	4.33±0.11	0.01±0.11	0.1±2.5
Low Mg Ca	4.46± 0.06	0.082±0.0a	1.93±0.13	2.28±0.03	0.038±0.01	4.52±0.01	4.25±0.11	0.27±0.11	6.5±2.5
High Mg Ca	4.42± 0.06	0.044±0.0b	1.81±0.13	2.23±0.03	0.034±0.01	4.46±0.01	4.07±0.11	0.39±0.11	8.5±2.5
ANOVA P Value									
Treatment	0.3465	<0.0001	0.5374	0.4393	0.7472	0.0560	0.4541	0.2077	0.2139

[a-c] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

The highest intake Ca was obtained in the 1417 ppm $CaSO_4$ treated water, while 708 ppm $CaSO_4$ treated water group also showed higher intake than the other treatments without Ca treatments. The Ca content in excreta, eggshell or egg content was not different among the treatments (P>0.05). The Ca output through the excreta ranged from 1.81 to 2.08 g/hen/day. A hen deposited about 2.20 to 2.28 g calcium into an eggshell and it was 49-51% of the total Ca intake. This eggshell Ca content is similar to the amount that is found in the literature (Hurwitz 1970; Neijat et al. 2011) and to the result of eggshell Ca in the pH balance study. The egg albumen and yolk contained 0.03 g of Ca in it. The Ca in egg content was mostly found in the yolk as reported by Stibilj et al. (2002).

The retention and retention percent of Ca was not affected by the high Ca, Mg and SO_4 in water (P>0.05). The retention percent of Ca ranged from 0.1 to 8.5% among the treatments. Um and Paik (1999) reported that hens supplied with 4% Ca in diets, similar to the current study, had 2.5 to 3.5 g/hen/day balance, without considering Ca excretion from egg. The age of hens were similar to the current study. The daily Ca retention in the egg and body of hen ranged from 2.4 to 2.5 g in the current study.

6.6.2.1.2 Magnesium

Mg retention and retention percent were not affected by water mineral treatments (P>0.05) (Table 6.15). Mg retention ranged from 0.07 to 0.09 g/bird/day, while retention percent ranged from 29 to 31% of total intake. The total intake of Mg was significantly different in the treatment groups, where high intake was observed in high Mg treatments. The intake from the feed was not different since the feed intake was not different among the groups. However, intake from water was different (P<0.05). Total output was not affected by high mineral content in water, since the outputs from excreta, or eggs were not affected.

Table 6.15. The effect of high mineral content in drinking water on Mg intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- ---------- g/bird/day----------								(%)
Control	0.24±0.00	0.002±0.001 d	0.15±0.01	0.02±0.00	0.01±0.00	0.24±0.01d	0.17±0.01	0.07±0.01	30.0±2.8
Low Mg	0.24±0.00	0.020±0.001 c	0.16±0.01	0.02±0.00	0.01±0.00	0.26±0.01c	0.18±0.01	0.08±0.01	29.6±2.8
High Mg	0.24±0.00	0.038±0.001 b	0.16±0.01	0.02±0.00	0.01±0.00	0.28±0.01b	0.19±0.01	0.09±0.01	31.5±2.8
Low Mg Ca	0.24±0.00	0.021±0.001 c	0.16±0.01	0.02±0.00	0.01±0.00	0.26±0.01bc	0.18±0.01	0.08±0.01	29.9±2.8
High Mg Ca	0.24±0.00	0.046±0.001 a	0.17±0.01	0.02±0.00	0.01±0.00	0.29±0.01a	0.20±0.01	0.09±0.01	31.8±2.8
ANOVA P Value									
Treatment	0.4478	<0.0001	0.3131	0.2236	0.7792	<0.0001	0.2883	0.3863	0.9682

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

Um and Paik (1999) found that the Mg retention in hen's body and egg at 40 weeks of age, that were fed diets with similar Ca and P contents to the current study, was about 0.2 g/hen/day. In the current study we found the retention of Mg in the body and egg per day as about 0.1 g.

6.6.2.1.3 Phosphorus

The total intake or output of P did not differ among treatment groups (P>0.05) (Table 6.16). The total intake came almost excessively from feed P since the content of P in water was negligible. Daily feed P intake was about 0.45 g by all groups. The birds were in positive P retention and retention percentage ranged from 15 to 29% of total intake (Table 6.16). Similarly, Pekel et al. (2012) showed that the P retention percent of Lohmann brown hens at 45 weeks age with intake of 0.5 g of P was 24%. The calculation of retention considered the total output of P in both excreta and egg. The daily output of P from excreta and egg were 0.19 and 0.23 g respectively (Pekel et al. 2012). The excreta P contents in the current study ranged from 0.22 to 0.24 g/hen/day while egg P content was 0.11g. Um and Paik (1999) found that the retention of P in body and eggs of hens at 42 weeks of age, which supplied similar diets as the current study (4% Ca, 0.4% P), was 0.21 g/hen/day. In the present study, the retention of P in the body and egg ranged from 0.18 to 0.23 g/hen/day which similar to the findings of Um and Paik (1999). The daily P requirement of a hen on 100 g feed intake is 0.25 g recommendation NRC (1994). Therefore, for the hens in current study require 0.23 to 0.24 g of P/hen/day as they consume 105 to 108 g of feed/hen/day.

Table 6.16. The effect of high mineral content in drinking water on P intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- ---------- g/bird/day----------								(%)
Control	0.46±0.01	ND	0.24±0.02	0.001±0.00	0.10±0.00	0.46±0.01	0.34±0.03	0.11±0.02	24.4±5.6
Low Mg	0.45±0.01	ND	0.27±0.02	0.001±0.00	0.10±0.00	0.45±0.01	0.37±0.03	0.08±0.02	17.8±5.6
High Mg	0.45±0.01	ND	0.24±0.02	0.001±0.00	0.10±0.00	0.45±0.01	0.35±0.03	0.10±0.02	21.8±5.6
Low Mg Ca	0.46±0.01	ND	0.28±0.02	0.001±0.00	0.10±0.00	0.46±0.01	0.39±0.03	0.07±0.02	15.3±5.6
High Mg Ca	0.45±0.01	ND	0.22±0.02	0.001±0.00	0.10±0.00	0.45±0.01	0.32±0.03	0.13±0.02	29.0±5.6
ANOVA P Value									
Treatment	0.4479		0.4421	0.7733	0.4333	0.4479	0.4291	0.4544	0.4524

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

6.6.2.1.4 Potassium

K retention of the hens in different treatment groups was not affected by the high mineral contents in water (P>0.05) (Table 6.17). K is important in blood acid base regulation (Mongin 1981). For the laying hen, appropriate acid base balance is important for the activity of carbonic anhydrase enzyme which are involved in production of sound eggshells (Keshavarz and Austic 1990). Since Na and K retention were not affected by high Ca, Mg and SO_4 water, it is speculated that the acid-base balance may not get affected. That would be supported by the unaffected eggshell quality during the production trial in period 3, with these water treatments as discuss in chapter 4.6.1.4. The percent retention of K ranged from 24 to 26% among the treatments. The NRC (1994) recommendation for daily K requirement of a hen on 100 g feed intake is 0.15 g. The hens in the current study consumed 105 to 108 g/day of feed, so the requirement should be 0.14 g/hen/day. In the current study, the hens were in 0.21 to 0.24 g/hen/day K retention. So these birds received adequate K from their diet.

6.6.2.1.5 Sodium

Na retention and retention percent were not affected by water mineral treatments (P>0.05). Na retention was negative in hens at 42 weeks of age (Table 6.18). The total intake of Na was 0.22 g/hen/day from both feed and water while total output was 0.23 g/hen/day. The high output is due to the increased Na content in the egg content. Na content in an egg is from 0.06 to 0.07 g/egg (Georgievskiĭ et al. (1981), but Na in the egg content was high at 0.12 g in the current study. That caused high output than the intake which lead to negative retention. Bielamowicz (2011) also reported that a 50 g egg contained 0.06 g Na where 0.05 g was found in egg white while 0.009 g in egg yolk.

Table 6.17. The effect of high mineral content in drinking water on K intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.89±0.01	ND	0.60±0.03	ND	0.065±0.004	0.89±0.01	0.67±0.04	0.22±0.03	25.9±3.9
Low Mg	0.89±0.01	ND	0.60±0.03	ND	0.064±0.004	0.89±0.01	0.66±0.04	0.22±0.04	25.3±3.9
High Mg	0.87±0.01	ND	0.59±0.03	ND	0.066±0.004	0.87±0.01	0.66±0.04	0.21±0.04	24.9±3.9
Low Mg Ca	0.90±0.01	ND	0.60±0.03	ND	0.062±0.004	0.90±0.01	0.66±0.04	0.24±0.04	26.4±3.9
High Mg Ca	0.89±0.01	ND	0.59±0.03	ND	0.065±0.004	0.89±0.01	0.66±0.04	0.23±0.04	26.0±3.9
ANOVA P Value									
Treatment	0.4478		0.9997		0.9662	0.4478	0.9999	0. 9940	0.9998

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected-below the minimum detectable limit of analytical equipment.

Table 6.18. The effect of high mineral content in drinking water on Na intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.21±0.00	0.009±0.00	0.10±0.01	0.008±0.00	0.12±0.00	0.22±0.00	0.23±0.01	-0.01±0.00	-3.5±2.4
Low Mg	0.21±0.00	0.009±0.00	0.10 ±0.01	0.008±0.00	0.11±0.00	0.22±0.00	0.23±0.01	-0.01±0.00	-3.5±2.4
High Mg	0.21±0.00	0.009±0.00	0.09±0.01	0.009±0.00	0.12±0.00	0.22±0.00	0.22±0.01	-0.01±0.00	-3.8±2.4
Low Mg Ca	0.21±0.00	0.008±0.00	0.10±0.01	0.008±0.00	0.13±0.00	0.22±0.00	0.23±0.01	-0.01±0.00	-4.3±2.4
High Mg Ca	0.21±0.00	0.010±0.00	0.09±0.01	0.008±0.00	0.13±0.00	0.22±0.00	0.23±0.01	-0.01±0.00	-0.8±2.4
ANOVA P Value									
Treatment	0.5197	0.2150	0.4506	0.4406	0.2460	0.2217	0.9686	0.8992	0.9603

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

Na is an important mineral that regulate acid base balance in body fluids (Mongin 1981). High Na content in water reduce the shell quality by affecting the activity of carbonic anhydrase, which is involved in the formation of carbonate ions for eggshell calcification (Yoselewitz and Balnave 1989a). Even though a number of studies conducted to evaluate to NaCl in water on laying hen performance, the data on Na retention was limited to compare the results of our study. The NRC (1994) recommendation for the daily Na requirement of a hen on 100 g feed intake is 0.15 g. In our study, negative balance occurred among all treatments.

6.6.2.1.6 Sulphate

SO_4 retention and percent retention were affected by the water mineral treatments ($P<0.05$) (Table 6.19). The total intake of SO_4 was high among the groups receiving high SO_4 ($P<0.05$). The excreta SO_4 content was greater in high Mg, low Mg Ca and high Mg Ca water groups when compared to the control. This would indicate poor absorption of SO_4 ions in the digestive tract of the hens when concentration was high in water. Therefore, the total output was higher in the high SO_4 water groups. The retention of SO_4 among the groups receiving highest SO_4 groups of low Mg Ca and high Mg Ca, in with more than 1000 ppm SO_4, increased compared to other three groups. The retention of SO_4 was not different among the latter three groups. However, when compared to the control, only high Mg Ca group was significantly different in retention percent. Anderson and Stothers (1978) found high scouring rate in swine when SO_4 content was high in drinking water. Poor absorption of SO_4 in the gut leads to osmotic effect in the gut of birds which can cause loss of nutrients through watery excreta (Cassidy 1999).

Table 6.19. The effect of high mineral content in drinking water on SO_4 intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- ---------- g/bird/day----------								(%)
Control	0.30±0.0	0.01±0.00 d	0.26±0.01c	0.01±0.0	0.01±0.0	0.31±0.0 d	0.28±0.02 c	0.03±0.01c	8.3±3 b
Low Mg	0.30±0.0	0.07±0.00 c	0.29±0.01 bc	0.01±0.0	0.01±0.0	0.37±0.0 c	0.31±0.02 bc	0.05±0.01bc	14.0±3 ab
High Mg	0.30±0.0	0.13±0.10 b	0.37±0.01 ab	0.01±0.0	0.02±0.0	0.43±0.0 b	0.40±0.02 ab	0.03±0.01 c	6.7±3 b
Low Mg Ca	0.30±0.0	0.24±0.10 a	0.42±0.01 a	0.01±0.0	0.01±0.0	0.54±0.0 a	0.44±0.02 a	0.09±0.01ab	17.1±3 ab
High Mg Ca	0.30±0.0	0.26±0.10 a	0.41±0.01 a	0.01±0.0	0.02±0.0	0.56±0.0 a	0.44±0.02 a	0.12±0.01 a	22.1±3 a
ANOVA P Value									
Treatment	0.1543	<0.0001	<0.0001	0.7095	0.3459	<0.0001	<0.0001	<0.0001	0.0063

[a-b] means±SEM with different letters in same column are significantly different (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

6.6.2.1.7 Copper

Cu retention and percent retention were not affected by the high mineral water treatments (P>0.05) (Table 6.20). The hens were in positive Cu retention and was from 0.46 to 0.87 mg/bird/day, indicating the hens received adequate Cu for their metabolic needs. From the total intake, only 9 to 14% was retained in the hens. The daily Cu retention of pH balance study hens was higher and varied from 2 to 3 mg/hen. Um and Paik (1999) found that the retention of Cu in hens at 40 weeks of age ranged from 0.66 to 0.98 mg/hen/day. These hens were supplied with similar diet to the diet used in the current study. Their results were only based on Cu excretion via feces and urine. There was no recommendation for Cu requirement for laying hens in NRC (1994).

Cu content in excreta and egg content were not affected by water mineral treatments (P>0.05). Eggshell Cu was below the detectable concentration of the analysis method. Dobrzanski et al. (2008) reported that Cu content for the eggs weighing 60 to 61 g, was 7 ppm on fresh matter. So that, a 60 g egg contain 0.42 mg of Cu. Similarly, in the current study we found 0.3 to 0.5 mg of Cu in the egg content.

6.6.2.1.8 Iron

Hens were in negative Fe retention in all treatment groups (Table 6.21). The reasons would be the low Fe content in the diet and high Fe content in the egg content. The analysed value of Fe concentration of the diet sample was low (0.59 ppm) when compared to the other feed samples used for the two balance studies conducted at 55 (0.85 ppm) and 70 weeks (1.05 ppm) of age. The sample analysed in duplicate had 0.59 and 0.58 ppm Fe concentrations. So that, the calculated Fe intake was low during this balance study.

Table 6.20. The effect of high mineral content in drinking water on Cu intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------								(%)
Control	4.56±0.54	ND	3.61±0.31	ND	0.30±0.12	4.56±0.54	3.91±0.38	0.67±0.38	14.6±8.2
Low Mg	4.58±0.54	ND	3.71±0.31	ND	0.31±0.12	4.58±0.54	4.03±0.38	0.55±0.38	12.4±8.2
High Mg	4.52±0.54	ND	3.20±0.31	ND	0.45±0.12	4.52±0.54	3.65±0.38	0.87±0.38	14.6±8.2
Low Mg Ca	4.65±0.54	ND	3.88±0.31	ND	0.32±0.12	4.65±0.54	4.19±0.38	0.46±0.38	9.6±8.2
High Mg Ca	4.62±0.54	ND	3.40±0.31	ND	0.54±0.12	4.62±0.54	3.94±0.38	0.69±0.38	14.8±8.2
ANOVA P Value									
Treatment	0.4984		0.5861		0.5631	0.5198	0.8936	0.9494	0.9425

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

Table 6.21. The effect of high mineral content in drinking water on Fe intake, output, retention and percent retention at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------	----------	mg/bird/day						(%)
Control	16.76±0.20	ND	21.06±2.38	1.32±0.59	12.57±0.84	16.76±0.20	34.96±1.46	-18.20±2.46	-109.3±15.6
Low Mg	16.75±0.20	ND	19.94±2.38	1.59±0.59	11.45±0.84	16.75±0.20	32.99±1.46	-16.24±2.46	-97.2±15.6
High Mg	16.54±0.20	ND	17.74±2.38	1.34±0.59	12.56±0.84	16.54±0.20	31.64±1.46	-15.10±2.46	-91.6±15.6
Low Mg Ca	17.01±0.20	ND	17.29±2.38	1.49±0.59	11.07±0.84	17.01±0.20	29.85±1.46	-12.84±2.46	-75.4±15.6
High Mg Ca	16.91±0.20	ND	17.71±2.38	0.97±0.59	10.89±0.84	16.91±0.20	29.56±1.46	-12.65±2.46	-74.7±15.6
ANOVA P Value									
Treatment	0.5197		0.7485	0.9567	0.4660	0.5197	0.5482	0.5152	0.4815

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

Fe content in the egg content was unacceptably high when compared with the balance studies at 55 and 70 weeks of age. The reason for these extreme values is not exactly known. According to Um and Paik (1999), Fe concentrations in egg yolk, white and shell were 93.7, 3.4 and 13.2 ppm respectively on a dry basis. The dry matter content of the egg content samples in our study was 24%. So, a 60 g egg contained 14 mg of dry matter. Therefore, the Fe contents in a 60 g egg was 1.5 mg. Miller and Nnanna (1983) also reported that a large egg contained about 1 mg of Fe. However, in the current study the Fe content in an egg was 11 to 13 mg/egg, which is too high compared to Miller and Nnanna (1983) and Um and Paik (1999). According to our calculations based on analysed Fe concentration in feed of 0.58 ppm, the intake of Fe from the feed in the current study was only about 16 to 17 mg/hen/day. The excreta Fe ranged from 17 to 21 mg and this range was similar to the range obtained in the pH balance study. NRC (1994) recommendation for daily Fe requirement for a hen consume 100 g feed is 4.5 mg. Since the hens in the current study consume 105 to 108 g of feed, the Fe requirement was from 4.1 to 4.3 mg/hen/day. Therefore the hens received adequate Fe from their diet based on NRC (1994) recommendations. However, in the current study we observed negative retention of Fe from -13 to -18 mg/hen/day. Skrivan et al. (2005) found that the retention of Fe in laying hens at 40 weeks was 14 to 19 mg/hen/day without including egg Fe content. When retention was calculated using only intake and excreta Fe contents, it ranged from -4 to 0 mg/hen/day. This would indicate inadequacy of Fe for the daily metabolic functions. Further, Georgievskiĭ et al. (1981) reported that a chicken carcass contain about 60 mg of Fe in a one kg defatted tissue. A hen body weight was 1.7 kg at 42 weeks age. Assuming that chicken carcass has 6% fat (Robinson et al. 2001), Fe content could be calculated as

96 mg for a 1.7 kg hen. However, the negative balance occurred at 42 week study was -13 to -18 mg/hen/day. If this negative balance continued, almost all body sources need to be depleted within 5 to 7 days to overcome this much of deficiency. However, unaffected performance of the hens reflected that the hens were not in Fe deficiency at this stage or later. Therefore, the high negative balance seems unrealistic.

6.6.2.1.9 Manganese

Mn retention and retention percent were not affected by the water mineral treatments (P>0.05) (Table 6.22). Only 14 to 18% of Mn intake was retained by the hen from total intake of 15 mg/hen/day. Mn content in eggshell and egg content was very low. Most of Mn ingested (81 to 86%) was excreted through feces and urine while deposition in egg was negligible. The intake from the water was negligible. The Mn content in a 60 g egg was 0.02 mg (Dobrzanski et al. 2008), which was below the detectable limit of Mn (0.05 ppm) in ICP-OES. NRC (1994) recommendation for daily requirement of Mn for a laying hen consuming 100g of feed is 2 mg. In our study, we found Mn retention ranged from 2. 24 to 2.79 mg/hen/day, which is similar to NRC (1994) recommendation.

Dietary or water Mg and Ca level can affect Mn absorption in the digestive tract. In a rat study, Van Barneveld and Van den Hamer (1984) found decreased absorption of Mn with high levels of Mg and Ca in water. Sanchez-Morito et al. (1999) found that low Mg in diet increased Mn absorption in rats. Kimura et al. (1996) reported decrease in Mn in body tissues of rats including plasma and tibia bone when Mg was low in diet of rats. These findings suggested that interactions with Ca, and Mg in the digestive tract can affect Mn absorption. However, the high concentrations used in our study did not affect Mn retention in laying hens.

Table 6.22. The effect of high mineral content in drinking water on Mn intake, output, retention and retention percent at 42 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------mg/bird/day----------								(%)
Control	15.66±0.16	ND	13.41±0.63	ND	ND	15.66±0.16	13.41±0.63	2.24±0.63	14.3±4.3
Low Mg	15.57±0.16	ND	13.16±0.63	ND	ND	15.57±0.16	13.16±0.63	2.41±0.63	15.6±4.3
High Mg	15.36±0.16	ND	12.57±0.63	ND	ND	15.36±0.16	12.57±0.63	2.79±0.63	18.2±4.3
Low Mg Ca	15.79±0.16	ND	13.25±0.63	ND	ND	15.79±0.16	13.25±0.63	2.54±0.63	16.0±4.3
High Mg Ca	15.64±0.16	ND	13.40±0.63	ND	ND	15.64±0.16	13.40±0.63	2.24±0.63	14.3±4.3
ANOVA P Value									
Treatment	0.4478		0.8983			0.4478	0.8983	0.9757	0.9654

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

6.6.2.1.10 Zinc

There was a negative Zn retention in hens of all treatment groups (Table 6.23). The retention ranged from -3.93 to -6.64 mg/hen/day. The negative retention percent was from -29.57% to -48.38%. Zn is important trace minerals for eggshell formation (Mabe et al. 2003) by involving carbonic anhydrase enzyme action in the shell gland of hen (Georgievskiĭ et al. 1981). Therefore, deficiency of Zn would cause defects in eggshell. Eggshell quality was not affected at 42 week as discussed in Chapter 4, although the negative Zn retention observed. During the pH mineral balance study, a similar negative balance was found in the hens at 69 weeks of age. As described section 6.6.1.9, Zn is difficult to estimate since there is a lot of Zn in the environment. From the galvanized cages and metal trays used to collect excreta, contamination could occurred which lead to higher Zn content in excreta.

Dobrzanski et al. (2008) reported that the Zn concentration in egg content was 16 ppm in fresh basis. The eggshell Zn concentration was reported in dry matter as 3 ppm. Therefore, an egg weighing 60 g weight contained about 1 mg of Zn. In the current study, the Zn content in egg (60 to 61 g) ranged from 1.40 to 1.73 mg/egg.

6.6.2.1.11 Summary of the balance study at 42 weeks of age

The apparent retention and percent retention of intake of Ca, Mg, P, Na, K, Cu, Fe, Mn and Zn were not affected by water treatments with high Ca, Mg and SO_4. However, these measurements for SO_4 were affected by water treatments. Hens given 1500 ppm SO_4 retained more SO_4 compared to the control treatment. Retention and percent retention for the Na, Zn and Fe were negative for all the treatments.

Table 6.23. The effect of high mineral content in drinking water on Zn intake, output, retntion and percent retention at 42 weeks of age in laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- ---------- mg/bird/day----------								(%)
Control	13.40±0.14	ND	17.75±0.86	0.31±0.08	1.71±0.17	13.40±0.14	19.77±0.92	-6.37±0.91	-47.6±6.7
Low Mg	13.63±0.14	ND	18.39±0.86	0.47±0.08	1.41±0.17	13.63±0.14	20.27±0.92	-6.64±0.91	-48.4±6.7
High Mg	13.60±0.14	ND	16.94±0.86	0.34±0.08	1.73±0.17	13.60±0.14	19.01±0.92	-5.41±0.91	-39.7±6.7
Low Mg Ca	13.35±0.14	ND	15.34±0.86	0.26±0.08	1.67±0.17	13.35±0.14	17.28±0.92	-3.93±0.91	-29.6±6.7
High Mg Ca	13.68±0.14	ND	16.85±0.86	0.34±0.08	1.40±0.17	13.68±0.14	18.59±0.92	-4.91±0.91	-36.1±6.7
ANOVA P Value									
Treatment	0.3509		0.1646	0.4810	0.4650	0.3509	0.2166	0.2409	0.2596

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

The egg content and eggshell mineral contents were not affected by mineral water treatments. This would suggest the high Ca, Mg and SO_4 levels used in water treatments did not affect egg formation process in laying hens. Excreta SO_4 content increased with water treatments which indicated poor absorption of SO_4 in the digestive tract of laying hens.

6.6.2.2. The mineral retention and percent retention at 55 weeks age of laying hens

Feed and water consumption, egg weight and shell weight during the balance study at the 55 weeks of age in laying hens were presented (Table 6.24). There were no water treatment effect on these parameters (P>0.05). The hens were at post peak production where hen day egg production was 95 to 96% among treatments. The water treatments supplied were the same as the balance study at 42 weeks of age of hens including, control (well water), low Mg (625 ppm $MgSO_4$), high Mg (1250 ppm $MgSO_4$), low Mg Ca (625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$) and high Mg Ca (1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$).

Table 6.24. The feed intake, water intake, egg weight and eggshell weight during balance study 2 at 55 weeks of age of laying hens

Treatment	Feed Consumption (g/bird/day)	Water consumption (mL/bird/day)	Egg weight (g)	Eggshell weight (g)
Control	117±2	180±7	62.33±0.6	8.34±0.3
625 ppm $MgSO_4$	113±2	170±7	61.65±0.6	8.12±0.3
1250 ppm $MgSO_4$	114±2	166±7	62.49±0.6	8.20±0.3
625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$	115±2	174±7	62.98±0.6	8.37±0.3
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	117±2	182±7	62.82±0.6	8.29±0.3
ANOVA P Value				
Treatment	0.2503	0.4379	0.6605	0.3448

6.6.2.2.1 Calcium

The Ca retention and percent retention were not affected by the high Ca, Mg and SO_4 content in water (P>0.05) (Table 6.25). The range of Ca balance was from 0.18 to 0.37 g/hen/day. Ca retention percent ranged from 5.1 to 8.3% among the treatments. At 55 weeks of age, the hens in all the groups had positive Ca retention. About 50% of total Ca intake was deposited in the egg of hens regardless of the water treatments. This was similar to the amount deposited in eggs in the balance study at 42 weeks of age (Table 6.14). Lim et al. (2003) found that Ca retention in the hen was 2.26 g (only based on excreta Ca output), while excreta Ca content was 2.02 g/hen/day. These hens were supplied with 4% Ca in the diet. In our study, we found similar Ca retention when consider only excreta Ca output for the calculation. Excreta, eggshell and egg content Ca content were not affected by water treatments (P>0.05).

6.6.2.2.2 Magnesium

Mg retention and percent retention were significantly affected by the water treatments at 55 weeks of age of hens (P<0.05) (Table 6.26). The birds were in positive Mg retention among all the treatments. When compared to the control water, retention and percent retention increased in high Mg group (1250 ppm $MgSO_4$). The low Mg group had lower Mg retention and percent retention when compared to the high Mg group and low Mg Ca treatment. Excreta Mg increased in high Mg and high Mg Ca groups when compared to low Mg group. Out of total Mg intake, only 9 to 12% of Mg deposited in the eggs. The Mg content in eggshell and egg content were not significantly affected by high Mg, Ca and SO_4 content in water.

Table 6.25. The effect of high mineral content in drinking water on Ca intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	g/bird/day								(%)
Control	4.42±0.1	0.014±0.002 c	2.01±0.09	2.20±0.03	0.03±0.00	4.43±0.10	4.25±0.1	0.18±0.11	4.2±2.5
Low Mg	4.25±0.1	0.011±0.002 c	1.89±0.09	2.12±0.03	0.03±0.00	4.26±0.10	4.04±0.1	0.22±0.11	5.2±2.5
High Mg	4.29±0.1	0.012±0.002 c	1.79±0.09	2.14±0.03	0.03±0.00	4.30±0.10	3.97±0.1	0.33±0.11	7.7±2.5
Low Mg Ca	4.35±0.1	0.068±0.002 a	1.94±0.09	2.21±0.03	0.03±0.00	4.42±0.10	4.19±0.1	0.23±0.11	5.1±2.5
High Mg Ca	4.40±0.1	0.036±0.002 b	1.85±0.09	2.18±0.0	0.03±0.0	4.44±0.10	4.07±0.1	0.37±0.11	8.3±2.5
ANOVA P Value									
Treatment	0.4353	<0.0001	0.6222	0.2341	0.6598	0.1228	0.3450	0.7452	0.7404

[a-c] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention= (intake - output).
[5]Retention % = retention in the body compared to the intake.

Table 6.26. The effect of high mineral content in drinking water on Mg intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------g/bird/day----------								(%)
Control	0.21±0.0	0.002±0.0 d	0.13±0.0 b	0.02±0.0	0.01±0.0	0.21±0.0 d	0.16±0.0 ab	0.05±0.00 bc	23.1±2.0b
Low Mg	0.20±0.0	0.002±0.0 d	0.13±0.0 b	0.02±0.0	0.01±0.0	0.20±0.0 d	0.15±0.0 b	0.05±0.00 c	22.7±2.0b
High Mg	0.20±0.0	0.059±0.0 a	0.15±0.0 a	0.02±0.0	0.01±0.0	0.26±0.0 a	0.18±0.0 a	0.08±0.00 a	32.2±2.0a
Low Mg Ca	0.20±0.0	0.020±0.0 c	0.13±0.0 b	0.02±0.0	0.01±0.0	0.22±0.0 c	0.16±0.0 b	0.07±0.00 ab	30.3±2.0ab
High Mg Ca	0.20±0.0	0.030±0.0 b	0.15±0.0 a	0.02±0.0	0.01±0.0	0.24±0.0 b	0.18±0.0 a	0.07±0.00 ab	27.3±2.0ab
ANOVA P Value									
Treatment	0.1723	<0.0001	0.0016	0.2392	0.8978	<0.0001	0.0012	<0.0001	0.0063

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05). .
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

Atteh and Leeson (1983a) found that increasing Mg in water up to 100 ppm increased bone Mg and P content which lead to bone deformation in broiler chicks. Tibia bones got shortened and hocks were swollen. However, when Ca was added into water up to 100 ppm the effects were not observed, suggesting reduced absorption due to competition between the two minerals for same absorption sites in hen's digestive tract. In our study we did not observe leg problems among hens that received Mg up to 234 ppm in water with no added Ca.

Um and Paik (1999) reported that the Mg retention in hen and egg at 40 weeks of age was 0.2 g/hen/day, but no literature was found to compare Mg retention of the laying hens during post peak production phase. Daily requirement of Mg of a hen at 100 g feed intake is 0.05 g (NRC 1994). In our study we observed 0.07 to 0.11 g of apparent Mg absorption in hens when only excreta loss was considered as an output. Therefore, the hens in the current study received adequate amount of Mg from their diet based on NRC (1994) recommendation for laying hens.

6.6.2.2.3 Phosphorus

P retention and percent retention were not affected by high Mg, Ca and SO_4 in water (P>0.05) (Table 6.27). The positive retention ranged from 0.08 to 0.11 g/hen/day while percent retention ranged from 19 to 23% among the treatments. Um and Paik (1999) found P retention of hens at 40 weeks of age ranged from 0.21 to 0.38 g/hen/day (Intake – excreta) which was similar to the current study. In the current study, P retention was 0.19 to 0.22 g/hen/day (Intake – excreta). The hens in this study require 0.24 to 0.23 g of P/hen/day based on NRC (1994) recommendation of 0.25 g/hen/day at 100 g of feed intake. However, positive P balance occurred among the treatments at 55 weeks of age.

Table 6.27. The effect of high mineral content in drinking water on P intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.43±0.01	ND	0.24±0.01	0.01±0.00	0.10±0.00	0.43±0.01	0.35±0.01	0.08±0.01	19.2±3.2
Low Mg	0.41±0.01	ND	0.21±0.01	0.01±0.00	0.10±0.00	0.41±0.01	0.32±0.01	0.09±0.01	22.8±3.2
High Mg	0.42±0.01	ND	0.22±0.01	0.01±0.00	0.10±0.00	0.42±0.01	0.33±0.01	0.09±0.01	21.3±3.2
Low Mg Ca	0.42±0.01	ND	0.22±0.01	0.01±0.00	0.10±0.00	0.42±0.01	0.33±0.01	0.10±0.01	22.7±3.2
High Mg Ca	0.43±0.01	ND	0.21±0.01	0.01±0.00	0.10±0.00	0.43±0.01	0.32±0.01	0.11±0.01	22.3±3.2
ANOVA P Value									
Treatment	0.1723		0.5196	0.9554	0.8586	0.1723	0.5227	0.7455	0.7473

Means±SEM.
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.
ND: not detected - below the minimum detectable limit of analytical equipment.

6.6.2.2.4 Potassium

Similar to the results in 42 week balance study, K retention and retention percent were not affected by the water treatments (Table 6.28). K retention ranged from 0.19 to 0.23 g/hen/day while percent retention ranged from 24 to 28%. K is the major cation in intracellular body fluid and important in maintaining osmotic pressure (Georgievskiĭ et al. 1981). Further, K involved in acid base balance in body fluids (Mongin 1981) Therefore the high Mg, Ca and SO_4 would not affect these body measures by affecting K retention in hens. K content in excreta and egg content were not affected by water treatments. Eggshell K content was very low and not detected by ICP-OES.

6.6.2.2.5 Sodium

Positive Na retention was occurred at 55 weeks of age among all the treatments (Table 6.29), unlike negative retention occurred at 42 week study. There was no effect of water treatments on Na retention and percent retention (P>0.05). The range of percent retention was from 5.3 to 10.3% among treatment groups. Eggshell, egg content and excreta Na content were not affected by the water treatments (P>0.05). Based on the results of the study, it would be suggested that Na ion concentrations in body tissues would not get affected by high contents of Ca, Mg and SO4 ions in drinking water of the laying hens at 55 weeks of age. Na ion concentration in body tissues is quite important in acid base balance, therefore effect of Na on acid base balance would not be changed due to high contents of Ca, Mg and SO4 ions in drinking water.

Table 6.28. The effect of high mineral content in drinking water on K intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.81±0.01	ND	0.55±0.02	ND	0.06±0.01	0.81±0.01	0.60±0.02	0.19±0.02	24.1±2.1
Low Mg	0.77±0.01	ND	0.50±0.02	ND	0.06±0.01	0.77±0.01	0.56±0.02	0.21±0.02	27.7±2.1
High Mg	0.79±0.01	ND	0.51±0.02	ND	0.06±0.01	0.79±0.01	0.58±0.02	0.21±0.02	26.6±2.1
Low Mg Ca	0.80±0.01	ND	0.51±0.02	ND	0.06±0.01	0.80±0.01	0.57±0.02	0.23±0.02	28.1±2.1
High Mg Ca	0.81±0.01	ND	0.53±0.02	ND	0.06±0.01	0.81±0.01	0.59±0.02	0.21±0.02	26.0±2.1
ANOVA P Value									
Treatment	0.1723		0.2044		0.7956	0.1723	0.2255	0.8292	0.6912

Means±SEM.
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake – output).
[5]Retention % = retention in the body compared to the intake.
ND: not detected- below the minimum detectable limit of analytical equipment.

Table 6.29. The effect of high mineral content in drinking water on Na intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.17±0.01	0.01±0.001	0.08±0.01	0.01±0.001	0.08±0.01	0.18±0.01	0.17±0.01	0.01±0.003	6.1±4.4
Low Mg	0.16±0.01	0.01±0.001	0.07±0.01	0.01±0.001	0.08±0.01	0.17±0.01	0.16±0.01	0.01±0.003	5.3±4.4
High Mg	0.17±0.01	0.01±0.001	0.07±0.01	0.01±0.001	0.07±0.01	0.17±0.01	0.16±0.01	0.01±0.003	7.3±4.4
Low Mg Ca	0.17±0.01	0.01±0.001	0.07±0.01	0.01±0.001	0.08±0.01	0.17±0.01	0.16±0.01	0.01±0.003	6.2±4.4
High Mg Ca	0.17±0.01	0.01±0.001	0.07±0.01	0.01±0.0	0.07±0.01	0.19±0.01	0.16±0.01	0.02±0.003	10.7±4.4
ANOVA P Value									
Treatment	0.1723	0.4568	0.9968	0.5171	0.8612	0.0576	0.9561	0.8737	0.9170

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake – output).

[5]Retention % = retention in the body compared to the intake.

6.6.2.2.6 Sulphate

SO_4 retention and percent retention were affected significantly by water treatments (P<0.05) (Table 6.30). Birds were in positive SO_4 retention among all treatments. However, SO_4 retention increased in the high Mg, low Mg Ca and high Mg Ca groups when compared to the control group. The retention was higher in high Mg treatment than low Mg treatment, which contained more SO_4 in water than the latter. SO_4 excretion was higher in low Mg, high Mg, low Mg Ca and high Mg Ca treatments compared to the control water treatment and increased with increasing SO_4 content in water. Egg content and eggshell SO_4 content were not affected by high SO_4 in water.

6.6.2.2.7 Copper

At 55 weeks of age, Cu, retention and percent retention were not significantly affected by the water treatments (Table 6.31). The Cu retention of control group was negative while in other groups was positive. The retention was from – 0.15 to 1.11 mg/hen/day among treatments. The percent retention ranged from – 2.8 to 26% in the other four groups except the control group. The Cu intake ranged from 4.06 to 4.29 while excreta Cu was from 2.26 to 3.40 mg/hen/day. The excreta Cu content remained in a wide range among treatments. Therefore, Cu retention and percent retention were stayed in a wide range.

6.6.2.2.8 Iron

Fe retention and percent retention were not affected by water mineral treatments (P>0.05) (Table 6.32). The intake of Fe from the feed was 24 to 25 mg/hen/day while excreta Fe was 16 to 17 mg/hen/day.

Table 6.30. The effect of high mineral content in drinking water on SO_4 intake, output, retention and percent retention at 55 weeks of age of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	g/bird/day								(%)
Control	0.22±0.01	0.001±0.01d	0.20±0.01d	0.01±0.0	0.005±0.0	0.22±0.01d	0.21±0.01d	0.005±0.01c	2.3±1.8c
Low Mg	0.21±0.01	0.078±0.01c	0.26±0.01c	0.01±0.0	0.005±0.0	0.29±0.01c	0.28±0.01c	0.01±0.01bc	4.9±1.8bc
High Mg	0.21±0.01	0.215±0.01ab	0.30±0.01bc	0.01±0.0	0.005±0.0	0.43±0.01ab	0.32±0.01bc	0.11±0.01a	15.5±1.8ab
Low Mg Ca	0.22±0.01	0.186±0.01b	0.32±0.01ab	0.01±0.0	0.005±0.0	0.40±0.01b	0.34±0.01ab	0.07±0.01ab	16.6±1.8a
High Mg Ca	0.22±0.01	0.226±0.01a	0.36±0.01a	0.01±0.0	0.005±0.0	0.45±0.01a	0.38±0.01a	0.07±0.01ab	15.5±1.8ab
ANOVA P Value									
Treatment	0.4207	<0.0001	<0.0001	0.1178	0.9555	<0.0001	<0.0001	<0.0001	0.0011

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake – output).
[5]Retention % = retention in the body compared to the intake.

Table 6.31. The effect of high mineral content in drinking water on Cu intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---mg/bird/day---								(%)
Control	4.29±0.08	ND	3.40±0.32	0.18±0.04	0.84±0.03	4.29±0.08	4.44±0.30	-0.15±0.30	-2.8±7.7
Low Mg	4.06±0.08	ND	2.68±0.32	0.07±0.04	0.81±0.03	4.06±0.08	3.58±0.30	0.48±0.30	11.7±7.7
High Mg	4.21±0.08	ND	2.53±0.32	0.04±0.04	0.85±0.03	4.21±0.08	3.42±0.30	0.79±0.30	18.8±7.7
Low Mg Ca	4.25±0.08	ND	2.26±0.32	0.05±0.04	0.84±0.03	4.25±0.08	3.14±0.30	1.11±0.30	25.8±7.7
High Mg Ca	4.19±0.08	ND	2.66±0.32	0.06±0.04	0.83±0.03	4.19±0.08	3.55±0.30	0.64±0.30	14.3±7.7
ANOVA P Value									
Treatment	0.4219		0.1770	0.2931	0.9061	0.4219	0.0848	0.1361	0.1446

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake – output).
[5]Retention % = retention in the body compared to the intake.
ND: not detected- below the minimum detectable limit of analytical equipment

Table 6.32. The effect of high mineral content in drinking water on Fe intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------								(%)
Control	24.84±0.34	ND	17.31±0.81	0.36±0.06	4.87±0.37	24.84±0.34	22.55±0.67	2.29±0.77	9.1±3.1
Low Mg	23.77±0.34	ND	16.25±0.81	0.49±0.06	4.57±0.37	23.77±0.34	21.33±0.67	2.44±0.77	10.3±3.1
High Mg	24.13±0.34	ND	16.74±0.81	0.34±0.06	4.33±0.37	24.13±0.34	21.41±0.67	2.72±0.77	11.2±3.1
Low Mg Ca	24.19±0.34	ND	16.17±0.81	0.30±0.06	3.77±0.37	24.19±0.34	20.25±0.67	3.94±0.77	16.2±3.1
High Mg Ca	25.04±0.34	ND	17.53±0.81	0.34±0.06	4.22±0.37	25.04±0.34	22.10±0.67	2.94±0.77	11.5±3.1
ANOVA P Value									
Treatment	0.1434		0.7486	0.2151	0.3516	0.1434	0.2108	0.5968	0.5683

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.
ND: not detected- below the minimum detectable limit of analytical equipment.

The Fe content in an egg ranged from 4 to 5 mg, which is higher than the range found in the literature(1-2 mg/egg) as described in pH balance study (section 6.6.1.7) and mineral balance study at 42 week (section 6.6.2.1.8). The Fe percent retention ranged from 9 to 16% among the groups.

During the first balance study at 42 weeks of age, retention was highly negative. However, excreta Fe content was similar to the first balance study, verifying the daily Fe loss via excreta per hen. No literature was found to compare the retention and percent retention at this age of laying hens. Skrivan et al. (2005) reported that the retention of Fe in laying hens at 40 weeks was about 14 to 19 mg/hen/day without including egg Fe content, which was higher compared to our retention result of 8 mg/hen/day (without considering loss via egg).

Amount of Fe losses through eggshell and egg contents highly varied in the studies at 42 and 55 weeks of age. During the balance study at 42 weeks of age, eggshell Fe content varied from 1.32 to 1.59 mg/hen/day while during this study at 55 weeks, Fe content in eggshells ranged from 0.30 to 0.49 mg/egg. Egg content Fe was changed from 3.77 to 4.87 mg/hen/day while during the 42 week study the range increased as 11.07 to 12.57 mg/hen/day. The reason for this variation could be the sample contamination during collection and preparation. A controlled environment is essential for the trace mineral studies since minerals such as Fe and Zn are commonly found in the environment. A little contamination of Fe can occur huge change to the result of analysis.

6.6.2.2.9 Zinc

Zn retention and percent retention were not affected by water treatments (P>0.05) (Table 6.33). The Zn percent retention ranged from 0.9 to 5.6%, unlike negative values occurred at the first balance study. The retention ranged from 0.1 to 0.7 mg/hen/day. The excreta,

eggshell and egg content Zn were not significantly different among the treatments. Therefore, total intake or total output of Zn was not affected by the water treatments (P>0.05). The Zn intake from feed was similar to the intake at the first balance study at 42 week (12.80 to 13.32 mg/hen/day), but excreta Zn loss was relatively lower (15 to 18 vs 11 to 12 mg/hen/day). Dobrzanski et al. (2008) reported that the Zn concentration in egg content was about 16 ppm in fresh basis. The eggshell Zn concentration was reported on a dry matter as 3 ppm. So that, egg of 60 g weight contain about 1 g of Zn. In the current study, the Zn content in egg (60 to 61 g) ranged from 1.40 to 1.73 mg/egg.

6.6.2.2.10 Manganese

The Mn retention and percent retention were not affected by water treatments (P>0.05) (Table 6.34). The hens in this study was in a positive Mn retention. Literature available on Mn retention or percent retention were limited for laying hens to compare the results of this study. Mn is important for eggshell quality by involving formation of proteoglycans in eggshell matrix (Zamani et al. 2005). Therefore, deficiency of Mn would cause eggshells defects, but the hens in the current study in a positive retention of Mn. That would indicate the adequacy of Mn intake for the Mn metabolic functions. Excreta Mn content was not significantly affected by water treatments. This would indicates high Ca and Mg levels used in our study did not affect absorption of Mn in the digestive tract of laying hens. However, no information was found in the literature related to Mn absorption in laying hens given high Mg and Ca levels in water or diet.

Table 6.33. The effect of high mineral content in drinking water on Zn intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------								(%)
Control	13.32±0.17	ND	11.31±0.00	0.43±0.03	0.84±0.02	13.32±0.17	12.58±0.40	0.74±0.40	5.6±3.6
Low Mg	12.80±0.17	ND	11.53±0.00	0.28±0.03	0.81±0.02	12.80±0.17	12.63±0.40	0.17±0.40	1.3±0.6
High Mg	12.98±0.17	ND	11.57±0.00	0.28±0.03	0.85±0.02	12.98±0.17	12.71±0.40	0.27±0.40	2.0±0.6
Low Mg Ca	13.11±0.17	ND	11.39±0.00	0.32±0.03	0.84±0.02	13.12±0.17	12.56±0.40	0.56±0.40	4.2±3.6
High Mg Ca	13.26±0.17	ND	11.98±0.00	0.31±0.03	0.83±0.02	13.26±0.17	13.13±0.40	0.13±0.40	0.9±0.6
ANOVA P Value									
Treatment	0.2306		0.8822	0.1975	0.9063	0.2306	0.9227	0.8772	0.8781

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

Table 6.34. The effect of high mineral content in drinking water on Mn intake, output, retention and percent retention at 55 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------								(%)
Control	14.18±0.29	ND	11.02±0.43	ND	ND	14.18±0.29	11.02±0.43	3.16±0.32	22.3±3.6
Low Mg	13.42±0.29	ND	9.93±0.43	ND	ND	13.42±0.29	9.93±0.43	3.48±0.32	25.8±3.6
High Mg	13.92±0.29	ND	9.85±0.43	ND	ND	13.92±0.29	9.86±0.43	4.06±0.32	29.2±3.6
Low Mg Ca	14.06±0.29	ND	10.05±0.43	ND	ND	14.06±0.29	10.05±0.43	4.01±0.32	28.2±3.6
High Mg Ca	13.85±0.29	ND	10.23±0.43	ND	ND	13.85±0.29	10.23±0.43	3.62±0.32	25.6±3.6
ANOVA P Value									
Treatment	0.4296		0.3539			0.4296	0.3539	0.7408	0.6963

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

6.6.2.2.11 Summary of the balance study at 55 weeks of age

Similar to the results that occurred at 42 week study, SO_4 retention and percent retention were affected by water treatments. Hens retained more SO_4 and Mg at higher SO_4 (1000-1500 ppm) and Mg (250 ppm) content in water. However, retention of Ca, P, Na, K, Cu, Fe, Mn and Zn were not affected by the high levels of Ca, Mg and SO_4 in water and birds were in positive balance of these minerals. These results would indicate unaffected absorption of the major and trace minerals in the digestive tract of hens with the given concentrations of Ca, Mg and SO_4 in drinking water. The mineral contents in eggshell and egg content were not affected by high levels of Ca, Mg and SO_4 in water, suggesting that egg formation process was not affected by these mineral levels in hens at post peak egg production. Mg and SO_4 excretion were high in hens fed with high Mg, Ca and SO_4 in water indicating poor absorption of $MgSO_4$ at higher concentration in the digestive tract of laying hens.

6.6.2.3 The mineral retention and percent retention at 70 weeks of age of laying hens

Feed and water consumption, egg weight and shell weight during the balance study 3 at 70 weeks of age in laying hens were presented (Table 6.35). The hens were at the end production where hen day egg production was 90 to 91% among water mineral treatments. There were no differences among water treatments of these parameters during 70 weeks of age of the hens. The water treatments were as same the previous mineral balance studies conducted at 42 ad 55 weeks.

Table 6.35. The feed intake, water intake, egg weight and shell weight during balance study 3 at 70 weeks of age

Treatment	Feed Consumption (g/bird/day)	Water consumption (mL/bird/day)	Egg weight (g)	Shell weight (g)
Control	105±1.5	183±5.5	62.48±0.72	8.10±0.14
625 ppm $MgSO_4$	110±1.5	180±5.5	61.38±0.72	7.99±0.14
1250 ppm $MgSO_4$	108±1.5	181±5.5	63.36±0.72	8.17±0.14
625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$	111±1.5	194±5.5	61.71±0.72	8.42±0.14
1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$	109±1.5	192±5.5	62.93±0.72	8.31±0.14
ANOVA P Value				
Treatment	0.1510	0.2697	0.2896	0.2599

6.6.2.3.1 Calcium

At 70 weeks, hens in all treatment groups were in positive Ca retention (Table 6.36). Ca retention and percent retention were not significantly affected by the water treatments. Ca intake from the water was significantly higher in the groups with high Ca in water. In the other 3 groups, without high water Ca, the intake of Ca was 0.013 to 0.015 g/hen/day. Therefore, total Ca intake was relatively higher in high Ca groups. However, the total intake was not significantly different among treatments.

Excreta Ca content was not significantly different among the treatments. Similar to the studies conducted at 42 and 55 weeks of age, eggshell Ca was 2.1 to 2.2 g/egg. Egg content Ca was 0.03 g/egg in all groups. At 70 weeks, hens retained 7.2 to 10.4% Ca from their intake.

Table 6.36. The effect of high mineral content in drinking water on Ca intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	g/bird/day								(%)
Control	4.09±0.06	0.01±0.001c	1.75±0.09	2.16±0.04	0.03±0.002	4.10±0.07	3.94±0.09	0.16±0.12	7.2±2.9
Low Mg	4.16±0.06	0.01±0.001c	1.66±0.09	2.15±0.04	0.03±0.002	4.17±0.07	3.84±0.09	0.34±0.12	8.1±2.9
High Mg	4.16±0.06	0.01±0.001c	1.64±0.09	2.14±0.04	0.03±0.002	4.17±0.07	3.81±0.09	0.37±0.12	8.8±2.9
Low Mg Ca	4.26±0.06	0.07±0.001a	1.64±0.09	2.22±0.04	0.03±0.002	4.33±0.07	3.89±0.09	0.45±0.12	10.4±2.9
High Mg Ca	4.21±0.06	0.04±0.001b	1.78±0.09	2.19±0.04	0.03±0.002	4.25±0.07	4.00±0.09	0.25±0.12	5.7±2.9
ANOVA P Value									
Treatment	0.4516	<0.0001	0.6600	0.6800	0.4210	0.1665	0.6236	0.4588	0.8645

[a-c] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retentiion = (intake - output).
[5]Retention % = retention in the body compared to the intake.

6.6.2.3.2 Magnesium

Similar to 55 week balance study, Mg retention and percent retention were affected by water treatments (P>0.05) (Table 6.37). The Mg retention ranged from 0.05 to 0.11 mg/hen/day while percent retention was from 18.1 to 32.9%. The daily Mg retention and percent retention increased in high Mg groups compared to low Mg and control groups.

Atteh and Leeson (1983b) found that Mg retention (Mg intake – Mg in excreta) was lower when dietary Mg level increased from 0.17% to 0.77%. Authors suggested that lower retention could be due to a cathartic effect of high Mg in the hen digestive tract. However, water Mg level from 115 ppm to 234 ppm increased Mg retention in laying hens in the current study. Further, they found increased Mg content and decreased Ca content in eggshells, which could affect eggshell quality. They also found increased plasma Mg content, which was highly correlated to eggshell Mg. Bone Ca and Zn contents were reduced by high Mg content in the diet. In our study, eggshell Mg content was not affected by high contents of Mg in water treatments up to 234 ppm.

The total intake of Mg was significantly different since the Mg intake from the water were different among groups and feed Mg intake was similar among the groups.

Excreta Mg content was higher in high Mg Ca treatment, when compared to the control treatment (P<0.05). There was a tendency for increased excreta Mg with high Mg in water.

Table 6.37. The effect of high mineral content in drinking water on Mg intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	g/bird/day								(%)
Control	0.25±0.01	0.002±0.0d	0.18±0.00b	0.02±0.00	0.005±0.00	0.25±0.01d	0.20±0.01b	0.05±0.01c	19.2±0.8 b
Low Mg	0.26±0.01	0.002±0.0d	0.19±0.00ab	0.02±0.00	0.005±0.00	0.26±0.01d	0.21±0.01ab	0.05±0.01c	18.1±0.8 b
High Mg	0.25±0.01	0.064±0.0a	0.19±0.00ab	0.02±0.00	0.003±0.00	0.32±0.01a	0.21±0.01ab	0.11±0.01a	32.9±0.8 a
Low Mg Ca	0.26±0.01	0.022±0.0c	0.19±0.00ab	0.02±0.00	0.004±0.00	0.28±0.01c	0.21±0.01ab	0.07±0.01b	23.6±0.8 b
High Mg Ca	0.25±0.01	0.043±0.0b	0.20±0.00a	0.02±0.00	0.005±0.00	0.30±0.01b	0.23±0.01a	0.07±0.01b	23.9±0.8 b
ANOVA P Value									
Treatment	0.2020	<0.0001	0.0533	0.5451	0.5590	<0.0001	0.0476	<0.0001	<0.0001

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

Eggshell and egg content of Mg were not affected by water treatments with high Mg, Ca or SO_4 compared to the control (P>0.05). Eggshell Mg was 0.020 g/egg and egg content Mg was 0.005 g/egg in all treatments.

6.6.2.3.3 Phosphorus

Similar to observation in the balance studies at 42 and 55 weeks, P retention and percent retention were not affected by high water Ca, Mg and SO_4 (P>0.05) (Table 6.38). The P retention percent was from 24.4 to 26.6% in the hens. The daily retention of P ranged from 0.13 to 0.14 g/hen. Kovács et al. (2006) found that P retention of hens was 0.09 g/hen/day when intake of P was 0.5 g/hen/day. Excreta P content was 0.3 g/hen/day while egg P loss was 0.1 g/egg. These findings were very similar to our findings, where intake, excreta and egg P were 0.5 g/hen/day, 0.3 g/hen/day and 0.1 g/egg, respectively. Excreta, eggshell or egg content P were not significantly different among treatment groups (P>0.05). Intake of P from water was negligible.

6.6.2.3.4 Potassium

K retention and percent retention were not affected by water treatments (P>0.05) (Table 6.39), similar to the results of balance studies at 42 and 55 weeks of age of hens. The birds were in positive K retention among all treatments and ranged from 0.13 to 0.16 g/hen/day. The percent retention ranged from 22.7 to 28.2 % among the treatments. The excreta, eggshell and egg content K were not affected by high Ca, Mg and SO_4 concentrations in the water.

Table 6.38. The effect of high mineral content in drinking water on P intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	g/bird/day								(%)
Control	0.52±0.01	ND	0.29±0.01	0.01±0.00	0.09±0.002	0.52±0.01	0.39±0.01	0.13±0.01	24.9±2.1
Low Mg	0.53±0.01	ND	0.29±0.01	0.01±0.00	0.09±0.002	0.53±0.01	0.39±0.01	0.14±0.01	26.6±2.1
High Mg	0.53±0.01	ND	0.29±0.01	0.01±0.00	0.10±0.002	0.53±0.01	0.40±0.01	0.13±0.01	24.8±2.1
Low Mg Ca	0.54±0.01	ND	0.30±0.01	0.01±0.00	0.09±0.002	0.54±0.01	0.40±0.01	0.14±0.01	26.1±2.1
High Mg Ca	0.53±0.01	ND	0.30±0.01	0.01±0.00	0.09±0.002	0.53±0.01	0.40±0.01	0.13±0.01	24.4±2.1
ANOVA P Value									
Treatment	0.2020		0.7412	0.8870	0.1993	0.2020	0.7733	0.8894	0.9370

Means±SEM.
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.
ND: Not Detected- below the minimum detectable limit of analytical equipment.

Table 6.39. The effect of high mineral content in drinking water on K intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention [4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.56±0.01	ND	0.37±0.01	ND	0.06±0.00	0.56±0.01	0.43±0.02	0.13±0.02	22.7±2.6
Low Mg	0.58±0.01	ND	0.36±0.01	ND	0.06±0.00	0.58±0.01	0.42±0.02	0.16±0.02	28.2±2.6
High Mg	0.57±0.01	ND	0.37±0.01	ND	0.06±0.00	0.57±0.01	0.43±0.02	0.14±0.02	24.4±2.4
Low Mg Ca	0.59±0.01	ND	0.37±0.01	ND	0.06±0.00	0.59±0.01	0.43±0.02	0.16±0.02	26.8±2.4
High Mg Ca	0.58±0.01	ND	0.37±0.01	ND	0.05±0.00	0.58±0.01	0.42±0.02	0.16±0.02	26.9±2.4
ANOVA P Value									
Treatment	0.2020		0.8123		0.0976	0.2020	0.8602	0.4632	0.5518

Means±SEM

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake – output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analytical equipment.

6.6.2.3.5 Sodium

Na retention and percent retention were not affected by water treatments (P>0.05) (Table 6.40). The positive retention ranged from 0.01 to 0.02 g/hen/day among the treatments while percent retention ranged from 9.2 to 15.0%. Na content in excreta, eggshell or egg content was not affected by water treatments (P>0.05). The Na percent retention was from 5 to 10% at 55 week study, while negative percent retention was occurred at 42 week study among all the treatments.

6.6.2.3.6 Sulphate

SO_4 retention and percent retention were significantly affected by the water treatments (Table 6.41). Increased retention and percent retention percent were observed in high SO_4 treatments associated with high Mg. The high content of SO_4 associated with Ca in water, did not increase the retention compared to the control water treatment. The reason could be the poor solubility of $CaSO_4$, which prevent dissolution of SO_4 for absorption in the digestive tract of birds. Negative retention and percent retention were occurred in the control treatment.

A similar range in SO_4 retention was observed at both 42 and 55 week studies. At 42 weeks of age, balance ranged from 0.03 to 0.12 g/hen/day among the treatments while at 55 week study, SO_4 balance ranged from 0.01 to 0.11 g/hen/day.

6.6.2.3.7 Copper

Cu retention and percent retention were not affected by water treatments (P>0.05) (Table 6.42). Hens were in daily positive balance of 0.45 to 0.88 mg/hen among all treatments. The percent retention ranged from 14.3 to 28.7%. Similar to the results observed at 55 week study, Cu retention is in a wide range.

Table 6.40. The effect of high mineral content in drinking water on Na intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- g/bird/day----------								(%)
Control	0.14±0.00	0.00±0.00	0.05±0.01	0.01±0.00	0.07±0.00	0.14±0.01	0.13±0.01	0.01±0.00	9.2±2.7
Low Mg	0.15±0.00	0.00±0.00	0.05±0.01	0.01±0.00	0.07±0.00	0.15±0.01	0.13±0.01	0.02±0.00	14.5±2.7
High Mg	0.15±0.00	0.00±0.00	0.04±0.01	0.01±0.00	0.07±0.00	0.15±0.01	0.13±0.01	0.02±0.00	15.0±2.7
Low Mg Ca	0.15±0.00	0.01±0.00	0.06±0.01	0.01 ±0.00	0.07±0.00	0.16±0.01	0.14±0.01	0.02±0.00	11.0±2.7
High Mg Ca	0.15±0.00	0.01±0.00	0.06±0.01	0.01±0.00	0.07±0.00	0.16±0.01	0.14±0.01	0.02±0.00	12.6±2.7
ANOVA P Value									
Treatment	0.3431	0.2541	0.4092	0.7928	0.8956	0.1040	0.4307	0.5188	0.5027

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

Table 6.41. The effect of high mineral content in drinking water on SO_4 intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Water	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------	----------	----------	----------	g/bird/day	----------	----------	----------	(%)
Control	0.28±0.00	0.01±0.00 d	0.27±0.00 c	0.01±0.00	0.005±0.00	0.28±0.01 d	0.29±0.01c	-0.01 ±0.0 c	-4.02±2.7 c
Low Mg	0.28±0.00	0.08±0.00 c	0.33±0.00 b	0.01±0.00	0.004±0.00	0.36±0.01 c	0.35±0.01b	0.02 ±0.00 c	5.2±2.7 bc
High Mg	0.28±0.00	0.23±0.00 a	0.34±0.00 b	0.01±0.00	0.004±0.00	0.51±0.01 ab	0.36±0.01b	0.16 ±0.00 a	30.7±2.7 a
Low Mg Ca	0.28±0.00	0.21±0.00 b	0.44±0.00 a	0.01±0.00	0.005±0.00	0.49±0.01 b	0.46±0.01a	0.03 ±0.00 c	6.0±2.7 bc
High Mg Ca	0.29±0.00	0.25±0.00 a	0.43±0.00 a	0.01±0.00	0.005±0.00	0.54 ±0.01 a	0.45±0.01a	0.09±0.00 b	16.3±2.7 b
ANOVA P Value									
Treatment	0.4604	<0.0001	<0.0001	0.8960	0.5889	<0.0001	<0.0001	<0.0001	<0.0001

[a-d] Means±SEM within same column with different letters are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake= from feed and water.
[3]output= total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.

Table 6.42. The effect of high mineral content in drinking water on Cu intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	---------- mg/bird/day----------							(%)
Control	3.04±0.04	2.59±0.10	ND	ND	3.04±0.04	2.59±0.10	0.45±0.10	14.3±3.3
Low Mg	3.10±0.04	2.38±0.10	ND	ND	3.10±0.04	2.38±0.10	0.72±0.10	23.3±3.3
High Mg	3.09±0.04	2.21±0.10	ND	ND	3.09±0.04	2.21±0.10	0.88±0.10	28.6±3.3
Low Mg Ca	3.13±0.04	2.43±0.10	ND	ND	3.13±0.04	2.43±0.10	0.70±0.10	22.4±3.3
High Mg Ca	3.13±0.04	2.56±0.10	ND	ND	3.13±0.04	2.56±0.10	0.57±0.10	18.3±3.3
ANOVA P Value								
Treatment	0.5476	0.0929			0.5476	0.0929	0.0731	0.0587

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: Not Detected- below the minimum detectable limit of analysis equipment.

This was due to the widely distributed range of excreta Cu content (2.2 to 2.6 mg/hen/day) compared to the similar intake of Cu (3.0 to 3.1 mg/hen/day) among the treatments.

Intake, excreta, eggshell or egg content Cu contents were not different among water treatments. Intake of Cu from water was negligible. These findings were similar to what was found at 42 and 55 week studies.

6.6.2.3.8 Zinc

Zn retention and percent retention were not affected by water treatments (P>0.05) (Table 6.43) at 70 weeks of age of the hens. Zn retention were negative for the control, low Mg and high Mg groups while positive for low Mg Ca and high Mg Ca groups. The total output was greater than the intake since excreta Zn content was higher than the intake in negative groups. During the study at 42 week, Zn balance was negative for all treatment groups. As described in that study results, excreta sample contamination could occur due to Zn coated cages and excreta collection metal trays that resulted higher Zn content in excreta than feed. Further, the birds could get Zn from the galvanised cages to affect intake. Therefore, Zn contamination could be occurred and intake can be affected by these sources and this would be the reason for the variability of Zn retention results from study to study and within a study.

Table 6.43. The effect of high mineral content in drinking water on Zn intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------mg/bird/day----------							(%)
Control	10.57±0.20	10.79±0.52	ND	0.68±0.02	10.57±0.20	11.47±0.51	-0.91±0.56	-9.2±5.8
Low Mg	10.77±0.20	10.75±0.52	ND	0.67±0.02	10.77±0.20	11.42±0.51	-0.65±0.56	-6.2±5.8
High Mg	10.77±0.20	10.97±0.52	ND	0.71±0.02	10.77±0.20	11.68±0.51	-0.91±0.56	-8.6±5.8
Low Mg Ca	10.86±0.20	9.75±0.52	ND	0.64±0.02	10.86±0.20	10.39±0.51	0.46±0.56	4.0±5.8
High Mg Ca	10.82±0.20	9.81±0.52	ND	0.68±0.02	10.82±0.20	10.50±0.51	0.32±0.56	2.8±5.8
ANOVA P Value								
Treatment	0.8699	0.3108		0.1900	0.8699	0.2731	0.3430	0.3477

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake= from feed and water.

[3]output= total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

ND: not detected- below the minimum detectable limit of analysis equipment.

6.6.2.3.9 Iron

Fe retention and percent retention were not affected by water treatments (P>0.05) (Table 6.44). Fe retention was positive in hens in all treatments. Hens maintained 4 to 7 mg daily positive Fe retention. The percent retention ranged from 16.9 to 25.7% of intake in the hens. Excreta, eggshell and egg content Fe were not affected by water treatments (P>0.05). When comparing results of previous balance studies, Fe content was highly varied in both eggshells and egg contents. The reported concentrations for Fe in eggshell or egg content in the literature were varied from study to study (Schaafsma et al. 2000; Dobrzanski et al. 2008; Abduljaleel et al. 2011) as discussed in section 6.6.1.7.

6.6.2.3.10 Manganese

Mn retention and percent retention were affected by water treatments (P<0.05) (Table 6.45). The birds were in positive Mn retention in all treatments. Mn retention and percent retention were higher in low Mg treatment when compared to the control water treatment. However, the reason for reduced Mn retention in control water treatment is not known. There was no significant difference among other treatments. High Ca and Mg can affect Mn absorption in laying hens as described in section 6.6.2.1.9. Mn content in eggshell and egg content were very little and not detected by the analysis equipment.

6.6.2.3.11 Summary of the balance study at 70 weeks of age

High Mg and SO_4 in water significantly increased the retention and percent retention of Mg and SO_4, respectively. Similar to 55 week study, Mg retention and percent retention were higher in the hens given highest (250 ppm) content of Mg in the water. Hens in the high Mg and high Mg Ca groups had higher SO_4 retention and percent retention.

Table 6.44. The effect of high mineral content in drinking water on Fe intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	----------mg/bird/day----------							(%)
Control	28.16±0.50	21.03±0.58	1.29±0.00	0.99±0.00	28.16±0.50	23.32±0.85	4.84±1.20	16.9±3.3
Low Mg	28.27±0.50	19.57±0.58	1.44±0.00	1.05±0.00	28.27±0.50	22.07±0.85	6.19±1.20	21.9±3.3
High Mg	28.26±0.50	19.58±0.58	0.44±0.00	0.98±0.00	28.26±0.50	21.00±0.85	7.26±1.20	25.7±3.3
Low Mg Ca	28.64±0.50	19.45±0.58	0.87±0.00	1.30±0.00	28.64±0.50	21.51±0.85	7.13±1.20	24.8±3.3
High Mg Ca	28.60±0.50	19.93±0.58	0.87±0.00	1.01±0.00	28.60±0.50	21.81±0.85	6.79±1.20	23.6±3.3
ANOVA P Value								
Treatment	0.9025	0.4845	0.5917	0.2448	0.9025	0.5470	0.5580	0.5152

Means±SEM.

[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

[2]Intake = from feed and water.

[3]output = total output from excreta, eggshell and egg content.

[4]Retention = (intake - output).

[5]Retention % = retention in the body compared to the intake.

Table 6.45. The effect of high mineral content in drinking water on Mn intake, output, retention and percent retention at 70 weeks of age of laying hens

Treatment[1]	Feed	Excreta	Eggshell	Egg content	Intake[2]	Output[3]	Retention[4]	Percent retention[5]
	mg/bird/day							(%)
Control	13.18±0.18	11.24±0.23	ND	ND	13.18±0.18	11.24±0.23	1.94±0.28 b	14.7±1.9 b
Low Mg	13.70±0.18	10.48±0.23	ND	ND	13.70±0.18	10.48±0.23	3.22±0.28 a	23.5±1.9 a
High Mg	13.52±0.18	10.90±0.23	ND	ND	13.52±0.18	10.90±0.23	2.62±0.28 ab	19.3±1.9 ab
Low Mg Ca	13.83±0.18	10.90±0.23	ND	ND	13.83±0.18	10.90±0.23	2.93±0.28 ab	21.1±1.9 ab
High Mg Ca	13.63±0.18	10.98±0.23	ND	ND	13.63±0.18	10.98±0.23	2.65±0.28 ab	19.4±1.9 ab
ANOVA P Value								
Treatment	0.1537	0.2931			0.1537	0.2931	0.0436	0.0456

[a-b] Means±SEM within same column with different letters are significantly different (α=0.05).
[1]Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$; Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]Intake = from feed and water.
[3]output = total output from excreta, eggshell and egg content.
[4]Retention = (intake - output).
[5]Retention % = retention in the body compared to the intake.
ND: Not Detected- below the minimum detectable limit of analysis equipment.

Mn retention was higher in low Mg treatment compared to the control treatment, but no differences were occurred among other treatments. The retention and percent retention of Ca, P, K, Na, Cu, Fe and Zn were not affected by high levels of Ca, Mg and SO_4 in water and hens were in daily positive retention of these minerals.

The mineral contents in eggs including eggshell and egg content were not affected by high levels of Ca, Mg and SO_4 in water similar to what had observed at 42 and 55 weeks balance studies. Mg and SO_4 excretion were high in hens supplied with high Mg and SO_4 in water similar to the results obtained at 55 week balance study proving the poor absorption of $MgSO_4$ in the digestive tract of the hens.

6.6.2.4 The effect of age on mineral retention of hens

To evaluate the age effect on mineral retention, mineral retention data from 3 studies at 42, 55 and 70 weeks of age of hens in water mineral production study (trial 1) were analyzed with repeated measures analysis. Since these 3 studies were conducted at different egg production stages of the laying cycle, mineral metabolism of hens could be affected by the age.

6.6.2.4.1 Calcium

There was no interaction between water mineral treatment and birds age on Ca retention (P>0.05) (Table 6.46). At all stages, in all treatment groups, hens had less than 1 g of positive daily Ca retention. Um and Paik (1999) found 2.5 to 3.5 g/hen/day of apparent Ca absorption in hens and Hurwitz (1970) and Neijat et al. (2011) found 2 to 2.2 g of Ca deposition in eggs. According to these findings, Ca balance could be in range from 0.3 to 1.5 g/hen/day in laying hens. The daily Ca retention of the hens in our study was in that range.

Table 6.46. Effects of water mineral treatments and age of bird on Ca, Mg and SO_4 retention of laying hens at 42, 55 and 70 weeks of age

	Mineral								
	Calcium			Magnesium			Sulphate		
	42 week	55 week	70 week	42 week	55 week	70 week	42 week	55 week	70 week
	g/hen/day								
Treatment[1]									
Control	0.16±0.11	0.25±0.11	0.16±0.11	0.07±0.01bcd	0.05±0.01cd	0.05±0.01cd	0.05±0.02 b	0.05±0.02 b	0.11±0.02 ab
Low Mg	0.29±0.11	0.22±0.11	0.33±0.11	0.08±0.01abc	0.05±0.01d	0.04±0.01d	0.04±0.02 b	0.04±0.02 b	0.07±0.02 ab
High Mg	0.41±0.11	0.44±0.11	0.44±0.11	0.09±0.01ab	0.08±0.01ab	0.11±0.01a	0.07±0.02 ab	0.06±0.02 ab	0.15±0.02 a
Low MgCa	0.00±0.11	0.44±0.11	0.36±0.11	0.08±0.01ab	0.07±0.01bcd	0.07±0.01bcd	0.08±0.02 ab	0.03±0.02 b	0.11±0.02 ab
High MgCa	0.29±0.11	0.44±0.11	0.39±0.11	0.09±0.01ab	0.07±0.01bcd	0.07±0.01bcd	0.05±0.02 b	0.05±0.02 b	0.05±0.02 b
Age mean	0.23±0.04	0.34±0.04	0.30±0.04	0.08±0.01	0.06±0.01	0.07±0.01	0.06±0.01	0.04±0.01	0.10±0.01
ANOVA P Value									
Treatment	0.5482			<0.0001			0.5362		
Age	0.2278			<0.0001			<0.0001		
Treatment ×Age	0.2264			0.0039			0.0316		

[a-b] Means±SEM with different letters in age effect for Mg and interaction effects for SO_4 are significantly different according to the Tukey-Kramer test (α=0.05).
[1]Water mineral treatments = Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$, Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

6.6.2.4.2 Magnesium

There was no water treatment by birds age interaction on Mg retention (P<0.05) (Table 6.46). At both 55 and 70 weeks of age, high Mg water treatment increased Mg retention compared to the low Mg and the control treatments, but not at 42 weeks of age. The Mg retention was similar in both low Mg and high Mg treatments at 42 weeks of age. That would suggest that similar Mg retention was occurred in hens at 42 weeks of age where egg production was high, regardless of the level of Mg in their drinking water. Mg can be found in eggshells and during peak production hens would need more Mg for eggshell formation. Therefore, absorption of Mg in the digestive tract of hens might get increased during their peak egg production phase similar to what has been found for Ca absorption. Ca absorption rate increases during peak egg production to ensure adequate Ca supply for eggshell formation and decline with the age of the hen where fewer number of eggs produced (Al-Batshan et al. 1994). Further, at 42 weeks of age Mg retention was similar among all treatments unlike at 55 and 70 week balance studies results. However, no information was found in the literature regarding Mg absorption rate in laying hens to support these findings.

6.6.2.4.3 Sulphate

There was treatment by birds age interaction on SO_4 retention (P<0.05) (Table 6.46). SO_4 retention increased in high Mg treatment than high Mg Ca treatment at 70 weeks of age of hens. For any age, the retention was positive. The reason for reduced retention in high Mg Ca treatment could be poor absorption of SO_4 at higher level when associated with Mg and Ca in the digestive tract of hens. High SO_4 content in the gut can cause osmotic effect which lead to watery feces. Further, there was a trend of increasing SO_4 balance in high $MgSO_4$

treatment compared to high Mg Ca treatment at any age of birds. High Ca levels in water can reduce the absorption of SO_4 by forming less soluble $CaSO_4$ in the digestive tract of hens (Hurwitz and Rand 1965).

6.6.2.4.4 Sodium

There was no water treatment by birds age interaction on Na balance of hens (P>0.05) (Table 6.47). Na balance was significantly affected by age of birds. The balance was negative and lower at 42 weeks of age compared to both 55 and 70 weeks of age. Na balance was not affected by high content of Ca, Mg and SO_4 at any of balance study conducted at 42, 55 and 70 weeks of age of hens during water mineral trial 1.

6.6.2.4.5 Potassium

There was no water mineral treatment by birds age interaction on K retention of hens (P>0.05) (Table 6.47). K retention was affected by age of birds (P<0.05). The retention increased at 42 and 55 weeks of age compared to 70 weeks of age of hens. The difference of K retention at these 3 stages of hens could be due to the change of K content in the diets used at different phases of production. The K content in the diets were 0.7%, 0.7% and 0.5% which used at 42, 55 and 70 weeks studies, respectively.

6.6.2.4.6 Phosphorus

There was no water treatment by birds age interaction on P retention of hens (P>0.05) (Table 6.47). The retention was higher at 70 weeks of age when compared to both 42 and 55 weeks of age (P<0.05). This change would reflect the increase in total P content in the diet at different phases. The total P content of the diets at 42, 55 and 70 weeks of age were 0.4%, 0.4% and 0.5% respectively.

Table 6.47. Effects of water mineral treatments and age of bird on Na, K and P retention of laying hens at 42, 55 and 70 weeks of age

	Mineral								
	Sodium			Potassium			Phosphorus		
	42 week	55 week	70 week	42 week	55 week	70 week	42 week	55 week	70 week
	g/hen/day								
Treatment[1]									
Control	-0.01±0.00	0.01±0.00	0.02±0.00	0.21±0.02	0.19 ±0.02	0.12±0.02	0.11±0.02	0.08±0.02	0.13±0.02
Low Mg	-0.01±0.00	0.02±0.00	0.03±0.00	0.22±0.02	0.21±0.02	0.16±0.02	0.08±0.02	0.09±0.02	0.14±0.02
High Mg	-0.01±0.00	0.02±0.00	0.02±0.00	0.21±0.02	0.20±0.02	0.14±0.02	0.09±0.02	0.09±0.02	0.13±0.02
Low Mg Ca	-0.01±0.00	0.02±0.00	0.02±0.00	0.23±0.02	0.22±0.02	0.15±0.02	0.07±0.02	0.09±0.02	0.14±0.02
High Mg Ca	-0.01±0.00	0.02±0.00	0.02±0.00	0.23±0.02	0.21±0.02	0.15±0.02	0.13±0.02	0.11±0.02	0.13±0.02
Age mean	-0.01±0.0 b	0.01±0.00 a	0.02±0.00 a	0.22±0.01 a	0.21±0.01 a	0.15±0.01b	0.09±0.02b	0.09±0.02 b	0.13±0.02 a
ANOVA P Value									
Treatment	0.8654			0.8252			0.9232		
Age	<.0001			<0.0001			<0.0001		
Treatment × Age	0.1839			0.9961			0.2080		

[a-b] Means±SEM with different letters in phase effect for K and P are significantly different (α=0.05).
[1]Water mineral treatments = Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$, Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.

6.6.2.4.7 Copper

There was no interaction between water mineral treatments and age of the bird on Cu retention of the hens (P>0.05) (Table 6.48). Cu retention was not affected by the age of bird. The retention ranged from 0.5 to 1 g in hens regardless of the treatment and age of bird. Hens were in positive balance in all phases for all treatments except for control treatment at 55 weeks. The high Cu content in excreta and eggshell in the control treatment caused that negative balance compared to other treatments at 55 weeks age.

6.6.2.4.8 Zinc

Zinc retention data were not analysed using repeated measures analysis since the data were negative at both 42 and 70 weeks of age. At 42 week study, excreta Zn content (17 mg/hen/ day) was higher than the intake (13 mg/hen/ day). This would lead to high negative retention at this study. At 70 week study, three treatments had negative Zn retention including control, low Mg and high Mg water treatments. Since these negative retentions occurred because of high content of Zn in excreta and it could possibly be due to sample contamination with Zn from the environment, data were not analysed to determine the age effect.

6.6.2.4.9 Iron

Fe retention was affected by age of the hen (P<0.05) (Table 6.48). The retention increased at 70 weeks of age compared to 55 weeks of age. The Fe retention at 55 and 70 weeks of age were 2.8 and 6.2 mg/hen/day, respectively. The Fe content in eggshell and egg content were highly varied from study to study. At 55 week study, Fe in egg content was higher than that of 70 week study (3.8 to 4.9 mg/egg vs 0.99 to 1.30 mg/egg, respectively).

Table 6.48. Effects of water mineral treatments and age of bird on Cu, Mn and Fe retention of laying hens at 42, 55 and 70 weeks of age

	Mineral								
	Copper			Manganese			Iron		
	42 week	55 week	70 week	42 week	55 week	70 week	42 week[2]	55 week	70 week
	mg/hen/day								
Treatment[1]									
Control	0.67 ±0.29	-0.15±0.29	0.45±0.29	2.24±0.52	3.16±0.52	1.94 ±0.52		2.29±0.91	4.84±0.91
Low Mg	0.55±0.29	0.48±0.29	0.72±0.29	2.41±0.52	3.48±0.52	3.22 ±0.52		2.44±0.91	6.19±0.91
High Mg	0.87±0.29	0.79±0.29	0.88±0.29	2.79±0.52	4.06±0.52	2.62 ±0.52		2.72±0.91	7.26±0.91
Low Mg Ca	0.46±0.29	1.11±0.29	0.70±0.29	2.54±0.52	4.01±0.52	2.93 ±0.52		3.94±0.91	7.13±0.91
High Mg Ca	0.69±0.29	0.64±0.29	0.57±0.29	2.24±0.52	3.62±0.52	2.65 ±0.52		2.94±0.91	6.79±0.91
Age mean	0.65±0.13	0.61±0.13	0.66±0.13	2.44±0.2b	3.67±0.2 a	2.67±0.23b		2.80±0.42b	6.17±0.42a
ANOVA P Value									
Treatment	0.3099			0.5593				0.0443*	
Age	0.9596			<0.0001				0.0002	
Treatment × Age	0.5637			0.3416				0.6909	

[a-b] Means±SEM with different letters in phase means of Mn and Cu and treatment means of Fe at 55 and 70 weeks are significantly different (α=0.05).
[1]Water mineral treatments = Control: well water; Low Mg: 625 ppm $MgSO_4$; High Mg: 1250 ppm $MgSO_4$, Low mg Ca: 625 ppm $MgSO_4$ plus 1417 ppm $CaSO_4$; High Mg Ca: 1250 ppm $MgSO_4$ plus 708 ppm $CaSO_4$.
[2]All balance values were highly negative at 42 week study and did not analysed using repeated measures.
*Tukey Kramer procedure did not identify significant differences among least square means

This could be the reason for increased retention occurred at 70 weeks study. Since the retention at the 42 weeks of age was highly negative among all the treatments and seemed to be unrealistic based on production performance data, those were not included to the repeated measure analysis to determine the age effect.

6.6.2.4.10 Manganese

There was no interaction between water mineral treatments and age of the birds on Mn retention of the hens (P>0.05) (Table 6.48). The retention was affected by the bird age (P<0.05). Higher Mn retention occurred at 55 weeks of age when compared to both 42 and 70 weeks of age. A reduced excreta Mn content was observed at 55 week study compared to the intake at 42 and 70 weeks. That caused increased retention of Mn at this week. The reason could be the low Mn content in the diet at this age. The Mn content in the diets given at 42, 55 and 70 weeks were 148 ppm, 122 ppm, and 138 ppm respectively. At low concentration of Ca in the digestive tract of hens, the absorption of minerals get enhanced (Hurwitz and Bar 1966). Similar effect could be applied to the increased retention at 55 weeks where low content of Mn in the diet.

6.7 Conclusions

Neither pH 6 nor 6.5 affected mineral retention of Ca, Mg, P, K, Na, Cu, Mn, and Zn, when compared to pH 7.9 water in hens during late production. A negative Fe retention occurred at pH 6 and eggshell Fe content increased.

The macro and micro mineral retention or percent retention were not negatively affected by the high water contents of Ca, Mg and SO_4, up to 487, 234 and 1317 ppm, respectively, at 42, 55 or 70 weeks of age in laying hens. Hens were in positive mineral retention at any age except for Fe and Zn. The negative retention of Zn and Fe could be due to the sample

contamination occurred during the studies. At higher levels of Mg (234 ppm) and SO_4 (1317 ppm), retention and percent retention of Mg and SO_4 were higher.

Egg content and eggshell mineral contents were not affected by low water pH (except for Fe) or high levels of Ca, Mg and SO_4 in water. Na, P, Fe and SO_4 balance of the laying hens were increased with the bird age while K and Mg balance were decreased. Mn balance was higher at 55 weeks of age of the hens. Changes occurred in concentrations of some minerals in diets, supplied during different production phases, affected mineral retention.

CHAPTER 7 CONCLUSION

7.1 Conclusion

pH, hardness, alkalinity, and SO_4 ion contents in water for the laying hens in Canadian egg production units were not completely within the current recommendations for poultry. The higher levels of Ca and Mg which was associated with hard water, would not be satisfactory when SO_4 concentration was high in the water. Experimental data are needed to determine impacts of the extremes of these parameters on laying hen performance.

The findings of the pH study concluded that, water pH 6 to 7.9 did not have negative impact on laying hen production performance, egg quality and mineral retention at late production. pH 8.2 had negative effects on hens performance when the pH was increased by sodium bicarbonate. Further studies need to be conducted in order to determine water pH effect with alternative sources to adjust the water pH.

The high contents of Ca, Mg and SO_4 up to 786, 562 and 1988 ppm, respectively, did not negatively affect pullet grower and developer production performance while these minerals up to 732 ppm, 508 ppm and 1818 ppm, respectively, did not negatively affect production performance and egg quality of the laying hens. Bone quality of the laying hens at the end of production cycle was not affected by Ca, Mg and SO_4 up to 487 ppm, 234 ppm and 1317 ppm, respectively, in water. Mineral retention and percent retention of Ca, K, Na, P, Cu, Fe, Zn and Mn were not negatively affected by Ca, Mg and SO_4 up to 487 ppm, 234 ppm and 1317 ppm, respectively, in water at 42, 55 and 70 weeks of age where their early and mid-post peak and late production phases occurred. Mg and SO_4 balance increased by high water contents of Mg and SO_4, respectively, but these changes occurred in mineral

metabolism did not affect production performance, egg quality or bone quality as observed during water mineral production trial 1.

Therefore, Lohmann-Lite laying hens tolerated higher concentrations of the Ca, Mg and SO_4 in drinking water than were recommended for poultry by Carter and Sneed (1996); Weltzien (2002) and Fairchild and Ritz (2012) at any production stage, when evaluated under given experimental conditions.

7.2 Future studies

The effect of water pH need to be evaluated in a full production cycle to determine impacts at different production stages of laying hens. Further, alternative sources to citric acid and sodium bicarbonate should be tested to change water pH. Organic acids such as citric acid found to have different modes of actions in the digestive tract of birds including enhancing nutrient absorption and reducing pathogenic bacterial count. Sodium bicarbonate is known to cause metabolic alkalosis when use in high amounts, which can affect performance of laying hens. Instead sodium hypochlorite could be used to increase the water pH.

The evaluation of Ca, Mg and SO_4 at higher concentrations than the levels used in this project would be useful to determine threshold levels of these minerals for laying hens.

Since alkalinity of water from some Canadian egg production units exceeded current recommendation for poultry and effects at higher alkalinity is not known for laying hens, future studies could be planned to evaluate effects of alkaline water on laying hens.

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APPENDIX 1. INFORMATION FORM –WATER QUALITY SURVEY ACROSS CANADA

Water Quality for Laying Hens
Water Survey
Information Form

Name	
Farm Name	
Farm Address	
Shipping Address (if different from farm address)	
e-Mail address	
Phone #	
Fax #	
Type of Water Supply (please check)	
Municipal	
Well	
Deep	
Shallow	
Surface	
Do you have results from a previous water test	**yes or no**
Are you results available	**yes or no**

***Return shipping courier charges will be covered by the project. Please use Purolator as the courier service. The appropriate Purolator account # to use is 8974303. The Instructions for sampling the water are available with the sample container.**

Please return form to:
Janice MacIsaac **e-mail address: Janice.MacIsaac@dal.ca**
APRI **Fax: 902-895-6734**
58 River Road **Phone: 902-896-2254**
Truro, Nova Scotia, B2N 5E3