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Evaluation of Supplemental Strontium on Eggshell Quality and Laying Hen Performance

Introduction

Strontium (Sr), like calcium (Ca) is a bone seeking mineral element, of which 99% is located in bone tissue (Schroeder et al., 1972). The biochemical physiology of Ca and Sr are closely integrated with Ca and Sr having similar metabolic pathways in bone deposition (Marie, 1996) and renal absorption (Walser, 1969). Strontium is a naturally occurring trace element found in relatively high concentrations of 400-450 ppm throughout the earth's crust, and around 8 ppm in seawater (Millero et al., 2008).

Strontium in the form of strontium ranelate at 2 g/day is a proven and effective supplement (Canalis *et al.* 1996, Buehler *et al.* 2001, Meunier *et al.* 2004, Reginster *et al.* 2005) for the treatment of osteoporosis in postmenopausal women. Strontium has been shown to increase bone formation by an increase in the number of bone producing osteoblast cells, while concurrently reducing bone resorption within the bone osteoclast cells (Marie, 2005, Marie et al., 2011). Strontium functions in an anabolic mode in respect to bone formation and maintenance.

The Sr content of commercial animal feeds normally ranges from 10 to 50 mg/kg and to-date Sr has not been shown to be an essential nutrient for vertebrates such that death will result from its absence (Pors Nielsen, 2004). To-date, there has been little research into the effects of feeding Sr at low supplemental doses to laying hens. In one recent study, there was a significant increase in total egg numbers and an improvement in feed conversion ratio (FCR) in those hens supplemented with Sr at 500 mg/kg (Browning and Cowieson, 2014). This was unexpected and to the authors knowledge it was the first time such an outcome has been documented in laying hens. In one other study, by Shahnazari et al. (2006) the effect of high doses of Sr on bone strength in laying hens was undertaken. They demonstrated a large positive effect on bone density, volume and micro-architecture however they did not produce any improvement in egg production or FCR, possibly due to the very high dose rates used (up to 6000 mg/kg Sr).

In Australia, table eggs are a major source of quality protein and fat soluble vitamins and with the rising population and changing ethnicity, the per capita consumption of table eggs is rising annually becoming an important source of quality protein and fat soluble vitamins. The current annual per capita consumption is currently 221.3 eggs and the gross value of egg production in Australia at the farm gate was A$625.5 m in 2014/2015, and at the retail level A$ 1.836 b in 2013/2014 (AECL, 2015). One of the major losses in commercial egg production is the large percentage of cracked eggs which represent on average about 12% of total production. There is currently an overall

shortage of commercial eggs further exacerbating the economic loss from cracked eggs. It may be hypothesized that Sr because of its unique ability to improve bone strength by directly replacing Ca may also be able to improve the density and breaking strength of eggshells and concurrently improve the skeletal integrity of the older laying hen. A reduction in the number of cracked eggs would represent a real benefit to the layer industry and associated stakeholders.

Objectives

The aim of this study was to evaluate the effect of feeding two low levels of Sr on egg quality particularly eggshell strength, bird performance, and bone composition in older laying hens. The older laying hen represents an animal model similar to that of the postmenopausal woman in which Sr supplementation at low levels has shown to produce significant benefit in bone density and reduce bone fractures.

Methodology

All experimental procedures conducted in this study were approved by the University of Sydney Animal Ethics Committee and were in accordance with the Australian code for the care and use of animals for scientific purposes (NHMRC, 2013).

A total of 144, 57-week-old ISA Brown laying hens were fed for 16 weeks on a mash layer diet supplemented with three levels of supplemental Sr (Treat A=0, Treat B=250 and Treat C=500 mg/kg Sr). For acclimatization purposes, the experimental diets were fed for 12 days after which egg production and feed intake were measured for performance calculations (101 days). Each treatment consisted of 48 birds (sixteen replicates of three birds per replicate). Each bird was housed separately in cages measuring 25 × 50 × 50 cm^2, with three adjacent cages forming the replicate unit and placed evenly throughout the experimental laying house. The photoperiod regimen was 16 h of light and 8 h of dark. Water and feed were supplied *ad libitum.* Throughout the trial all eggs were collected and weighed for each replicate group. Feed consumption for each replicate group of three birds was recorded throughout the experiment. On day 105 of this study, a 48 h total collection procedure was undertaken for six of the 16 treatment replicate groups for each treatment (18 birds per treatment), whereby the feed input and faecal output were measured to determine energy, nitrogen and mineral retention.

In order to determine egg quality a total of 48 eggs per treatment group (three hens per replicate and 16 replicates) were collected on the final day of the trial and their egg quality characteristics analyzed at University of New England (UNE) the following day. Also, on the last day of the study, six birds were humanely euthanized from each treatment group and both tibia bones excised, with the right tibia sent for bone composition analysis at New South Wales ICP unit and the left tibia bone sent for breaking strength analysis at New England University egg testing laboratory.

Composition of the diets

The ingredient composition and nutrient details of the mash basal layer diet are shown in Table 1. The nutrient specifications which are shown in Table 1 meet the recommended requirements of ISA Brown laying hens at 50 weeks of age (ISA Brown Nutritional Management Guide 2010).

Table 1. Dietary ingredients and calculated nutrient composition of the control diet.

Ingredients	(g/kg)	Calculated Nutrient Composition	
Wheat	488.251	Crude Protein g/kg	17.64
Sorghum	2 00.00	Poultry ME kcal/kg	2802
Soybean meal	135.0	Crude fat g/kg	33.51
Limestone powder	74.468	Calcium g/kg	40.0
Meat meal 50%	56.670	Avail Phosphorus g/kg	0.355
Canola meal solv	26.660	Lysine Dig g/kg	7.79
Soybean oil	1.100	Methionine Dig g/kg	3.97
Salt	16.668	Met & Cys Dig g/kg	6.42
Sodium Bicarbonate	17.670	Threonine Dig g/kg	5.05
DL Methionine	1.567	Isoleucine Dig g/kg	5.98
Lysine HCL	0.733	Tryptophan Dig g/kg	2.0
Threonine	0.033	Valine Dig g/kg	7.6
Layer Premix	1.5	Sodium g/kg	1.7
Choline Chloride	0.210	Potassium g/kg	6.87
Potassium Carbonate	0.467	Chloride	1.73
		Barium mg/kg	14.4
Total	1000 g	Strontium mg/kg	30.5

Provided the following per kg diet: ethoxyquin 25 mg; biotin 0.1 mg; calcium pantothenate 9.0 mg; cyanocobalamin 0.015 mg; folic acid 1.0 mg; menadione 2.5mg; niacin 30 mg; pyridoxine 3.5 mg; Vitamin A acetate 10,000 IU; Vitamin D3 2,500 IU; 1,25-Hydroxyvitamin D3 69 mg; riboflavin 5.0 mg; thiamine 2.5 mg; dl-α-tocopheryl acetate 25 mg; Co 0.4 mg; Cu 5 mg; Fe 60 mg; I 1 mg; Mn 50 mg; Mo 0.5 mg; Se 0.2 mg; Zn 60 mg, Canthaxanthin 3.1 mg: Apo-ester 2.9 mg; xylanase 300 mg.

A summary of the three dietary treatments are provided in Table 2. The treatment level for Sr at 500 ppm was chosen because of previous positive outcomes at 500 ppm in laying hens. The lower dose of 250 ppm was chosen to help ascertain a lower dose response. The diets were blended on 2 separate occasions at the beginning (1st blend) and midway (2nd blend) through the trial. The strontium carbonate was supplied by Sigma-Aldrich at Anella Ave Castle Hill, NSW 1765 with a potency of 580 mg/kg Sr.

Table 2. Summary of dietary treatments

Treatments	Added (Sr) mg/kg	Added strontium carbonate mg/kg
A. no added Sr	0	0
B. 250ppm Sr	250	440
C. 500ppm Sr	500	880

Egg quality measurements

Prior to the commencement of the trial 48 eggs were randomly selected and analyzed at the Egg Quality Laboratory at the University Of New England (UNE), Armidale Australia for basic egg quality parameters. Following completion of the trial, 144 eggs (three eggs from each replicate group and there were 48 replicates) were transported by car to UNE and analyzed the following day. Eggs were scored for translucency (0 lowest – 5 highest) using an egg candler and analysed for egg shell quality measurements, shell colour by reflectivity, egg weight, egg shell breaking strength by quasi-static compression, shell deformation to breaking point and shell weight (egg quality equipment, Technical Services and Supplies, U.K.). Shell thickness was measured using a custom-made gauge based on a Mitutoyo Dial Comparator gauge (Model 2109-10). Percentage shell was calculated from shell weight and egg weight. Egg internal quality was measured as albumen height, Haugh Units and yolk colour (TSS equipment).

Shell reflectivity (%) – this is a measure of the lightness of shell colour – the lower the measurement, the lighter the brown colour of the shell.

Egg weight (g) – this is the weight of the egg in grams.

Shell breaking strength (N) – this is the force in Newtons that needs to be applied to the egg at a controlled speed to cause it to break. It is a measure of the strength of the eggshell.

Shell Deformation (μm) – the distance in micrometers that the egg is compressed by the shell breaking strength machine before the egg breaks. It is a measure of egg elasticity.

Albumen height (mm) – this is the height in millimeters that the egg white stands up from a flat surface when an egg is carefully broken out. It is a measure of the freshness of the egg.

Haugh Unit – this is an empirical measure which uses the egg weight and albumen height and is the industry measurement of egg freshness.

Yolk colour score – the colour of the yolk on a scale developed initially by Roche (now DSM)

Percentage shell (%) – the shell weight divided by the egg weight expressed as a percentage. It gives an indication of the amount of shell present in proportion to the size of the egg.

Shell thickness (μm) – the thickness in micrometers of the shell at the equator of the eggshell.

Bone quality measurements

The left and right tibia bones were excised post mortem from 18 birds (6 left and 6 right tibia bones per treatment group) on completion of the trial. The right tibia bones were analyzed for their total ash content and mineral content. The percentage tibia ash content was measured at Sydney University Poultry Research laboratory and the percentage mineral components were analyzed at the University of New South Wales by inductively coupled plasma optical emission spectrometry (ICP). The breaking strength of the left tibia bones were measured at the UNE by the application of a force at controlled speed sufficient to break the bone. The force measured in Newtons (N), is a measure of the strength of the bone.

Chemical analyzes

The gross energy (GE) of the diets and dried faeces were determined using a Parr 1281 adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA), which was standardized with benzoic acid. Nitrogen concentration of samples was determined by the Dumas method using an FP-428 nitrogen analyzer (LECO®Corp., St Joseph, MI, USA) as described by Sweeney (1989). The mineral composition of the feed, faeces, and tibia ash were determined by inductively coupled plasma optical emission spectrometry (ICP) using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltham, MA, USA) following digestion with nitric acid and hydrogen peroxide beforehand (Peters et al., 2003).

Calculations

The calculations for apparent metabolizable energy (AME), apparent coefficients for dry matter (ACDM) and nitrogen (ACNR), and the retention values for the specific minerals, were calculated as (intake−output) / intake where *intake* represents total gross energy, DM, N and mineral quantity consumed and *output* is the total gross energy, DM, N and mineral excretion over the 48 h collection period.

Statistical analysis

Statistical analysis of the data was performed using JMP v. 9.0 (SAS Institute, Carey, NC, USA) and subjected to analysis of variance. Correlation coefficients were analyzed for any possible interrelationship between the variants. In all instances differences were reported as significant if $P<0.05$ and trends noted between P-values of 0.05 and 0.10.

Results

Composition of the diets

The analyzed nutrient levels for Sr, Ca, P Na and barium (Ba) in the three treatment diets and their respective separate blended samples are shown in Table 3. The analyzed values for Ca, Sr and sodium (Na) in the three treatment diets content do not agree well with the theoretical nutrient values for Ca, Sr and Na provided in Table 1. In addition, there were major differences in the Ca, Sr and Na content of the same treatment diet manufactured on separate occasions. The Ca in treatment A sample3 ; treatment B sample3 and treatment C or samples 2 and 3, were well below the expected (4%) and also below recommended intake for ISA Brown (4.3 g/hen/day after 50 weeks). As all the limestone was in powder form (not the ⅔ as 2 to 4mm recommended for ISA Brown) the treatment C hens in particular may have been Ca deficient. In respect to protein, there were only minor variations between the three treatment diets, however there was a marked difference in protein content between the first and second blended batches of the control diet (Diet A -17.31% vs 17.96%).

The average non-supplemented or “inherent” Sr content in the control diet (no Sr added) was 31.2 ppm which is in agreement with published data indicating a natural background range of 20 to 50 ppm for Sr in most animal feeds. The Sr level in treatment C in the first sample analyzed was excessive at 1037 ppm, being more than twice the formulated level of 500 ppm which may have reduced production. The level of Sr in the feed should have been the only difference between treatment diets. The experimental feeds at best appear to have been poorly blended giving rise to gross nutrient variation in the one feed. The fact that the diets were in mash form may have contributed to laboratory subsamples of only ½ gram as being unrepresentative of feed composition.

The inherent Ba content of the control diet was 14.3 ppm. There was a close alignment between the Ba content of the feed and the Sr content of the feed (R2=0.83) indicating higher levels of strontium carbonate in the feed also increased the barium content of the feed proportionately. The strontium content of the strontium carbonate salt was analyzed at the start of the trial to be 58.0% and the barium content was 0.7%.

Table 3. Summary of protein, strontium (Sr), calcium (Ca), phosphorous (P), sodium (Na) and barium (Ba) results for each dietary treatment for multiple samples.

Treatment	Protein 2 samples$^{\beta}$ (%)		Analyzed Sr 3 samples* (ppm)			Analyzed Ca 3 samples* %			Analyzed P 2 samples* %		Analyzed Na 3 samples* %			Analyzed Ba 3 samples* ppm		
	1	**2**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**3**	**1**	**2**	**3**
A. no added Sr	17.31	17.96	32.5	37	24	4.01	4.35	2.85	0.60	0.48	0.20	0.23	0.14	14.0	16.0	12.8
B. 250 ppm added Sr	18.07	18.19	361	410	255	4.51	5.20	3.25	0.69	0.57	0.22	0.28	0.15	19.1	26.1	19.9
C. 500 ppm added Sr	18.09	18.26	**1037**	469	475	5.18	3.29	3.29	0.59	0.56	0.25	0.16	0.15	28.0	23.0	22.8

$^{\beta}$ 2 samples analyzed for protein on trial completion Jan 2016

**1st sample analyzed at commencement of trial July 2015. *2nd & 3rd sample analyzed completion of trial Jan 2016*

The 1st and 2nd samples above were from the same batch of feed..

Production outcomes

The percentage hen housed production, average egg weight and feed conversion efficiency (FCR) results, per treatment, over the 101 day collection period are shown in Table 4 (12 days of acclimatization not included). The addition of supplemental Sr at either 500 mg/kg or 250 mg/kg did not increase total egg numbers, average egg weight, feed intake per 60 g egg, FCR, nor produce any change in feed intake per bird per day as compared to the control feed. In respect to mortality, only one bird died on day 69 as a result of a broken neck when it caught its head between cage bars. The production details for the replicate group affected were adjusted accordingly.

The percentage hen housed production rate was very high at 94 - 95.5% which is significantly higher than the ISA Brown standard laying rate of 80 - 87% for 57 to 71 week old birds. The achieved production rate equated to birds being about 24 - 38 weeks of age according to the ISA Brown standard laying rate.

Table 4. Effect of added Sr on egg production, egg weight (g), feed intake per 60g egg (g), feed conversion efficiency (FCR) and daily feed intake (g).

Added Sr (mg/kg)	Hen Housed Production (Aug-Nov)- 101 days (%)	Average Egg Wt (g)	Feed Intake per 60g egg(g)	FCR per g egg	Daily Feed intake/bird(g)
0 (n=48)	95.5	62.3	125.1	2.09	124
250 (n=48)	94.0	64.0	123.2	2.05	123
500 (n=48)	93.9	63.6	125.4	2.09	124
Pooled SEM*	±1.23	*± 0.8*	*±2.5*	*±0.04*	*±2.0*
P-values	*P=0.599*	*P=0.321*	*P=0.797*	*P=.797*	*P=0.952*

SEM= standard error mean

Egg Quality Results

The results for egg quality characteristics on a total of 144 eggs as determined by UNE are shown in Table 5 and Table 6. There was no difference in egg quality parameters such as egg weight, albumin height, haugh units, yolk colour or egg deformity when Sr was added at either 250 mg/kg or 500 mg/kg as compared to the control diet with no added Sr. The average egg weight on the 500 mg/kg Sr diet was greater than the 250 mg/kg Sr diet.

Table 5. Effect of Sr supplementation on egg quality: egg weight, albumin height, haugh units, yolk colour and egg deformity.

Treatment	Added Sr mg/kg	Egg wt (g)	Albumin Height (mm)	Haugh Units	Yolk Colour	Egg Deformity (µm)
A (n=48)	0	65.6^{ab}	6.41	77.65	10.50	246.7
B (n=48)	250	64.8^{b}	6.31	76.51	10.79	282.7
C (n=48)	500	66.9^{a}	6.62	78.68	10.44	311.5
Pooled SEM*		0.73	0.158	1.272	0.163	32.27
P-values		*P=0.1322*	*P=0.374.*	*P=0.4822*	*P=0.264*	*P=0.3661*

SEM= standard error mean. *Levels not connected by same letter are significantly different*

The results for the eggshell quality characteristics such as eggshell breaking strength, eggshell reflectivity, % eggshell of total egg and average eggshell thickness for those eggs analyzed at UNE are shown Table 6. There were no significant differences between treatments for any of the eggshell quality parameters.

Table 6. Effect of Sr supplementation on eggshell quality: eggshell strength, eggshell weight, eggshell reflectivity, % eggshell and average eggshell thickness.

Treatment	Added Sr (mg/kg)	Eggshell wt (g)	Eggshell Breaking Strength (N)	Eggshell Reflectivity (%)	% Eggshell of total egg (%)	Average Eggshell thickness (µg)
A (n=48)	0	6.17	38.62	26.06	9.42	415
B (n=48)	250	5.99	36.65	26.69	9.24	409
C (n=48)	500	6.23	39.34	26.35	9.33	416
*Pooled SEM**		*0.111*	*1.325*	*0.556*	*0.146*	*5.6*
P-values		*P=0.293*	*P=0.333*	*P=0.7295*	*P=0.682*	*P=0.626*

*SEM= standard error mean

Bone Characteristics

The analytical results for the left tibia bones are shown in Table 7. The Sr content of tibia bone and significantly increased with increasing levels of Sr in the diet (R^2=0.94) however this increase was independent of the Ca and Na levels in the bone. The Ba content of tibia bone significantly increased in treatment C (500 ppm Sr) however the deposition of Ba into bone was not proportional to the amount in the feed (R^2=0.57). The mean tibia bone strength and total ash values showed no significant improvement when Sr was added at either 250 or 500 ppm. The Ca, P, Na, Mg and K content of the tibia bones were not statistically different from control treatment A.

Table 7. Effect of Sr supplementation on tibia bone mineral composition; Tibia strength, Tibia ash, Calcium (Ca), Phosphorous (P), Strontium (Sr), Sodium (Na),Magnesium (Mg),Potassium (K),Barium (Ba).

Added Sr mg/kg	Tibia strength (N)	Tibia ash (g)	Tibia (Ca) (%)	Tibia (P) (%)	Tibia (Sr) (%)	Tibia (Na) (%)	Tibia (Mg) (%)	Tibia (K) (%)	Tibia (Ba) %
0 (n=6)	242.6	39.2	35.23	18.71	0.025^{c}	1.09	0.56	0.51	0.019^{b}
250 (n=6)	248.4	43.3	35.67	18.71	0.090^{b}	1.11	0.60	0.49	0.022^{b}
500 (n=6)	232.3	42.8	35.58	18.74	0.167^{a}	1.12	0.61	0.52	0.026^{a}
Pooled SEM*	29.07	2.01	0.207	0.114	0.0065	0.018	0.018	0.042	0.0012
P values	*P=0.925*	*P=0.327*	*P=0.311*	*P=0.979*	*P<0.0001*	*P=0.452*	*P=.224*	*P=0.873*	*P=0.0017*

*SEM = standard error mean Levels not connected by same letter are significantly different

Eggshell Composition

The mineral composition for the combined eggshells within each replicate group of three birds were analyzed and the results shown in Table 8. The addition of strontium carbonate to the diet increased the Sr and Ba content of the eggshell and decreased the Ca content of the eggshell. The increase in Sr within the eggshell was directly proportional to the Sr level in the diet (R^2=0.98) and Sr appeared to replace Ca in the shell. There was no effect of strontium carbonate supplementation on the eggshell mineral content for P, Na, Mg and K.

Table 8. Effect of Sr supplementation on eggshell mineral composition, specifically; Calcium (Ca), Phosphorous (P), Strontium (Sr), Sodium (Na), Magnesium (Mg), Potassium (K) and Barium (Ba).

Treatment	Added Sr mg/kg	Eggshell (Ca) (%)	Eggshell (P) (%)	Eggshell (Sr) (%)	Eggshell (Na) (%)	Eggshell (Mg) (%)	Eggshell (K) (%)	Eggshell (Ba) (%)
A (n=16)	0	35.77[a]	0.13	0.030[c]	0.12	0.40	0.050	0.0054[c]
B (n=16)	250	35.45[ab]	0.13	0.160[b]	0.11	0.39	0.049	0.0075[b]
C (n=16)	500	35.23[b]	0.13	0.292[a]	0.12	0.40	0.049	0.0083[a]
*Pooled SEM**		*0.146*	0.0026	*0.0039*	*0.0015*	*0.006*	*0.00058*	*0.00018*
P-values		*P=0.0411*	*P=0.952*	*P<0.001*	*P=0.626*	*P=0.802*	*P=0.709*	*P<0.0001*

*SEM = standard error mean. Levels not connected by same letter are significantly different

Nutrient Retention

The results for energy (AME), protein ((ACNR) and dry matter (ACDMR) are found in Table 9. There was no effect of Sr supplementation on the energy, protein or dry matter retention of the feed digestibility within the birds.

Table 9. Effect of Sr supplementation on apparent metabolizable energy (AME), nitrogen retention (ACNR) and apparent dry matter retention (ACDMR) in the laying hen.

Treatment	Added Sr mg/kg	AME (MJ)/kg	ACNR (%)	(ACDMR) (%)
A (n= 6)	0	12.53	43.65	76.19
B (n=6)	250	12.48	49.02	76.59
C (n=6)	500	12.41	47.13	75.38
*Pooled SEM**		*0.110*	*2.343*	*0.782*
P-values		*P=0.742*	*P=0.288*	*P=0.551*

**SEM = standard error mean.*

Bodyweight change

The effect on individual bird bodyweight change during the 100 day trial is shown in Table 10. The change in body weight for each bird was statistically analyzed on individual bird replicates (48 replicates per treatment). There was no difference in bodyweight change with Sr supplementation at either 250 ppm or 500 ppm as compared to the control diet. The individual variation in bird bodyweight for each treatment group was considerable as indicated by the large standard error mean value.

Table 10. Effect of Sr supplementation on bird bodyweight

Treatment	Added Strontium mg/kg	Bird Bodyweight Change (g)
A (n=48)	0	56.7
B (n=48)	250	15.0
C (n=48)	500	53.3
Pooled SEM*		*21.19*
P-values		*P=0.304*

*SEM = standard error mean.

Discussion

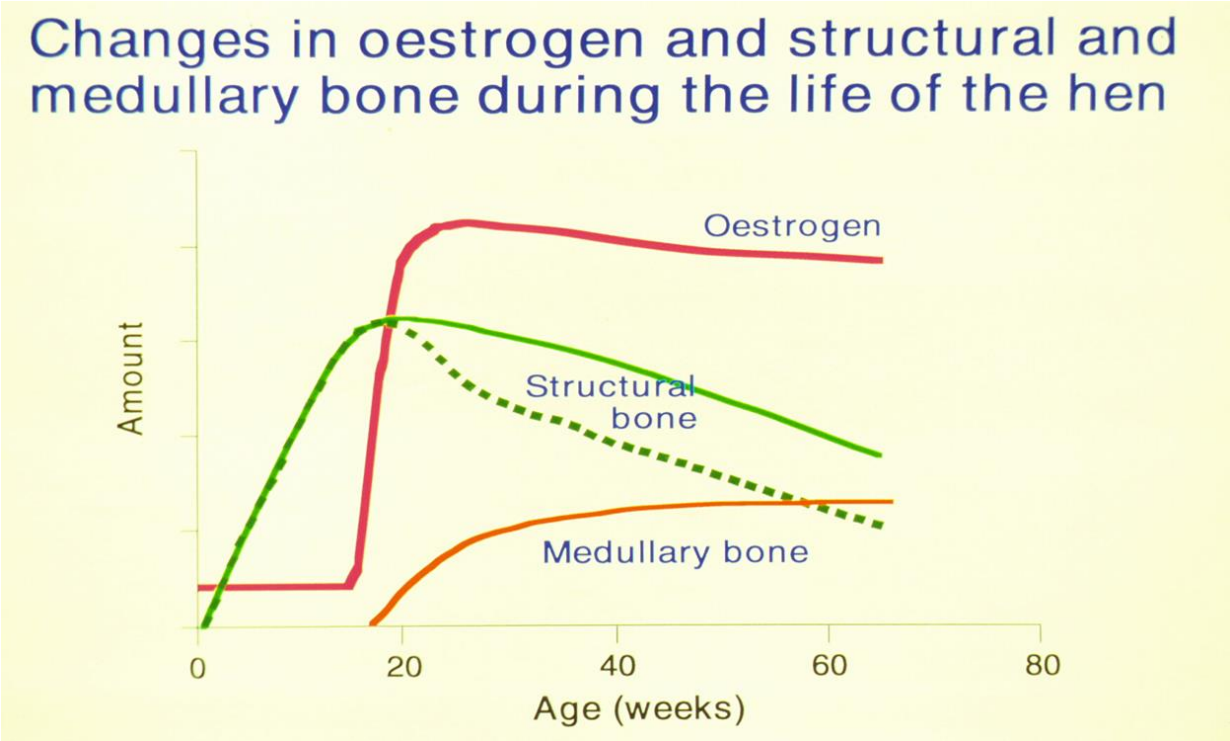
The outcome of no improvement in egg production, tibia bone strength or egg shell strength with Sr supplementation in this study was unexpected. The fact that egg production did not increase with Sr supplementation was contrary to a previous study at Sydney University which showed Sr supplementation at 500 ppm improved egg production and feed efficiency in laying hens (Browning and Cowieson, 2014). Also, the finding that tibia bone strength did not improve with Sr supplementation is in contrast to a previous study by Shahnazari et al. (2006) who produced an improvement in bone mineral density, bone volume and architecture in laying hens fed supplemental Sr. In fact, the lack of improvement in bone breaking strength in those tibia bones which contained significantly higher levels of Sr is contrary to the results of numerous experiments in many vertebrate species which conclusively showed Sr improved bone mineral density, strength and in human trials actually reduced bone fractures (Meunier et al., 2004, Reginster et al., 2005, Reginster et al., 2008). This is exemplified by the fact that Sr is a prescribed treatment in human medicine for the improvement of bone density and bone strength in clinical cases of osteoporosis.

A positive outcome from this study was the finding that eggshell concentrations of Sr increased with increasing levels of Sr addition to the diet, in the same manner as the concentration of Sr in bone increased. Because higher levels of Sr in bone have been proven to increase bone strength, it is a justifiable extrapolation to hypothesize that higher levels of Sr would also increase eggshell strength particularly older birds even if strontium supplementation is undertaken before point of lay.

In respect to “older birds”, the birds in this study were supposed to be 57 weeks of age at the start of the trial after a request for birds about 50+ weeks of age. However, the percentage hen housed egg production rates were very high, up to 95% and it is unlikely that the birds were 57 weeks of age as advised at the commencement of the trial. The birds were most likely to be considerably younger at possibly about 28 - 30 weeks of age. Proof of age will be required in any future studies in laying hens.

As stated above, the level of Sr in both tibia bone and eggshell increased in proportion to the concentration of Sr in the feed which suggests Sr has the potential to improve bone and eggshell density if fed over a longer time period possibly commencing before point of lay when physiological changes in bone structure are most rapid (Whitehead, 2004). Please refer to Diagram 1

Diagram 1. Changes in bone structure during the life of the hen



One possible reason for the lack of improvement in bird performance with Sr supplementation may have been the large variation in the nutrient composition as exemplified by the Ca, P, Na and Sr levels shown in Table 3. The excessive Sr level of 1037 ppm for treatment C (rather than 500 ppm) may explain why this treatment group did not improve egg production. Also the daily intake of calcium on treatment C may have been deficient further exacerbating a reduced potential for optimum egg production. This variability in respect to actual daily nutrient intake per hen is illustrated in Table 11 which provides details of the actual daily nutrient intake per hen across the three treatment diets relative to the ISA Brown recommended daily intake (RDI) for Ca and Na. The levels of Ca and Na in the three treatment diets should have been the same, only the Sr level

should have been different. Unfortunately, there was also a variation within treatment diets in their separately blended batches of feed, particularly in treatment C. In fact, most of the daily nutrient intakes for Ca and Na were either in excess or deficient, relative to the RDI for the ISA Brown hen. These nutritional irregularities may have affected the response to Sr supplementation in this trial.

Table 11. Actual daily nutrient intake compared to that recommended daily intake (RDI) by ISA Brown Breeder Company for birds 50 weeks+.

Nutrient	Isa Brown RDI[#]	Treatment A Av. daily intake			Treatment B Av. daily intake			Treatment C Av. daily intake		
Feed Samples		1	2	3	1	2	3	1	2	3
Actual average daily feed intake/bird (g)	125	124			123			124		
Ca (g)	**4.45**	4.96	5.44*	3.53*	5.64	6.40*	4.00*	6.42*	4.05*	4.05*
Na (mg)	**180**	250	285*	174	271	344*	186	314*	198	186
Sr (mg)	N.A.	4.06	4.59	2.98	44.28	50.43	31.37	**169**	58.16	58.90
Ba (mg)	N.A	1.75	1.98	1.59	2.34	3.21	2.45	3.47	2.85	2.83

*Values in excess or deficient compared to ISA Brown RDI in the ISA Brown Nutritional Management Guide 2010
Sample 1 and 2 represent the same feed made July 2015 but analyzed before and after the trial. Sample 3 is the second batch of feed blended midway through the trial.

In this study, the large nutrient variation between feed batches blended on separate occasions, indicates a strong preference for all future experimental diets to be pelleted and blended in sufficient quantities to meet the total feed requirements for the full trial period. Furthermore, in this study the experimental diets were in a mash form which may have contributed to a certain amount of separation of key nutrients, both at the time of laboratory sampling and also in the everyday feeding by the birds. For greater consistency of feed sampling and daily nutrient intake by the birds, it would be preferable for the feed to be pelleted. In any future research very close supervision by the project leader needs to be undertaken at the time experimental diets are blended.

Strontium in the form of strontium carbonate is a naturally occurring mineral, currently mined widely throughout the world. Unfortunately, a common contaminant of strontium carbonate is barium carbonate which can be present up to 3% in strontium carbonate. Barium is present in all soils and plants with the Ba content of different plant species grown on different soils ranges from 0.5 to 40 ppm with a mean value of about 10 ppm (Underwood, 1977). Unlike Sr, Ba is extremely toxic when absorbed (Sollmann, 1957) with characteristic systemic action marked by stimulation of the muscles of all types, regardless of innervation. Based upon extrapolation of available data, the level of soluble Ba in a diet probably should not exceed 20 ppm. (N.R.C., 1980), however higher levels of less-soluble forms of Ba found in natural substances may be tolerated (N.R.C., 1980). In this study, the analyzed Ba concentration in strontium carbonate was 0.7% and the Ba content of

the treatment ranged from 12 to 28 ppm. Although 20 ppm of Ba has been stated as the maximum tolerable level in animal nutrition because of the inherent insolubility of barium carbonate, it is unlikely that 12 to 28 ppm of Ba would have produced negative responses within the laying birds in this study. This statement is substantiated by the fact that there were no differences in egg production, feed intake or general health of all birds in this trial. However, the extreme toxicity of Ba is a concern and barium carbonate contamination may pose a major limiting factor to the widespread use of strontium carbonate in animal feeds. Because even small quantities of Ba are toxic, it may represent a generally undiagnosed anti-nutritional factor if it is a significant contaminant in commonly used raw materials particularly those of mineral origin, even salt

Conclusion

The addition of Sr in the form of strontium carbonate at 250 and 500 ppm to the diet of laying hens 50 weeks of age, did not change eggshell quality characteristics, egg production, feed efficiency or bone strength. This finding is in contrast to previous published research which showed dietary Sr at 500 ppm improved egg production and feed utilization. Strontium has been proven in other vertebrate species to improve bone density, bone strength and reduce bone fractures.

The level of Sr in both tibia bone and eggshell increased in proportion to the concentration of Sr in the feed which suggests Sr has the potential to improve bone and eggshell density if fed over a longer time period possibly commencing before point of lay when physiological changes in bone structure are most rapid.

It was found that the Ba content of the diet appeared to be directly related to the Sr content of the diet, and close monitoring of the Ba content of strontium compounds should always be undertaken.

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Sub-Project	**Evaluation of Supplemental Strontium on Eggshell Quality and Laying Hen Performance**
Poultry CRC Sub-Project No. 2.1.18	
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Sub-Project Overview	Previous research showed layer hens improved their egg production and feed utilization with dietary strontium supplementation. The aim of this sub-project was to confirm these findings and in addition measure the effects of strontium on tibia bone and egg shell strength because strontium has been proven to increase bone density and bone strength in vertebrate animals.
Background	Up to 12% of eggs are cracked at point of sale and this represents a huge loss for Australian egg producers. Strontium is a trace mineral which has been proven to improve bone strength in many vertebrate species by increasing bone deposition and at the same time, reducing bone resorption. Strontium is currently prescribed for the treatment of osteoporosis in older adults because it significantly increases bone density and reduces bone fractures. A recent study in laying hens at Sydney University showed strontium supplementation significantly improved egg production and feed conversion efficiency. This CRC research project proposed to investigate the feeding of supplemental strontium, at two dose rates, to older laying hens in order to evaluate the ability of strontium to improve eggshell strength, increase egg production and improve feed utilization.
Research	For 16 weeks, supplemental strontium in the form of strontium carbonate was fed at three levels, namely 0, 250 or 500 ppm to 144 laying hens of 57 weeks of age. Egg production, feed intake, bone strength, eggshell strength and overall egg quality for each dietary treatment was measured over 14.4 weeks (101 days) following an acclimatization period of 12 days. Each treatment group was fed to 48 laying hens birds which were divided into 16 replicates of 3 birds. There was no change in egg production, feed efficiency, bone strength or eggshell quality with the feeding of strontium at either 250 ppm or 500 ppm. However, the level of strontium in tibia bone and eggshell significantly increased in proportion to the concentration of strontium in the feed, indicating a potential for strontium to improve bone and eggshell strength if supplemented for a longer period of time. Barium is a toxic trace mineral and it can be found as a contaminant in strontium carbonate salt. The barium levels in feed, bone and eggshell were closely aligned to the strontium content of the feed. The barium content of strontium carbonate should always be monitored prior to feeding.
Implications	As a result of this study and its lack of positive outcomes for strontium supplementation, strontium carbonate currently cannot be recommended for addition to commercial layer diets. However, more research should be undertaken, to investigate the real potential of supplemental dietary strontium to improve bone and eggshell strength in older laying hens. It maybe hypothesized that dietary strontium supplementation would be most effective if fed to laying hens at the onset of lay because at this time the laying hen is undergoing the greatest physiological change in bone structure and eggshell gland development.
Publications	Nil