

Chapter 4

Influence of Ca and P in the drinking water on egg production, egg quality, bone integrity and shell strength.

Introduction

Good shell quality is assumed to result from feeding diets high in calcium and low in phosphorus (Hartel 1990). Findings contest this assumption. Diets high in calcium are detrimental to egg weight (Ousterhout 1980) and sometimes to rates of lay (Moran et al. 1970). Insufficient dietary phosphorus depresses egg production and raises mortality (Singsen et al. 1962 and 1969, Harms and Miles 1977). It follows that there can be no single diet capable of supplying the amounts of calcium and phosphorus required for both maximal egg production and optimal shell quality.

Most nutritional studies with minerals have been carried out using dietary supplements. Little attention has been given to the role of minerals in drinking water. Underground water supplies, often containing high concentrations of dissolved salts, are a common source of drinking water for poultry in South Africa. Recent evidence suggests that some minerals in drinking water may exert adverse effects on the performance of laying hens when present at concentrations similar to those found in natural sources (Balnave and Scott 1986).

Water samples taken at poultry producers in certain areas of South Africa contained high levels of Ca and P (up to 291 and 32 mg/l respectively). This water may contribute significantly to the calcium and phosphorus status of layers. Establishing the contribution of Ca and P in the drinking water to egg shell quality and general egg production thus has immediate practical application.

The aim of this study was to establish the effect of different levels and combinations of Ca and P through the drinking water on growth, production and eggshell characteristics.

Materials and methods

The experimental animals were 720 Amber Link point of lay hens (20 weeks old), reared and vaccinated by a reputable organization to standard practices of the poultry industry. Water was administered to each repetition (20 birds) from a nipple drinker system connected to a calibrated 15 l Perspex cylinder via 5 nipples on a 3 m long pipe. Each nipple had the capacity to supply water to 12 layers. This nipple gives adequate amounts of water, yet maintains dry litter and is maintenance free. Lids on cylinders were removable for easy access and treatment administration. An outlet at the bottom simplified cleaning and refilling.

Hens were kept in a mechanically ventilated broiler house on a floor system with sawdust as bedding material. The house was divided into 36 pens of 2x3 m. Each pen housed 20 hens and was fitted with 5

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wire nest boxes with wooden lids and hay as nesting material, placed on the floor of the broiler house. The temperature was measured every day in 5 evenly distributed spots throughout the house with twin bulb minimum/maximum thermometers. The thermometers were suspended about 1.5 m above floor level at the entrance, in the middle and at the end of the house. Ventilation shafts were opened and electric fans functioned for the duration of the trial to curb ammonia poisoning. The lighting program during lay was according to supplier specification. A commercial laying diet with a vitamin and mineral premix was fed throughout the laying period. The Ca level in the feed was 36.89 g/kg and the P level was 5.05 g/kg.

Two round pan feeders were suspended from the roof of each cage. The brim of the feeder was kept at the same height as the backs of the birds. The hens were subjected to different levels and combinations of Ca and P through the drinking water (Table 4.1).

Calcium lactate was tested as a source of feed calcium for hens by several workers (Heywang 1946, Essary and Holmes 1966). They determined that it was equivalent to ground limestone and precipitated calcium carbonate for supporting whole egg weight and eggshell quality. Calcium lactate was therefore used as Ca source in this trial and P was supplied with Potassium phosphate.

The trial design was 4 levels of Ca and 3 levels of P as well as 6 combinations of both. There were 3 repetitions and 20 birds per replicate. The water from the Pretoria municipal source was used and the Ca and P present in the water was taken into account when formulating the inclusion levels. Chickens were housed in an environmentally controlled broiler house, on a floor system.

Water intake, feed intake, body weight, egg production, egg weight and temperature were measured weekly. Egg yolk colour was measured using the Roché Colour Fan. Egg-breaking strengths, eggshell thickness and the plasma Ca contents of representative samples of hens were established after 6 and 12 weeks.

After 12 weeks the trial was terminated. Mortalities were recorded and post mortem reports acquired. A representative sample of hens from each treatment group was sacrificed at the end of the trial period according to The Slaughter of Poultry (Humane Conditions) Regulations (Amendment) 1990. Kidney, liver, spleen and pancreatic samples were examined histopathologically. The breaking strength of the femora was determined on raw bones using the Allo Kreamer Shear Press (Rowland et al. 1967). Pieces of representative samples of eggs from each treatment were mounted on buttons and covered with Gold Palladium for Electron Microscopic Investigation.

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Table 4.1. Inclusion levels of Ca and P:

Treatment	Ca addition mg/l	P addition mg/l
1	0	0
2	100	0
3	200	0
4	300	0
5	0	150
6	100	150
7	200	150
8	300	150
9	0	300
19	100	300
11	200	300
12	300	300

Results and Discussion

Several factors are involved in eggshell formation and its subsequent quality (Butcher 1996). Major factors include, but are not limited to, the source and level of calcium in the diet, phosphorus level in the diet and temporal intake of these minerals.

Phosphorus is an important mineral for eggshell formation. Eggshells contain little phosphorus (Ca : P in eggshell is approximately 100 : 1), but this element interacts with calcium in bone formation. Calcium is stored in the skeleton almost entirely as calcium phosphate; synthesis of medullary bone requires dietary phosphorus. This phosphorus is, however, essentially superfluous, because if the calcium is used for shell formation, the phosphorus must be excreted.

Nutritional interest in phosphorus has been stimulated by several observations that dietary excess of this element has a detrimental effect on shell quality (Arscott et al. 1962, Taylor 1965, Harms 1982a and 1982b). It is not clear whether this phosphorus excess, by accumulating in the blood, interferes with mobilization of skeletal reserves of calcium phosphate during shell formation, or whether there is a direct antagonistic effect of blood phosphorus on the shell forming process. Whatever the mechanism, there is no doubt that diets which lead to an increase in plasma phosphate cause a decline in egg specific gravity and thus in shell quality. Miles and Harms (1982) showed a clear negative linear correlation between specific gravity and plasma phosphate over a range of treatments.

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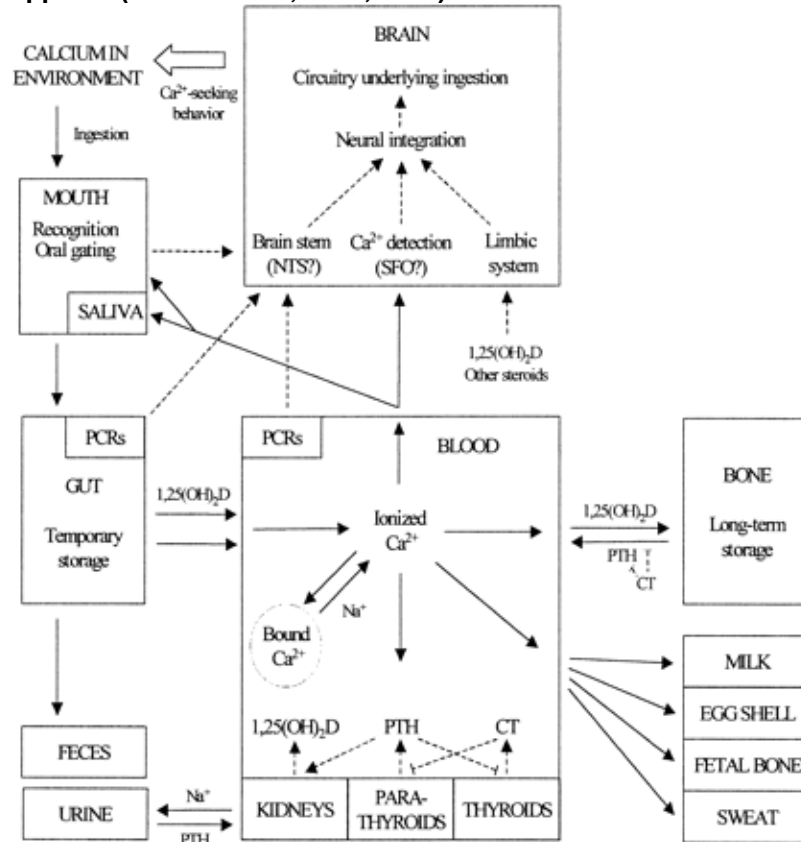
Peak plasma and organic phosphorus concentration 15 hours after ovulation may be attributable to medullary bone resorption during shell formation (Van de Velde et al. 1986). It is speculated that this rise in blood phosphorus level interferes with the mechanisms of eggshell calcification, on one hand, and taxes the hen's body through excess excretion, on the other (Anwar and Balander 2004).

The fraction of dietary Ca absorbed varies with body Ca requirements, daily Ca intake and age. The factors that increase or decrease intestinal Ca absorption are presented in Table 4.2 (Favus 1992).

Table 4.2. The factors that increase or decrease intestinal Ca absorption

<u>Increase</u>	Decrease
Vitamin D	Aging
Parathyroid hormone	Glucocorticoids
Low-Ca diet	Thyroid hormone
Growth	Phytate
Lactation	Oxalate
Pregnancy	Thiazide diuretics
Lactose	Gastric surgery
Estrogen	Metabolic acidosis
Alkalosis	

Figure 4.1. A simplified model of the relationship between calcium homeostasis and calcium appetite (Tordoff et al.; 1998, 2001)



According to this model, the primary signal for calcium appetite is derived from ionized calcium levels in the blood, which are detected by calcium receptors, perhaps in the subfornical organ (SFO). Signals concerning calcium status also arise from the oral cavity and peripheral pre- and post absorptive calcium receptors (PCRs). These may be integrated in the nucleus tractus solitarius (NTS) or other early brain stem nuclei. The influences of parathyroid hormone (PTH), calcitonin (CT) and 1,25-dihydroxyvitamin D [1,25-(OH)₂D] are mostly secondary to their actions on ionized calcium in the blood. However, 1,25(OH)₂D and other hormones may exert a direct effect on the brain to influence calcium appetite (Tordoff et.al. 1998, 2001, Figure 4.1).

In laying domestic hens (*Gallus domesticus*) 125 mg of calcium are deposited every hour (Reynolds 1997). This mobilization represents a total clearance of blood calcium every 12 min. Each eggshell requires approximately 2 g of calcium. A digestive bottleneck restricts the amount of calcium available from dietary sources to approximately 1 g per day. The shortfall is met by mobilization of calcium from the medullary bone. In extreme cases, as much as 10% of the skeletal mass can be mobilized in less than 24 hours. Although medullary bone has been reported on in other species, its role as a calcium source during egg production in small birds is poorly understood.

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The calcium content of the blood of normal chickens, except hens producing eggs, was found to be practically the same as that of other animals. As pullets matured, indicated by comb development, the calcium content of the blood increased. During egg production the calcium content of the blood remained high, being from two to three times the ordinary amount. When laying ceased, either from molting or setting, the calcium content dropped to the normal level. It rose to a high level again when egg laying resumed. During egg production the amount of calcium in the blood did not remain constant, as is usually the case, but fluctuated as much as 10 milligrams from week to week. The cause of the fluctuation has not been determined.

Both the calcium and phosphorus contents of the blood are higher for hens than for cocks (Kansas State College of Agriculture and Applied Sciences, Technical Bulletin 34, 1933). Moreover, bone stores of adult hens are substantial, and calcium is conserved efficiently, making a long period of deprivation necessary (Hughes and Wood-Gush 1971).

Ninety-seven percent of the eggshell consists of calcium carbonate. The shell weighs approximately 6.0 g, so almost 6.0 g of calcium carbonate must be synthesized and deposited on the shell each time the hen produces an egg. For many hens, this is almost daily for long sequences. Calcium carbonate is 40% calcium, thus about 2.5 g of elemental calcium must be found and transported to the shell gland in the 18-20 hours it takes to form the eggshell. The calcium content of blood at any given time is no more than 30 mg. Thus the shell contains over 80 times more calcium than the blood (Hunton 2005).

In a study done by Scheideler et al. (1995) the serum Ca levels of broilers fed 140% of the NRC recommendation was 9.21 mg/dl. In this study the Ca level in the blood ranged from 195.833 mg/l in treatment 8 to 267.917 mg/l in treatment 5. These differences were, however, not significant ($P = 0.2394$, Table 4.3).

Eggshell is a relatively constant proportion of egg weight (Djader 1982). Lennards et al. (1981) found no relationship between serum calcium and shell weight or egg weight. They concluded that the normal variation in serum calcium is not related to the hen's ability to produce eggshell.

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Table 4.3 Blood Ca levels of hens receiving different levels and combinations of Ca and P (mg/l) P = 0.2394

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean blood Ca levels after 12 weeks	SD
1	0	0	260.000 ^a	±38.058
2	100	0	228.750 ^a	±55.043
3	200	0	214.167 ^a	±23.596
4	300	0	223.750 ^a	±26.605
5	0	150	267.917 ^a	±34.286
6	100	150	224.583 ^a	±21.878
7	200	150	196.250 ^a	±14.416
8	300	150	195.833 ^a	±32.890
9	0	300	212.500 ^a	±45.208
10	100	300	228.333 ^a	±41.727
11	200	300	248.333 ^a	±12.521
12	300	300	219.583 ^a	±22.512

Ca and P treatment had a significant influence ($P = 0.0001$) on weekly body weight (Table 4.4). The mean body weights, measured over the whole period were, however, not significantly affected by Ca and P administration ($P = 0.7624$). There were no significant interactions between Ca and P levels and the duration of exposure to treatments, 6 or 12 weeks, on body weight ($P = 0.3534$).

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Table 4.4. LS Means of body weight (kg) of hens receiving different levels of Ca and P in the drinking water.

LS Means for weekly body weight (kg)														
Treat ment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
1 ±SD	1.438 0.066	1.760 0.221	1.674 0.059	1.691 0.059	1.699 0.058	1.727 0.051	1.749 0.045	1.763 0.040	1.764 0.040	1.782 0.041	1.786 0.023	1.789 0.029	1.786 0.017	1.812 0.006
2 ±SD	1.476 0.019	1.757 0.335	1.713 0.016	1.726 0.016	1.717 0.017	1.759 0.007	1.782 0.009	1.791 0.018	1.805 0.011	1.811 0.011	1.829 0.003	1.820 0.007	1.838 0.011	1.865 0.021
3 ±SD	1.441 0.045	1.535 0.041	1.672 0.034	1.706 0.041	1.718 0.042	1.737 0.035	1.756 0.039	1.765 0.033	1.773 0.032	1.790 0.042	1.795 0.035	1.792 0.050	1.825 0.055	1.856 0.046
4 ±SD	1.488 0.039	1.562 0.042	1.669 0.052	1.695 0.025	1.720 0.046	1.749 0.036	1.769 0.052	1.775 0.042	1.785 0.054	1.801 0.038	1.814 0.034	1.806 0.039	1.822 0.034	1.844 0.056
5 ±SD	1.446 0.027	1.537 0.027	1.666 0.017	1.700 0.025	1.727 0.042	1.754 0.036	1.763 0.019	1.764 0.015	1.772 0.023	1.789 0.027	1.801 0.036	1.796 0.039	1.810 0.043	1.815 0.050
6 ±SD	1.430 0.022	1.584 0.098	1.669 0.035	1.671 0.045	1.685 0.044	1.713 0.042	1.752 0.010	1.767 0.027	1.775 0.022	1.807 0.054	1.797 0.020	1.799 0.013	1.811 0.023	1.833 0.027
7 ±SD	1.492 0.040	1.571 0.037	1.680 0.027	1.709 0.031	1.744 0.016	1.762 0.020	1.792 0.042	1.787 0.022	1.798 0.027	1.786 0.057	1.823 0.041	1.826 0.040	1.844 0.029	1.834 0.023

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Table 4.4. LS Means of body weight (kg) of hens receiving different levels of Ca and P in the drinking water (continued).

LS Means for weekly body weight (kg)														
Treat ment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
8 \pm SD	1.504 0.021	1.592 0.035	1.710 0.022	1.730 0.015	1.747 0.028	1.763 0.020	1.787 0.029	1.783 0.022	1.797 0.024	1.831 0.029	1.815 0.037	1.811 0.030	1.830 0.028	1.837 0.026
9 \pm SD	1.439 0.017	1.556 0.013	1.671 0.031	1.690 0.035	1.703 0.044	1.732 0.033	1.754 0.056	1.763 0.045	1.770 0.046	1.784 0.046	1.787 0.056	1.787 0.051	1.797 0.048	1.809 0.055
10 \pm SD	1.409 0.058	1.523 0.043	1.666 0.038	1.680 0.043	1.697 0.048	1.719 0.049	1.738 0.040	1.749 0.037	1.764 0.050	1.803 0.064	1.786 0.052	1.785 0.048	1.805 0.049	1.818 0.045
11 \pm SD	1.449 0.076	1.562 0.090	1.681 0.062	1.701 0.055	1.719 0.063	1.747 0.054	1.766 0.061	1.746 0.047	1.776 0.041	1.790 0.036	1.807 0.049	1.805 0.048	1.821 0.046	1.833 0.040
12 \pm SD	1.468 0.014	1.556 0.040	1.663 0.045	1.716 0.013	1.725 0.008	1.752 0.018	1.765 0.002	1.770 0.007	1.779 0.016	1.788 0.017	1.794 0.016	1.797 0.018	1.802 0.012	1.821 0.012

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Table 4.5. Mean body weight of birds over time (kg). $P = 0.7624$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Body weight	SD
1	0	0	1.730	0.228
2	100	0	1.763	0.024
3	200	0	1.726	0.036
4	300	0	1.736	0.041
5	0	150	1.724	0.024
6	100	150	1.721	0.012
7	200	150	1.746	0.025
8	300	150	1.753	0.018
9	0	300	1.717	0.04
10	100	300	1.710	0.044
11	200	300	1.729	0.052
12	300	300	1.728	0.01

Ca and P treatment had a significant influence on egg production in terms of eggs/hen/week or % (Table 4.6, $P = 0.0004$), but no significant influence on egg mass (Table 4.7, $P = 0.4175$). Interactions between Ca and P levels and exposure time to the treatments did not affect egg production ($P = 0.8838$) and egg weight ($P = 0.4747$) significantly. Mean egg production and egg weight over the trial period were not affected by Ca and P administration (Table 4.8).

Dietary phosphorus appears to have a biphasic effect on eggshell quality. An inadequate level of P in the diet reduces eggshell quality; high dietary P also has detrimental effects. The mechanism by which a high level of dietary P adversely affects eggshell quality has not yet been determined.

Possible mechanisms have been suggested (Keshavarz and Austic 1990). Calcium absorption may be reduced because of the formation of insoluble calcium phosphate in the gastrointestinal tract. An increased level of P may reduce the mobilization of Ca from the bones for shell formation. P ions could inhibit normal precipitation of calcium carbonate under physiological conditions.

There was a significant ($P = 0.0001$) interaction between P levels in the eggshells and exposure time to P administration (Table 4.9). The P levels in the shells decreased as the P levels in the water were increased. The Ca content of the shells was not significantly influenced by Ca and P addition to the drinking water (Table 4.10).

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As age advances, proportion of yolk increases, whereas proportions of albumen and shell thickness decrease (Akbar et al. 1983, Fletcher et al. 1983).

There was no significant ($P = 0.2261$) interaction between eggshell thickness and the exposure time to the Ca and P treatments (Table 4.11).

Although Ca and P are two major macro-minerals involved in bone formation (Frost and Roland 1991), strength or weakness of eggshell is more directly related to carbonic anhydrase activity than to Ca-ATPase, calcium-binding protein in shell gland +2 (Balnave et al. 1992) and serum Ca concentration (Lennards et al. 1981).

In the case of alkalosis, decreased concentration of ionized Ca in serum negatively affects shell formation (Odom et al. 1986). Lower solubility of dietary Ca and slower rate of passage limit the formation of eggshell (Gordon and Roland 1997). Skeletal and urinary Ca metabolism does not affect eggshell quality (Buss et al. 1980).

Eggshell strength depends on its thickness, weight and structure. The mineral content of the diet influences those parameters more than the breed does (Lennards et al. 1981, Junqueira et al. 1984, Clunies and Leeson 1995). Eggshell is a relatively constant proportion of egg weight (Djader 1982).

In this experiment there was a significant ($P = 0.0268$) interaction between eggshell breaking strength and the exposure time to the Ca and P administration (Table 4.12). There was no significant ($P = 0.1963$) interaction between the Roché colour score of egg yolks and the exposure time to the treatments. The average score was between 8 and 9 during the first 6 weeks, and between 7 and 8 during the second 6 weeks (Table 4.13).

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Table 4.6. LS Means of egg production (eggs /hen/week) of hens receiving different levels of Ca and P in the drinking water (SD± 0.2127).

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
1 ±SD	0.33 0.225	1.87 0.928	4.30 1.146	5.77 0.153	6.20 0.278	6.38 0.058	6.40 0.050	6.60 0.259	6.37 0.340	6.75 0.350	6.45 0.346	6.62 0.231	6.67 0.115
2 ±SD	0.15 0.132	1.70 0.950	4.28 0.711	5.38 0.500	5.87 0.520	6.3 0.450	6.57 0.208	6.63 0.231	6.45 0.278	6.60 0.346	6.45 0.436	6.5 0.132	6.58 0.333
3 ±SD	0.25 0.312	1.72 0.725	3.92 0.448	5.57 0.535	5.76 0.577	6.27 0.501	6.38 0.548	6.28 0.375	6.65 0.265	6.58 0.765	6.52 0.231	6.62 0.513	6.25 0.841
4 ±SD	0.52 0.369	3.00 0.737	4.75 0.180	5.05 0.477	5.95 0.391	6.32 0.104	6.60 0.229	6.23 0.473	6.60 0.458	6.77 0.126	6.60 0.180	6.65 0.100	6.83 0.189
5 ±SD	0.22 0.029	1.95 0.650	4.07 0.454	5.93 0.454	5.85 0.695	6.38 0.369	6.47 0.126	6.62 0.379	6.68 0.202	6.78 0.115	6.62 0.318	6.43 0.225	6.75 0.132
6 ±SD	0.10 0.132	1.95 0.300	4.53 0.284	6.02 0.306	5.80 0.577	6.43 0.161	6.50 0.350	6.41 0.429	6.69 0.056	6.89 0.367	6.65 0.342	6.60 0.266	6.95 0.087

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Table 4.6. LS Means of egg production (eggs /hen/week) of hens receiving different levels of Ca and P in the drinking water (SD± 0.2127) (continued).

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
7 ±SD	0.30 0.361	2.45 1.117	3.95 0.650	5.32 0.675	5.47 0.597	6.15 0.132	6.37 0.318	6.35 0.278	6.42 0.293	6.70 0.312	6.52 0.351	6.58 0.208	6.80 0.477
8 ±SD	0.30 0.229	2.38 0.225	4.52 0.843	5.62 0.076	5.85 0.265	6.08 0.525	6.47 0.225	6.63 0.104	6.57 0.153	6.65 0.100	6.48 0.275	6.48 0.251	6.77 0.202
9 ±SD	0.25 0.304	2.10 0.901	4.08 0.575	5.37 0.551	6.07 0.633	6.22 0.104	6.35 0.132	6.45 0.350	6.55 0.563	6.62 0.375	6.55 0.200	6.67 0.153	7.05 0.278
10 ±SD	0.22 0.333	2.07 0.551	4.42 0.592	5.22 0.750	5.98 0.635	6.48 0.076	6.52 0.355	6.37 0.480	6.48 0.553	6.88 0.104	6.68 0.340	6.58 0.379	6.85 0.173
11 ±SD	0.27 0.202	2.22 0.729	4.35 0.608	6.13 0.231	5.75 0.444	6.15 0.377	6.52 0.257	6.43 0.437	6.53 0.437	6.67 0.407	6.45 0.225	6.64 0.295	6.81 0.185
12 ±SD	0.35 0.132	2.65 0.229	5.10 0.132	6.13 0.231	6.05 0.312	6.57 0.189	6.73 0.340	6.67 0.058	6.77 0.289	6.95 0.100	6.75 0.100	6.48 0.104	6.88 0.404

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Table 4.7. LS Means of egg weight (g) of eggs produced by hens receiving different levels of Ca and P in the drinking water (SD \pm 2.3615).

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
1 \pm SD	37.94 1.257	47.62 1.852	53.77 15.95	48.61 0.896	50.80 1.05	52.27 0.607	53.37 0.226	54.60 0.809	54.71 0.537	55.70 0.546	56.10 0.404	56.67 0.283	56.53 0.238
2 \pm SD	23.58 20.44	49.44 3.149	46.18 2.170	48.22 1.104	50.77 0.044	54.22 0.614	54.34 0.839	55.01 1.066	54.82 0.759	55.91 0.706	56.35 0.687	57.14 0.683	56.55 1.153
3 \pm SD	23.55 20.40	44.99 2.501	45.59 1.429	48.41 1.493	51.39 0.838	52.60 0.452	54.08 0.264	54.43 1.036	54.59 0.857	55.40 0.571	56.09 0.706	56.33 0.837	58.31 2.989
4 \pm SD	37.68 1.477	43.93 1.823	44.95 2.486	47.53 1.076	50.05 1.014	51.68 0.849	52.43 1.262	53.05 0.833	53.59 1.487	54.75 0.946	55.47 1.062	55.53 1.458	56.42 0.814
5 \pm SD	35.99 2.267	44.45 1.822	44.44 0.709	47.97 0.740	50.57 1.277	52.05 0.704	53.28 1.189	53.70 1.006	54.02 0.418	55.13 0.571	55.44 1.119	55.93 0.833	56.16 0.347
6 \pm SD	20.50 19.25	46.57 1.002	44.94 1.636	48.08 2.687	51.75 0.277	53.23 0.494	54.22 0.358	54.52 0.453	54.76 0.916	56.45 0.978	56.51 0.978	56.85 0.716	57.12 0.762

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Table 4.7. LS Means of egg weight (g) of eggs produced by hens receiving different levels of Ca and P in the drinking water ($SD \pm 2.3615$) (continued).

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
7 $\pm SD$	25.79 22.40	46.25 2.579	44.75 0.503	48.33 0.832	50.38 1.331	52.42 1.741	52.97 1.119	53.69 1.036	53.74 1.593	55.04 0.712	55.65 1.158	55.96 1.616	56.34 1.550
8 $\pm SD$	36.28 2.362	46.17 1.583	44.70 0.905	47.83 0.624	50.22 0.724	52.24 0.657	52.97 0.841	54.12 0.856	54.40 0.322	54.91 0.375	55.50 0.654	55.86 0.460	56.26 0.635
9 $\pm SD$	39.66 4.969	47.86 0.758	45.13 1.994	48.10 0.787	50.42 0.918	52.21 1.026	54.38 1.938	53.58 1.131	53.94 1.832	54.61 1.515	55.00 1.389	54.32 3.247	55.79 1.346
10 $\pm SD$	24.79 21.52	48.51 0.625	45.02 0.547	48.64 0.775	50.73 0.723	53.12 0.739	53.45 1.086	54.19 0.809	54.48 0.904	55.31 1.400	54.34 3.574	56.58 1.335	56.88 1.117
11 $\pm SD$	39.54 5.537	47.87 3.209	45.91 4.401	49.38 0.133	52.00 0.434	53.43 0.493	54.10 0.365	55.09 0.912	55.03 0.785	55.67 1.243	56.64 1.020	56.60 0.959	57.04 0.856
12 $\pm SD$	40.16 0.370	46.81 1.125	42.40 2.013	48.63 0.734	51.54 1.494	52.44 0.445	53.09 0.423	53.50 0.428	54.04 1.059	55.31 1.178	55.14 1.431	55.57 1.056	55.90 0.814

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Ca and P treatment had a significant influence ($P = 0.0004$) on egg production (eggs/hen/week or %) but no significant influence on egg mass ($P = 0.4175$). There were no significant interactions between Ca and P administered and exposure time to the treatments on egg production ($P = 0.8838$) and egg weight ($P = 0.4747$).

Table 4.8. Mean egg production and egg weight per treatment over weeks.

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Egg Production (Eggs/hen/week) $P = 0.7496$	Egg production (%) $P = 0.7761$	Egg weight (g) $P = 0.6842$
1	0	0	5.438	77.563	52.206
2	100	0	5.344	76.337	50.964
3	200	0	5.289	75.549	50.443
4	300	0	5.528	78.718	50.544
5	0	150	5.442	77.747	50.702
6	100	150	5.502	77.754	50.424
7	200	150	5.336	76.099	50.099
8	300	150	5.446	77.802	50.88
9	0	300	5.409	77.271	51.155
10	100	300	5.442	77.747	50.464
11	200	300	5.455	77.817	52.176
12	300	300	5.699	81.41	51.118

Table 4.9. P contents of the egg shells (%)

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean P content (%) after 6 weeks.	SD	Mean P contents (%) after 12 weeks.	SD
1	0	0	0.119a	0.005	0.115a	0.005
2	100	0	0.123a	0.007	0.120ab	0.004
3	200	0	0.120a	0.007	0.116ab	0.009
4	300	0	0.130a	0.009	0.126ab	0.002
5	0	150	0.122a	0.011	0.115ab	0.005
6	100	150	0.123a	0.001	0.116ab	0.001
7	200	150	0.120a	0.01	0.111ab	0.006
8	300	150	0.125a	0.007	0.108ab	0.006
9	0	300	0.126a	0.005	0.123ab	0.003
10	100	300	0.113a	0.009	0.113ab	0.006
11	200	300	0.129a	0.008	0.119ab	0.005
12	300	300	0.123a	0.006	0.114b	0.002

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Table 4.10. Ca contents of the egg shells (%)

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean Ca content (%) after 6 weeks	± SD	Mean Ca contents (%) after 12 weeks.	± SD
1	0	0	30.947	1.167	31.053	1.372
2	100	0	30.79	0.79	29.213	2.298
3	200	0	31.333	0.577	31.23	0.488
4	300	0	31.06	0.567	30.897	0.179
5	0	150	31.35	0.488	30.163	0.545
6	100	150	29.833	0.951	30.493	0.43
7	200	150	30.793	0.845	30.503	0.775
8	300	150	29.567	2.072	30.497	0.556
9	0	300	29.403	0.438	29.473	0.607
10	100	300	30.067	0.634	29.833	0.951
11	200	300	30.43	1.444	30.69	0.1
12	300	300	29.803	0.195	29.733	0.94

Table 4.11. Eggshell thickness of hens receiving different levels and combinations of Ca and P (mm) $P = 0.4213$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean shell thickness (mm) after 6 weeks.	± SD	Mean shell thickness (mm) after 12 weeks.	± SD
1	0	0	0.423a	0.035	0.369a	0.034
2	100	0	0.424a	0.03	0.391ab	0.018
3	200	0	0.429a	0.036	0.381ab	0.035
4	300	0	0.419a	0.037	0.381ab	0.027
5	0	150	0.422a	0.037	0.379ab	0.037
6	100	150	0.426a	0.035	0.379ab	0.029
7	200	150	0.447a	0.042	0.374ab	0.03
8	300	150	0.447a	0.033	0.379ab	0.034
9	0	300	0.419a	0.036	0.361ab	0.025
10	100	300	0.432a	0.044	0.368ab	0.036
11	200	300	0.419a	0.041	0.355ab	0.032
12	300	300	0.436a	0.037	0.373b	0.027

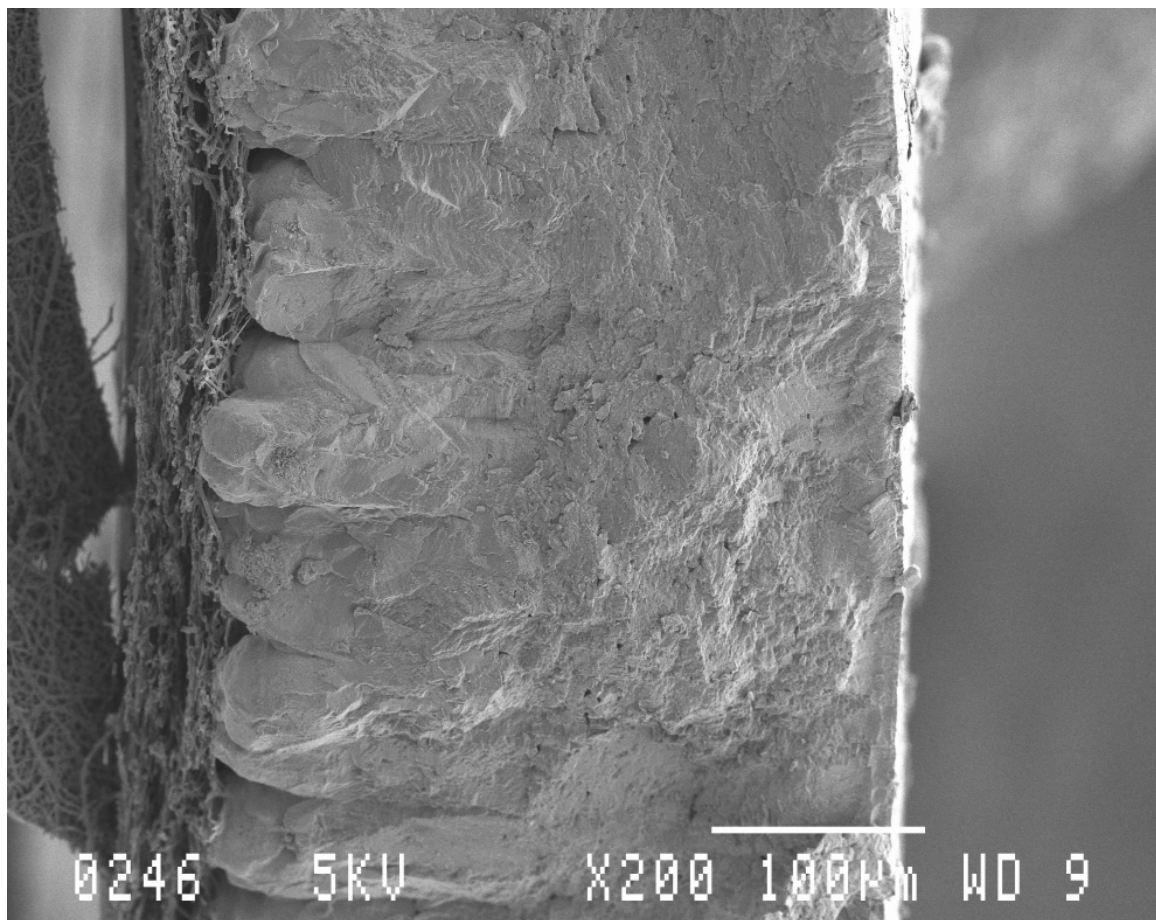
Table 4.12. Eggshell breaking strength of hens receiving different levels and combinations of Ca and P (N) $P = 0.4213$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean shell breaking strength (N) after 6 weeks. $P = 0.5254$	± SD	Mean shell breaking strength (N) after 12 weeks. $P = 0.0820$	± SD
1	0	0	36.898a	9.884	38.934a	6.988
2	100	0	36.145a	8.046	39.993a	6.211
3	200	0	37.998a	10.671	38.232a	8.635
4	300	0	38.492a	8.57	34.977a	7.899
5	0	150	43.532a	12.283	35.545a	11.579
6	100	150	35.789a	8.572	41.181a	6.634
7	200	150	38.771a	12.669	42.642a	8.999
8	300	150	38.610a	8.803	37.165a	8.784
9	0	300	40.605a	7.794	34.088a	11.673
10	100	300	36.183a	9.271	38.184a	11.066
11	200	300	39.487a	9.446	34.686a	9.054
12	300	300	38.175a	7.263	39.038a	6.583

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Images from the scanning electron microscope taken from eggs in the control group, the treatment with the lowest (Treatment 7) and highest (Treatment 9) eggshell breaking strengths are presented in Figures 4.2, 4.3 and 4.4 below. As can be seen, the shell of Treatment 7 is much more crystalline and the higher breaking strength is explained.

Figure 4.2. Lateral view of the eggshell from an egg of Treatment 1 (control) (x200).



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Figure 4.3 Lateral view of the eggshell of an egg from Treatment 7 (x200).

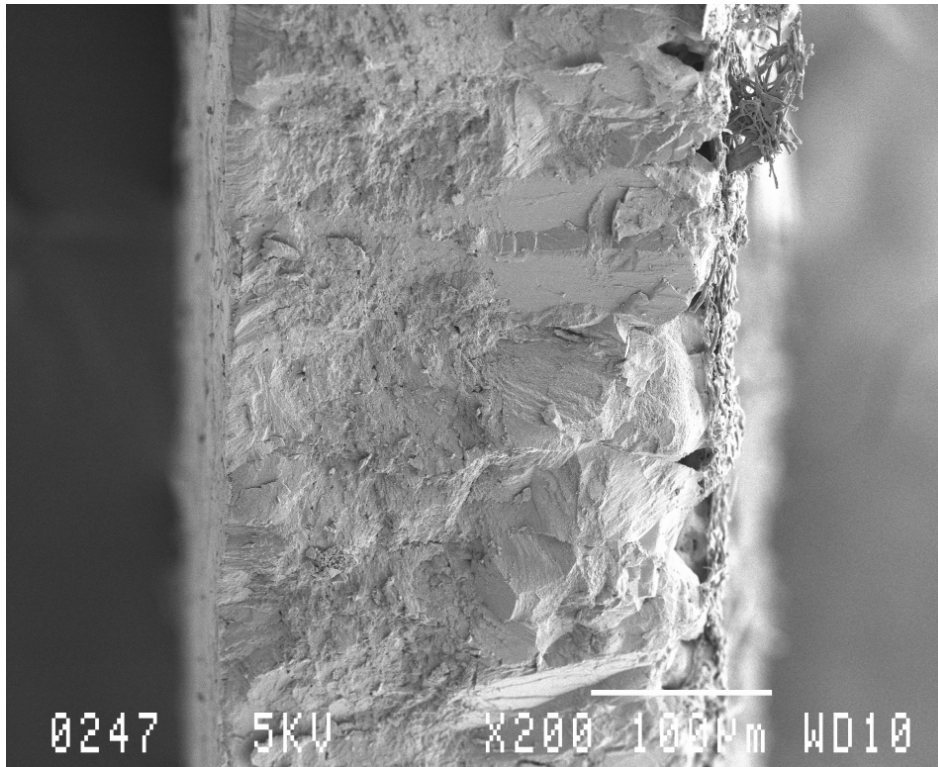
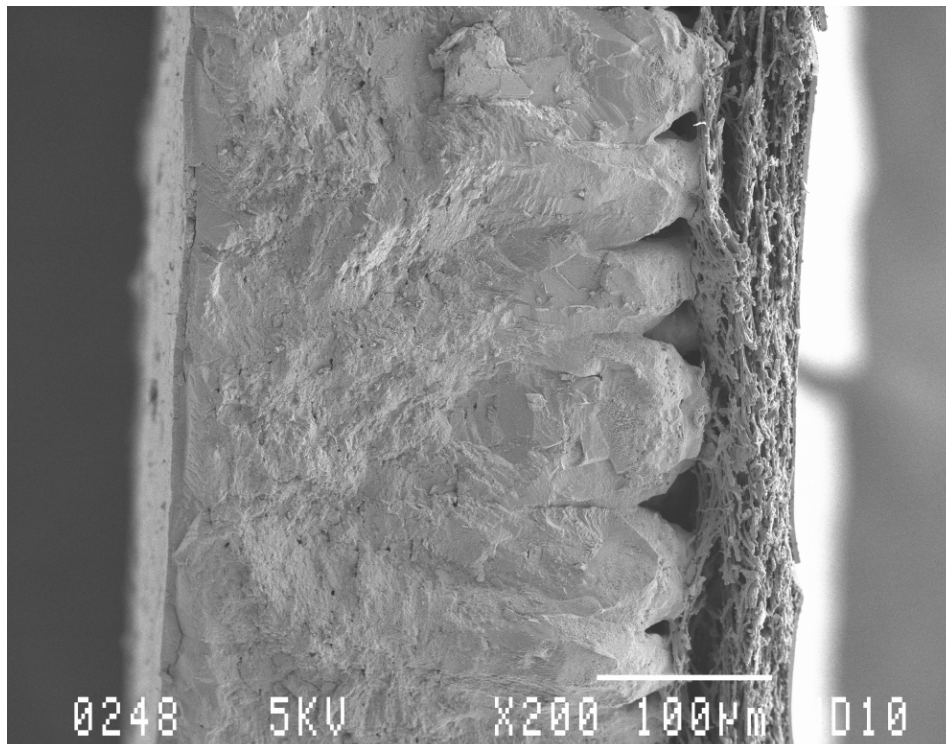
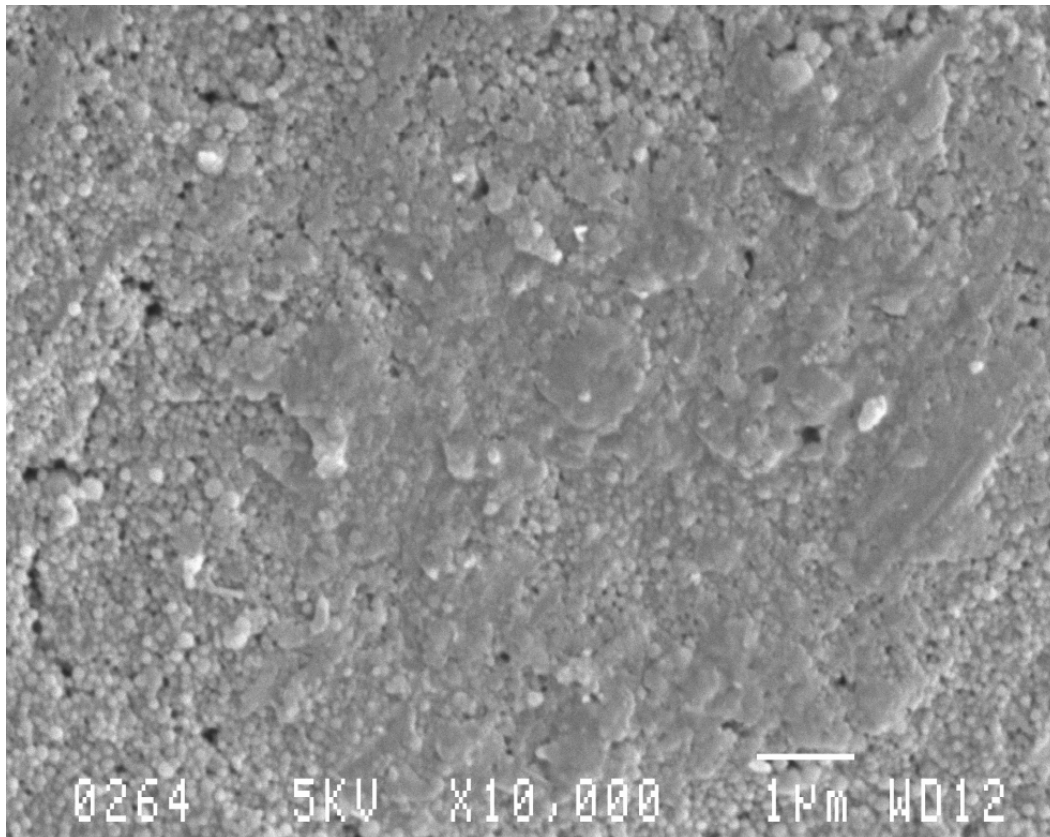


Figure 4.4. Lateral view of the eggshell of an egg from Treatment 9 (x200)



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Figure 4.5 Outer shell of Treatment 1. (x 10 000)



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Figure 4.6. Outer shell of Treatment 7. (x 10 000)

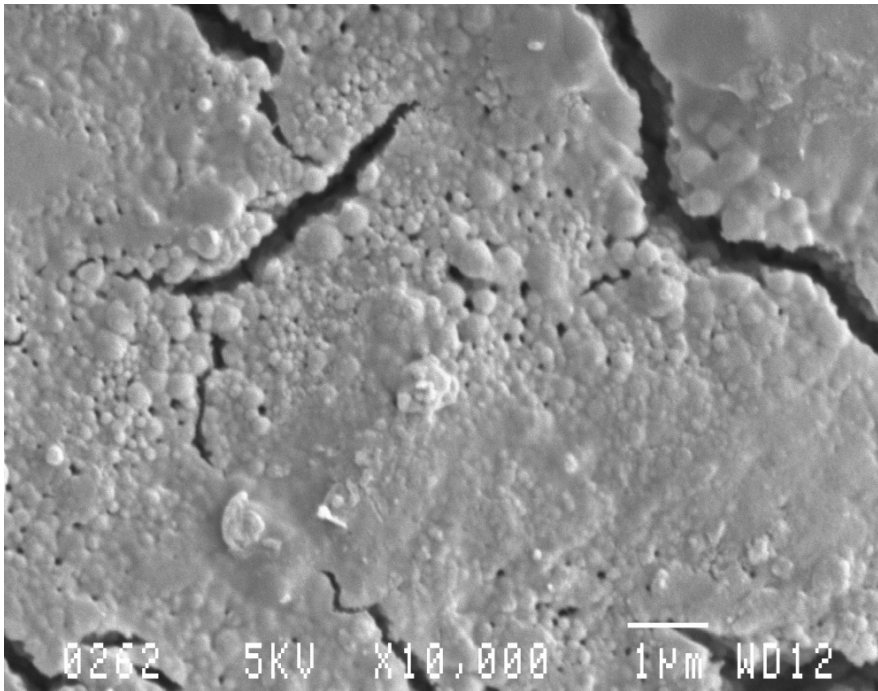
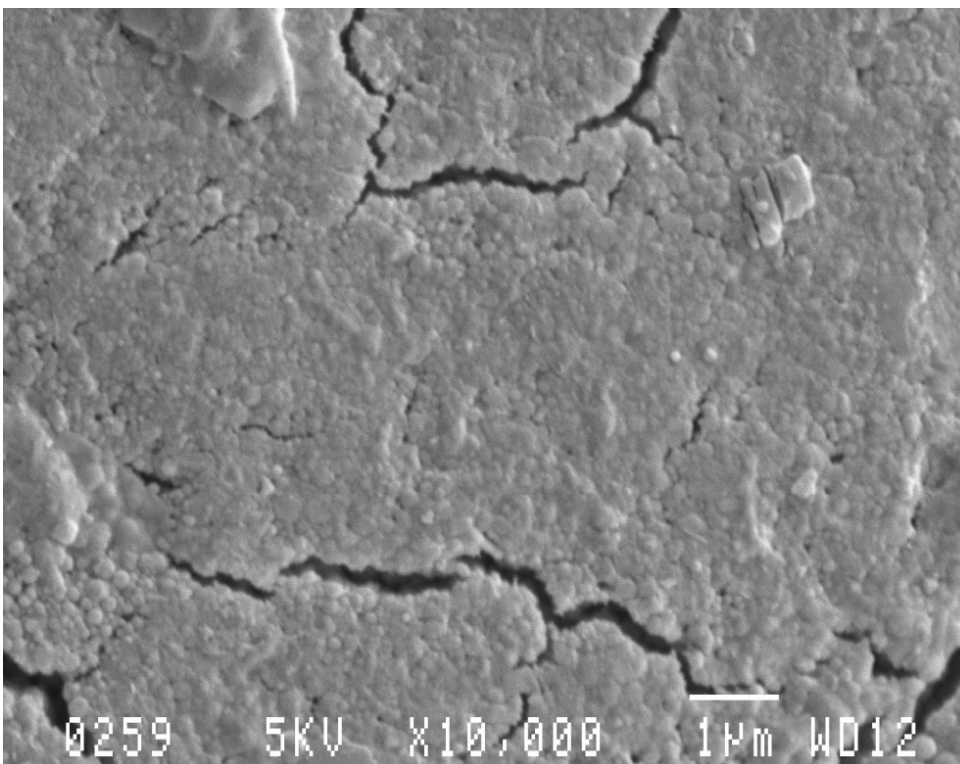


Figure 4.7. Outer shell of Treatment 9. (x 10 000)

Looking at the outer surface of the eggs the shells in Treatments 1, 7 and 9, a marked difference in the



outer shell appearance and structural soundness can be observed (Figures 4.5, 4.6, and 4.7, above). This corresponds to the treatments administered.

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Table 4.13. Roché egg yolk colour score of hens receiving different levels and combinations of Ca and P (P = 0.4213)

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Mean Roché colour score after 6 weeks. P = 0.1111	± SD	Mean Roché colour score after 12 weeks. P = 0.0190	± SD
1	0	0	9.222a	0.808	8.500a	0.924
2	100	0	9.000a	0.686	7.722a	1.018
3	200	0	9.222a	0.732	8.556a	0.984
4	300	0	8.556a	0.783	7.889a	1.079
5	0	150	9.000a	0.594	8.056a	0.802
6	100	150	9.056a	0.539	8.167a	1.043
7	200	150	9.000a	0.594	8.722a	0.669
8	300	150	9.056a	0.416	7.778a	1.166
9	0	300	8.833a	0.618	8.111a	0.963
10	100	300	9.056a	0.416	8.389a	0.85
11	200	300	9.111a	0.583	7.944a	0.802
12	300	300	9.111a	0.583	8.000a	1.085

Ca and P treatment had a significant influence (P = 0.0001) on feed intake (Table 4.14). There was however no significant interaction between Ca and P administered and exposure time to the treatments on feed intake (P = 0.7835).

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Table 4.14. LS Means of daily food intake (g) of hens receiving different levels of Ca and P in the drinking water

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
1 ±SD	96 10.46	135 1.74	134 10.25	141 10.30	149 4.91	146 2.70	151 4.04	155 1.08	156 3.91	155 3.62	159 3.50	167 10.97	170 4.91
2 ±SD	98 6.71	132 4.19	129 6.98	135 2.50	145 6.50	140 3.69	144 5.81	148 1.71	143 8.14	144 9.39	150 5.20	154 6.88	150 0.44
3 ±SD	99 4.43	133 1.93	131 2.77	137 1.62	147 3.18	148 2.62	150 4.96	151 6.69	156 6.40	152 1.85	151 2.21	158 5.47	156 3.12
4 ±SD	97 9.39	131 6.97	137 7.74	140 13.31	149 11.54	151 14.57	150 15.21	153 14.25	153 11.33	152 8.08	153 13.26	157 14.72	157 10.60
5 ±SD	99 11.61	130 3.97	133 9.64	140 7.45	144 6.36	150 10.37	145 7.30	149 4.72	147 6.03	150 8.43	149 2.23	154 2.66	153 3.57
6 ±SD	96 9.815	129 12.74	131 9.32	145 9.47	152 13.80	152 11.74	141 1.08	154 14.52	157 17.30	159 16.91	163 19.91	168 19.75	166 17.38
7 ±SD	100 12.27	126 4.30	129 4.53	135 3.84	142 2.74	144 3.68	142 7.90	146 9.12	151 7.07	150 6.90	151 6.81	156 11.08	154 8.90

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Table 4.14. LS Means of daily food intake (g) of hens receiving different levels of Ca and P in the drinking water (continued).

Treatment	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32
8 <i>±SD</i>	106 10.11	139 6.19	136 4.96	147 8.54	148 5.80	151 10.09	149 12.51	152 11.57	156 10.83	155 12.18	162 10.01	164 6.33	164 10.19
9 <i>±SD</i>	99 5.93	126 2.76	129 8.98	133 7.08	138 7.20	139 10.61	142 8.63	142 12.97	145 11.82	145 12.48	148 13.52	155 13.93	155 9.27
10 <i>±SD</i>	101 9.17	135 10.74	136 12.62	131 10.57	144 13.68	141 11.36	139 7.56	144 8.15	146 6.00	149 5.31	151 7.59	150 7.27	155 11.57
11 <i>±SD</i>	100 15.99	133 8.59	129 10.93	133 10.49	146 11.73	145 10.93	140 8.36	146 9.23	148 5.13	147 6.36	153 5.19	156 7.18	157 8.37
12 <i>±SD</i>	103 1.46	130 7.00	134 5.09	143 7.79	150 6.94	150 5.97	149 7.68	153 11.03	152 6.90	152 2.27	152 4.17	154 2.91	154 2.26

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Schedeler et al. (1995) found that excessive Ca intake decreases growth and feed efficiency in broiler chickens. In this experiment Ca and P treatment ($P = 0.7351$) and exposure time to the treatments ($P = 0.7835$) both had no significant influence on feed intake (Table 4.15).

Table 4.15. Mean food intake of birds over time (g/hen/day). $P = 0.7351$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Food intake	\pm SD
1	0	0	147a	3
2	100	0	139a	3
3	200	0	144a	2
4	300	0	145a	11
5	0	150	142a	5
6	100	150	147a	11
7	200	150	140a	6
8	300	150	148a	9
9	0	300	138a	9
10	100	300	140a	7
11	200	300	141a	8
12	300	300	144a	3

Ca and P treatment had no significant influence ($P = 0.8833$) on water intake (Table 4.16) and exposure time to the treatments on water intake ($P = 0.9992$) (Table 4.17).

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Table 4.16. LS Means for daily water intake (ml/hen/day) (SD \pm 0.004)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
1 \pm SD	149 10.87	177 3.38	203 21.97	192 21.90	189 13.07	193 9.10	188 15.75	214 19.91	208 9.25	211 4.96	202 8.43	195 11.54	251 12.10
2 \pm SD	147 14.17	179 3.79	199 0.41	188 1.22	185 7.58	191 7.95	188 8.44	210 2.30	204 8.50	220 10.82	204 5.76	198 6.82	252 9.97
3 \pm SD	154 4.32	180 4.29	196 7.03	194 9.91	190 9.96	191 5.00	191 6.48	210 12.82	205 10.83	213 12.17	206 12.04	200 12.36	254 15.48
4 \pm SD	148 7.88	182 5.00	205 11.69	194 6.29	188 6.61	188 3.65	186 3.38	205 8.57	205 8.67	209 6.29	202 4.45	198 2.07	250 2.29
5 \pm SD	144 11.84	177 3.24	198 8.03	192 4.26	185 2.18	189 4.85	187 6.16	200 7.06	201 10.52	204 6.28	197 8.36	193 4.99	237 11.32
6 \pm SD	155 5.42	181 3.41	201 7.68	190 9.76	186 6.60	193 1.15	188 7.25	212 8.16	212 8.40	221 5.83	209 10.54	203 9.62	257 9.23
7 \pm SD	151 12.61	178 1.97	203 5.39	197 10.14	193 4.83	191 1.44	189 2.17	213 1.29	213 2.53	216 2.73	210 2.15	204 3.51	255 1.86

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Table 4.16. LS Means for daily water intake (ml/hen/day)(SD \pm 0.004) (continued)

Treatm ent	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
8 \pm SD	152 11.12	179 4.34	190 5.57	188 7.22	183 6.51	188 5.04	186 4.59	204 11.81	205 18.41	210 19.27	203 20.29	197 18.82	251 27.92
9 \pm SD	152 9.96	180 3.78	203 8.44	193 12.74	191 9.80	190 2.58	192 2.61	211 7.84	209 12.45	215 13.38	205 11.63	204 12.64	258 17.30
10 \pm SD	152 9.70	179 2.30	201 6.07	192 4.76	190 8.37	192 5.07	191 6.95	210 13.93	210 9.51	228 8.07	213 10.69	202 10.32	255 17.18
11 \pm SD	149 21.11	179 1.97	201 4.88	198 5.56	194 5.42	191 6.70	194 6.59	208 11.51	203 8.69	214 8.07	205 9.69	201 6.62	254 6.66
12 \pm SD	157 7.90	177 6.07	207 18.74	198 12.48	191 5.20	191 3.40	197 2.38	216 4.95	214 11.84	212 5.49	204 0.48	199 0.72	256 2.65

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Table 4.17. Mean water intake of birds over time (ml/hen/day). $P = 0.8833$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Water intake	± SD
1	0	0	198a	12
2	100	0	197a	7
3	200	0	199a	9
4	300	0	197a	3
5	0	150	193a	6
6	100	150	201a	3
7	200	150	201a	3
8	300	150	195a	9
9	0	300	200a	8
10	100	300	201a	7
11	200	300	199a	6
12	300	300	202a	5

In the poultry industry, processing of spent hens, especially caged birds, often results in many broken and shattered bones. Downgrading is so severe that in some cases the processors refuse to buy the hens because of the danger of bone fragments in their products. The breaking strength of bones is used as a criterion for assessing the value of both diet and cage design for preventing bone breakage (Wilson 1991).

In this experiment the breaking strength of the femora increased to a maximum (279.067 N) in Treatment 4 (maximum Ca addition, no P). The lowest breaking strength was found in treatment 1 (68.427 N) where no Ca or P was added to the water (Table 4.18).

Table 4.18. Mean breaking strength of femora (N). $P = 0.0615$

Treatment	Ca inclusion in water (mg/l)	P inclusion in water (mg/l)	Breaking strength	± SD
1	0	0	68.427	±42.860
2	100	0	199.6	±42.860
3	200	0	102.3	±42.860
4	300	0	279.067	±42.860
5	0	150	107.8	±42.860
6	100	150	255.9	±42.860
7	200	150	191.2	±42.860
8	300	150	134.967	±42.860
9	0	300	172.183	±42.860
10	100	300	146.407	±42.860
11	200	300	132.683	±42.860
12	300	300	137.04	±42.860

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No histological abnormalities were evident in the gizzards, spleens, proventriculi and spleens examined. One or more lymphoid foci were present in the hearts of samples from treatments 2, 4 and 8. Scattered lymphoid foci were noted in the kidneys of treatments 1, 3, 4, 5, 6, 7, 8 and 9. In the kidney of treatment 11 there was a large, diffuse area of lymphocyte infiltration into the renal parenchyma. This lesion was interpreted as neoplastic (cancerous), ie renal lymphoma.

Egg yolk peritonitis was noted in a few of the specimens. Judged to be mild in treatments 3 and 11; it was regarded to be moderate in numbers 1, 8, 9 and 10. One or more lymphoid foci were found in the eggshell glands in treatments 4, 9 and 11.

The lymphoid foci found in various organs are indicative of an immune response to a persistent antigen. Most probably the antigen is a virus or mycoplasma of low pathogenicity; one that does not cause overt clinically recognizable disease. The histopathological lesions found in the tissue samples were therefore not linked to the addition of Ca and P in the drinking water.

Ninety-seven percent of the eggshell consists of calcium carbonate. The shell weighs approximately 6.0 g, so almost 6.0 g of calcium carbonate must be synthesized and deposited on the shell each time the hen produces an egg. For many hens, this is almost daily for long sequences. Calcium carbonate is 40% calcium, thus about 2.5 g of elemental calcium must be found and transported to the shell gland in the 18-20 hours it takes to form the eggshell. The calcium content of blood at any given time is no more than 30 mg. Thus the shell contains over 80 times more calcium than the content of the blood.

Calcium is obtained by the hen for shell formation from two sources (Hunton 2005) :

- Firstly, from the feed, via the intestine and the blood stream.
- Secondly, from reserves stored in the medullary bone. These reserves are replenished during the time eggshells are not being formed.

Mueller et al. (1964) found that of the calcium intake of laying hens, 78% was absorbed, 8% was excreted as endogenous calcium and 70% was retained. From 4.3 to 4.9 g of the skeletal calcium participated in eggshell formation, of which 1 g was turned over daily. The size of the exchangeable bone calcium pool was related to the quantity of shell produced and was larger in pullets with a negative calcium balance than in pullets with a positive balance.

Changes in the calcium source or its particle size have been tested as ways of improving shell quality

THE DEVELOPMENT OF WATER QUALITY STANDARDS FOR POULTRY PRODUCTION IN SOUTHERN AFRICA
Chapter 4 Influence of Ca and P in the drinking water on egg production, egg quality, bone integrity and shell strength

(Makled and Charles 1987). Guenter (1980) reported data from two long-term experiments, which indicated that continuous feeding of low levels of dietary phosphorus were more beneficial to egg shell quality, than the continuous feeding of higher levels of phosphorus. Although differences between the Ca and P treatments in this experiment did occur, both egg shell thickness and egg shell breaking strengths were not significantly increased by increased Ca or P administration.

The plasma calcium contents of hens receiving different levels of both Ca and P in the water did not differ significantly. This confirmed the findings of Hester et al. (1980) that hens which laid soft shelled eggs had plasma calcium and magnesium concentrations comparable to hens which produced hard shelled eggs. Lennards and Roland (1981) also found no relationship between serum calcium and shell weight or egg weight.

In 1961 Taylor reported that a substantial increase in eggshell thickness occurred when hens were transferred from a high phosphorus (0,8% P) to a low phosphorus (0.1% P) diet.

If the sole source of calcium is from the diet, then it is apparent that not only is the bird limited by the time taken to consume the mineral for direct use, but that substantial amounts are taken in when shell demands are non-existent. Fortunately, the long bones may act as a depot during this relatively short period of abundance until the dietary source later proves inadequate for shell formation (Hurwitz and Bar 1969). Bone breaking strengths were significantly lower in treatments receiving no Ca in the water.

Connor et al. (1969) reported reduced growth and increased mortality in chickens given CaCl_2 in the water. In this experiment levels of up to 300 mg/l of CaCl_2 did not adversely affect body weights, feed intake or water intake.

Reddy et al. (1968) found that the amount of calcium in the laying ration has a marked effect on shell quality and egg production. Ca and P treatment had a significant influence on egg production but no significant influence on egg mass.

Conclusion

The results show that water can be a valuable asset to increase eggshell integrity, but waterline maintenance may be increased because of the tendency of calcium to precipitate. Although calcium is one of the most studied minerals involved in laying hen nutrition, it does not seem to have been used to any extent as a drinking water supplement. This may be the result of a universal feeling that waterborne minerals are detrimental to equipment operation. Water should be seen as a dietary source of minerals (Ca + P) and should be taken into consideration when nutrient specifications are set for feed formulations to be used in the various poultry production systems.