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ACIDOGENIC CALCIUM SALTS AS Partial SUBSTITUTE FOR CALCIUM CARBONATE IN DIETS OF AGED LAYERS

By

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Abstract: *An experiment was conducted to investigate the effect of substituting calcium sulfate or calcium citrate for a part of dietary calcium carbonate on the performance of aged layers, eggshell quality, calcium retention, intestinal pH and blood calcium. One hundred and fifty Hy Line white (W-36) layers from 61 to 70 week of age were randomly distributed into five groups of 30 layers each. In four experimental groups each of calcium sulfate or calcium citrate was used to supply 0.3% or 0.6% of dietary calcium instead of calcium carbonate. Egg production was improved significantly by 4.1% and 3.5% due to adding 0.3% or 0.6% calcium in citrate form, while calcium sulfate had no effect. Average egg weight was not affected significantly by supplementing either calcium sulfate or calcium citrate into diets. Daily feed intake was reduced significantly by adding calcium citrate into diets while calcium sulfate had no significant effect. Feed conversion ratio significantly improved as a result of including calcium citrate into diets. The pH values of ileal content were reduced by supplementing calcium sulfate or calcium citrate into diets. Blood calcium levels of layers fed calcium citrate or calcium sulfate were slightly higher than the control. Calcium retention values of layers fed 0.3% (54.1%) or 0.6% (63%) calcium citrate were higher than that of control (49%). Egg shell quality was insignificantly improved by supplementing calcium citrate at 0.3% or 0.6% into the diets. Calcium sulfate at high level (0.6%) caused a slight impairment in egg shell quality.*

INTRODUCTION

Aged layer at later stage of production lay eggs with inferior eggshell quality causing an economic loss for egg producers (Roland 1979). This degradation in eggshell quality may be related to the natural decline of calcium absorption with the proportion increase in egg size at later age of laying hens,(Al Batshan et al 1994).

Calcium absorption of laying hens is affected mainly by its solubility rate in intestinal tract (Gheng and Coon 1990; Keshavarz et al 1993). Chemical forms of dietary calcium is one of the major factors affecting calcium solubility and absorption (Bronner 1993; Henry and Pesti 2002). Calcium carbonate form has been used as the major source of calcium in laying hen diets, due to its high calcium content and its low cost. However calcium carbonate is an alkaline basis and poorly soluble salt at near-neutral pH (6-7) of intestine (Bronner 1993). The solubility of calcium carbonate may enhance with low pH or slightly acidic media of the intestine (Bronner 1993; Guinotte et al 1995). Therefore, theoretically it can be hypothesized that, increasing the acid load of diet to create a slight acidic media in the intestinal chyme may improve the solubility and absorption of calcium carbonate which enhance egg shell quality (El Afifi and El Alaily 2001).

This hypothesis can be applied by supplementing laying hen diets with an acidogenic calcium salts such as calcium sulfate (Keshavarz 1994) or calcium citrate (Harvey et al 1990). Rulz - lopez and Austic (1993) proved that incorporating calcium sulfate into growing chick diets increased blood concentration of hydrogen ion (decrease pH value).

Van der Klis and Kemme (2002) stated that, mineral compounds of sulfate form are well utilized by animals and have a higher availability than those of carbonate. Hurwitz and Rand (1965) reported that replacing graded levels of calcium sulfate by calcium carbonate in laying hen diets, didn't affect egg shell quality while tibia calcification was improved. Keshavarz (1991), indicated that egg shell quality wasn't influenced by graded substitution of calcium sulfate with calcium carbonate in laying hen diets.

Henry and Pesti (2002) compared between calcium citrate and limestone in starting broiler chick diets and concluded that calcium citrate is a better calcium source due to its effect in improving tibia measurements.

In experiment with rats Kochanowski (1990) reported that, absorbed calcium citrate is better than that of calcium carbonate. Additionally similar results of calcium citrate are well documented in experiments with human (Harvey et al 1990, Kochanowski 1990, Sakhaee et al 1999 and Hanzlik et al 2005). In this connection, information on laying hens is still limited. Therefore this study aimed to partial substitute of dietary calcium carbonate by calcium sulfate or calcium citrate in layers at old age. The effect of this substitution was studied on laying performance, eggshell quality, intestinal pH, blood calcium and calcium retention.

MATERIALS AND METHODS

This study was carried out at Layer Nutrition Research Unit, Faculty of Agriculture, Ain-Shams University.

Experimental design and Management.

One hundred and fifty Hy-line White (W-36) layers were randomly housed from 61 to 70 week of age in individual battery cages located in open sided laying house. The birds were distributed into five treatments each of 30 hens. The five groups of birds were fed on corn soy diet which contained a constant ratio of calcium (3.96%) and supplied with all nutrients needed for layers according to the Hy-line (2000) management guide.

In four experimental diets, calcium sulfate ($CaSO_4.2H_2O$) or calcium citrate [$(CH_3COO)_2$ Ca] were used to supply 0.3% and 0.6% of dietary calcium instead of calcium carbonate, respectively. The fifth experimental diet was used as control. Because of calcium content of carbonate form (38%) was higher than those of calcium sulfate (22.6%) or calcium citrate (33.89%), soft sand was used to adjust diets for 100%. The experimental diets are shown in (Table 1).

Each individual hen was represented as experimental unit. Feed was provided in an individual feeder *ad lib* and water supplied through automatic nipples. Lighting hours were scheduled to be 17 hours per day. The ten week experimental period was divided into two stages of 5 weeks each. The traits were calculated at each interval and for the entire experimental period.

Performance Traits.

Egg weights in grams were recorded daily for each hen throughout all the experimental period. Average egg weight and egg production percentage were calculated for each hen and treatment group. Average egg mass was calculated as g. egg/hen/day. Feed consumption in grams per hen was recorded weekly and average feed consumption per treatment group was calculated. Feed efficiency ratio (g feed/g egg) was calculated as gram feed consumed per gram egg.

Eggshell Quality.

Eggshell quality was assessed every five weeks as eggshell percentage, thickness and strength. For this purpose, 12 eggs per treatment represent 6 hens as two consecutive eggs per hen were used. Eggs were weighed and broken using an appropriate apparatus to determine eggshell-breaking strength as kg/cm^2 according to the method described by **Fathi and El-Sahai** (1996). Egg contents were separated and shells with its membrane were cleaned and dried at 100C for 24hours. Eggshell percentage was calculated by dividing dry shell weight (g) by egg weight (g) and multiplying by 100.

Eggshell thickness (millimeter) was determined on dry shell using a micrometer.

Calcium Retention (balance).

Calcium retention was determined at the end of the experiment. For this purpose 30 birds (70 week of age) were chosen to represent the five treatments (6 birds/treatment). After 12 hours of fasting, the layers individually received diets for 24 hours then the feeders were removed 12 hours before collecting the excreta to allow intestinal tract to be empty. Feed consumption was recorded and excreta were weighed and dried. Moisture content of excreta was calculated as percentage of total weight. Calcium content of diets and excreta were determined by using a sample of about 1.5g. which was ashed in a muffle furnace at 600C for 3hours and prepared for calcium determination according to A.O.A.C. (1980) using chemical kit (Bicon, Germany).

Calcium retention as percentage of ingestion was calculated as follows:

Ca intake (g) - Ca in excreta (g) / Ca intake × 100

Slaughter Data.

At the end of the experiment, 6 hens per experimental group were slaughtered, blood samples were centrifuged for 15 minutes to separate serum that was decanted and frozen. Serum calcium was determined spectophotometerically by using chemical kit (Bicon, Germany). Ileal content samples were collected by pressing the outer wall of cut ileum to push its contents into clean glass bottle.

The pH values of the diets and ileal contents were determined by mixing 2g of diet or ileal samples with 8ml distilled water and pH meter was used.

Statistical Analysis

Statistical analysis was computerized by statistical program SAS (1994), with using Duncan’s multiple range tests to separate means.

RESULTS AND DISCUSSION

Laying performance. The overall mean of egg production percentage (Table 2) was significantly enhanced by 4.1% and 3.9% due to adding 0.3% and 0.6% of calcium citrate to the diets respectively. This improvement may be related to the enhanced effect of calcium citrate on absorption of dietary nutrients, which saving more protein, energy and calcium needed for egg production. Henry and Pesti (2002) reported that, calcium citrate improved chicks growth due to some specific action of citrate conjugate on nutrients absorption and energy metabolism.

Calcium sulfate had no significant effect on egg production. This result is in consistent with the previous results of Keshavarz (1994) who indicated that, egg production was not affected by supplementing diets with 1.44% calcium sulfate, furthermore, the higher level beyond 2.88% had an adverse effect on laying performance.

Average egg weights were not significantly affected by using calcium sulfate or calcium citrate either at 0.3% or 0.6% level. These results are in a good agreement with those obtained by Keshavarz (1991 and 1994) who didn't observed any appreciable influence on egg weight by enriching laying hen diets with different calcium sulfate levels. Regarding calcium citrate the knowledge on laying performance is still lacking, however the early work of Russell and McDonald (1929) didn't find any effect on egg weight by substituting calcium carbonate by calcium citrate. Average egg mass recorded a non significant improvement due to adding calcium citrate into diets.

Feed consumption and conversion. Overall values of feed intake were significantly reduced as a result of incorporating 0.6% calcium citrate into diets, while the addition of calcium sulfate had not effect. (Table 2).

The dramatic effect of calcium citrate on feed intake may be due to its high solubility of calcium citrate in water (Harvey et al, 1990). Therefore, it can soluble in aquatic phase of crop and then citrate ions release forming citric acid which creating acidic media and lowering feed intake. Falkowski and Aherne (1984) and Radecki et al (1988) showed that feed intake can be depressed as a result of low palatability of acidic diets containing citric acid.

Calcium sulfate is not soluble in water and need strong acidic media in stomach and small intestine for its ionization (Hurwitz and Rand 1965), so it doesn't affect feed intake . Keshavarz (1991) reported that, feed consumption was not affected by substitution of one third of calcium carbonate with gypsum (calcium sulfate).

Feed conversion ratio was significantly improved as a result of incorporating calcium citrate at level of 0.3% or 0.6% into diets respectively, compared with control. While addition of calcium sulfate to the diets had less effective in this respect. (Table 3). This improvement in feed conversion ratio resulting from calcium citrate addition may be related to the fast solubility of calcium citrate, which slightly reduced pH value of intestinal tract, causing an enhancement of dietary nutrients absorption. Giesting et al (1991) suggested that, reducing pH value of digestive tract led to a great improvement in feed utilization by reducing microbial survival, increasing pepsin activation and slowing passing rate to allow more time for digestion.

Calcium sulfate had no significant effect on feed conversion ratio. It may be due to slow ionization of sulfate in gastrointestinal tract (Hurwitz and Rand, 1965) with delayed acid formation. In addition to the effect of sulfate ion in increasing water consumption and excreta moisture (Table 4). This in turn might reduce the transit time of chyme causing a less absorption rate of nutrients (Keshavarz, 1991).

pH values. The pH values of diets (Table 4) were not altered by including calcium sulfate into diets while calcium citrate addition reduced dietary pH. That could be due to the fact that calcium sulfate is not soluble in water (Keshavarz, 1991), therefore its acidogenic effect did not appear during dietary pH determination which depended upon adding water into diet samples. In contrast calcium citrate is readily soluble and ionized in water so its acidic effect can be reflected on dietary pH value.

The pH values of ileal content is slightly reduced by supplementing calcium sulfate and citrate into diets (Table 4). Furthermore the high level of calcium citrate had a significant reduction effect on pH value. Burnell et al (1988) stated that, addition of citric acid to diets reduced the pH value of small intestine. Appearance the acidogenic effect of calcium sulfate in ileal pH may be related to the acidic media of upper intestine which can dissolve calcium sulfate. Keshavarz (1991) and Rulz-Lopez and Austic (1993) reported that, calcium sulfate had an acidogenic effect and can reduce blood pH.

Blood calcium. Blood calcium levels were slightly increased as a result of adding calcium citrate or calcium sulfate to diets (Table 4). That may be because of blood content of calcium is highly depended on calcium absorption (Hurwitz 1987). Therefore the slight increment in blood calcium can be considered as a good indicator for enhancement in calcium absorption and utilization by adding calcium citrate or sulfate to the diets.

Calcium retention percentage. Calcium retention values of layers fed diets composed of 0.3% and 0.6% calcium citrate were 54.1% and 63.0%, respectively which were higher than that of control (49.8%). This result is in harmony with the result of Genetesse et al (1994), El Afifi and El Alaily (2001) who showed that, calcium retention and absorption can be enhanced by adding citric acid into poultry diets. These increments in calcium retention and absorption are seemly related to the acidic media (low pH) created by feeding calcium citrate, the acidic media may enhance calcium absorption by two manners :-

1- increasing the solubility of calcium carbonate (Bronner 1993).

2- slowing passing rate of digesta, allowing more time for calcium absorption (Low 1990, Giesting et al 1991).

Calcium retention percentage of layers fed calcium sulfate were not well observed as those of calcium citrate. Furthermore the level of 0.6% calcium sulfate caused a slight impairment in calcium retention (47.0% *vs.* 49% for control). This result disagreed with that of Patton and Sutton (1952) who carried out an experiment with human subjects and reported that, calcium sulfate salt was absorbed as good as calcium in carbonate or lactate salts. It can be suggested that, addition of calcium sulfate into laying hen diets enhanced calcium absorption which reflect on blood calcium level (Table 4) while urinary excretion of calcium increase to get off blood acidosis or sulfate ions (Austic and Keshavarz 1988). Keshavarz (1991) stated that, the impairment in calcium retention of sulfate form doesn't necessarily indicate a lower calcium availability (solubility and absorption). Because birds feces and urine are voided together the lower calcium retention can be due to higher calcium excretion through kidney.

Moisture content of excreta increased from 72% to 76.7 and 79.1% due to using calcium sulfate by 0.3% and 0.6% into diets. This finding is in a good agreement with the results of Austic and Keshavarz (1988) who observed an increase in moisture content of excreta as a result of increase volume of urine.

Egg shell quality. The values of egg shell percentage, thickness (mm) and strength (kg/cm2) recorded a slight improvement due to adding calcium citrate into laying hen diets compared with control (Table 5). This improvement is mainly related to increase calcium absorption and retention as feeding calcium citrate containing diets. These results are in harmony with the finding of El Afifi and El Alaily (2001) who observed an enhancement in egg shell quality due to adding citric acid into diets. As well, Vogt and Harnisch (1989), observed an improvement in egg shell quality of layers fed diets containing of calcium fumarate. The high level (0.6%) of calcium sulfate slightly impaired egg shell quality, compared with control. These results were compatible with and attributed to calcium retention percentage which was negatively affected by high level of calcium sulfate. Keshavarz (1994) reported that, high dietary levels of sulfate had a detrimental effects on shell quality. He hypothesized that, reduced shell quality is due to increasing calcium excretion, resulting from the dietary changes of acid-base balance.

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Table (1): Composition of different experimental diets

Ingredients	Calcium carbonate (control)	Calcium sulfate (0.3%)	Calcium sulfate (0.6%)	Calcium citrate (0.3%)	Calcium citrate (0.6%)
Yellow corn	58.50	58.51	58.51	58.51	58.51
Soy been meal 44%	27.63	27.63	27.63	27.63	27.63
Di-calcium phosphate	1.81	1.81	1.81	1.81	1.81
Calcium Carbonate	9.20	8.40	7.60	8.40	7.60
Calcium sulfate	–	1.3	2.6	–	–
Calcium citrate	–	–	–	0.89	1.77
Common salt	0.30	0.30	0.30	0.30	0.30
Methionine H. A*	0.10	0.08	0.08	0.08	0.08
Lysine	0.12	0.12	0.12	0.12	0.12
Premix**	0.40	0.40	0.40	0.40	0.40
Oil	0.94	0.94	0.94	0.94	0.94
Sand	1.00	0.50	0	0.92	0.83
Total	**100**	**100**	**100**	**100**	**100**
			Calculated analysis		
ME (kcal/kg)	2640	2640	2640	2640	2640
Protein %	17.17	17.17	17.17	17.17	17.17
Calcium %	3.99	3.99	3.99	3.99	3.99
Av. Phosphorus %	0.46	0.46	0.46	0.46	0.46
Methionine + Cys. %	0.66	0.66	0.66	0.66	0.66
Lysine	1.00	1.00	1.00	1.00	1.00

*Methionine hydroxy analogue

**each one kg of premix contain the following:-

vit. A; 3750000Iu, vit. $D_{3;}$ 825000Iu, vit E 16.5g, vit. K 1.25g vit B_{12} 0.02mg, vit B_1 0.625g, vit. B2 2.475g Pantothenic acid 4.500g Nicotinic acid 10.0g vit. B6 1.650g vit B12 0.005g Biotin 0.040g Folic acid 0.20g Choline 165g, Cupper 2.0g, Iron 20g, Manganèse 25g, Zinc 15g, and Selenium 0.120g Iodine 0.20g.

Table (2): Effect of feeding different calcium salts on laying performance

	Calcium sulfate			Calcium citrate	
Age in week	**Control**	**0.3%**	**0.6%**	**0.3%**	**0.6%**
			Egg Production (%)		
61 - 65	81.4±1.5	83.8±1.3	84.4±1.1	85.7±1.6	85.4±1.3
66 - 70	80.6±1.6	81.8±1.5	81.5±1.7	84.6±1.3	84.5±1.1
Overall	81.0^{b}±1.6	82.8^{ab}±1.4	83.0^{ab}±1.2	85.1^{a}±1.1	84.9^{a}±1.1
			Egg weight (g)		
61 - 65	62.81±0.64	62.38±0.75	62.64±0.57	62.41±0.77	62.17±1.14
66 - 70	63.29±0.68	62.46±0.74	63.33±0.67	61.83±0.67	62.52±1.10
Overall	63.10±0.63	62.42±0.71	62.98±0.59	62.12±0.7	62.34±0.87
			Egg mass (g/ hen/ day)		
61 - 65	51.12±0.9	52.25±0.81	52.84±0.75	53.45±0.88	53.10±1.5
66 - 70	51.10±1.0	50.90±0.81	51.61±0.98	52.34±0.71	52.70±1.1
Overall	51.11±0.91	51.57±0.61	52.22±0.71	52.89±0.65	52.90±1.00

a-b within rows, means with no common superscripts differ significantly ($p \leq 0.05$)

Table (3): Effect of feeding different calcium salts on feed consumption and conversion.

		Calcium sulfate		Calcium citrate	
Age in week	**Control**	**0.3%**	**0.6%**	**0.3%**	**0.6%**
		Feed consumption (g/hen/day)			
61 - 65	92.6^{a}±1.9	91.1^{a}±1.2	94.2^{a}±1.4	90.6^{a}±1.5	85.7^{b}±1.4
66 - 70	96.3±1.2	95.6±1.1	94.6±1.4	93.8±1.3	92.4±1.0
Overall	94.5^{a}±1.5	93.4^{ab}±0.9	94.4^{a}±1.2	92.2^{bc}±1.4	89.1^{c}±1.3
		Feed conversion (g feed/g egg)			
61 - 65	1.81^{a}±0.031	1.75^{ab}±0.028	1.79^{ab}±0.032	1.70^{bc}±0.034	1.62^{c}±0.04
66 - 70	1.88±0.036	1.87±0.029	1.84±0.044	1.80±0.03	1.77±0.037
Overall	1.85^{a}±0.03	1.81^{ab}±0.025	1.82^{ab}±0.031	1.75^{b}±0.026	1.70^{b}±0.038

a-b-c within rows, means with no common superscripts differ significantly ($p \leq 0.05$)

Table (4): Effect of feeding different calcium salts on pH values, calcium retention, blood calcium and excreta moisture

		Calcium sulfate		Calcium citrate	
Treatments	**Control**	**0.3%**	**0.6%**	**0.3%**	**0.6%**
Dietary pH	6.28^{a}±0.06	6.25^{a}±0.07	6.23^{a}±0.09	6.10^{ab}±0.06	5.90^{b}±0.04
Ileal pH	6.30^{a}±0.10	6.16^{ab}±0.1	6.12^{ab}±0.07	6.20^{ab}±0.07	5.86^{b}±0.09
Blood calcium mg/dL	11.10±0.45	11.95±0.43	12.42±0.45	12.40±0.48	12.72±0.61
Calcium retention	49.8±3.2	51.0±7.6	47.8±5.3	54.1±3.7	63.0±6.3
Excreta moisture %	72.0±2.2	76.7±3.0	79.1±1.9	74.0±1.8	74.4±1.0

a-b within rows, means with no common superscripts differ significantly ($p \leq 0.05$))

Table (5): Effect of feeding different calcium salts on egg shell quality

Age in week		Calcium sulfate		Calcium citrate	
	Control	**0.3%**	**0.6%**	**0.3%**	**0.6%**
			% Egg shell		
65 week	8.46±0.151	8.59±0.222	8.26±0.117	8.59±0.205	8.85±0.255
70 week	7.93±0.297	8.50±0.452	7.59±0.219	8.59±0.452	8.42±0.364
Overall	8.19^{ab}±0.173	8.55^{a}±0.253	7.92^{b}±0.145	8.59^{a}±0.152	8.63^{a}±0.218
			Egg shell thickness (mm)		
65 week	0.348^{ab}±0.007	0.343^{ab}±0.007	0.339^{b}±0.008	0.344^{ab}±0.005	0.363^{a}±0.007
70 week	0.341±0.011	0.350±0.014	0.337±0.009	0.347±0.009	0.358±0.008
Overall	0.345^{ab}±0.006	0.347^{ab}±0.008	0.338^{b}±0.006	0.346^{ab}±0.005	0.361^{a}±0.005
			Egg shell strength (Kg/cm^2)		
65 week	4.16±0.539	4.29±0.419	4.29±0.311	4.47±0.399	4.57±0.329
70 week	4.38±0.436	4.49±0.300	3.91±0.377	4.49±0.300	4.54±0.464
Overall	4.27±0.335	4.39±0.249	4.10±0.242	4.48±0.218	4.56±0.272

a-b within rows, means with no common superscripts differ significantly ($p \leq 0.05$)

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استبدال أملاح الكالسيوم الحامضية بجزء من كربونات الكالسيوم في علائق الدجاج البياض المسن

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أجريت هذه التجربة لمعرفة تأثير استبدال كبريتات الكالسيوم أو سترات الكالسيوم بجزء من كربونات الكالسيوم في العليقة على الأداء الإنتاجي وجودة قشرة البيضة ونسبه الكالسيوم المحتجز بالجسم وكالسيوم الدم وكذلك على درجة الحموضة في محتويات الأمعاء. استخدم في هذه الدراسة عدد 150 دجاجة بياضة من نوع هاى لاين الأبيض (w-36) من 61 حتى 70

أسبوع من العمر. وتم تقسيمها عشوائيا إلى خمس مجاميع من 30 دجاجه في أربع مجاميع، تم استخدام كبريتات الكالسيوم أو سترات الكالسيوم لإمداد الدجاجات ب 0.3٪ أو 0.6٪ من كالسيوم العليقة بدلا من كربونات الكالسيوم.

أظهرت الدراسة تحسن معنوي في معدل إنتاج البيض بنسبه 4.1٪ و 3.9٪ نتيجة إضافة 0.3٪ أو 0.6٪ من الكالسيوم في صورة سترات على التوالي بينما لم يكن هناك تأثير واضح لكبريتات الكالسيوم.

متوسط وزن البيضة لم يتأثر معنويا بإضافة كبريتات الكالسيوم أو سترات الكالسيوم بالعليقة.

انخفض معدل الاستهلاك الغذائي وتحسن التحويل الغذائي معنويا نتيجة لإضافة سترات الكالسيوم للعلائق.

انخفضت درجة pH لمحتويات الأمعاء كنتيجة لإضافة الكالسيوم سترات أو كبريتات للعلائق.

نسبة الكالسيوم بالدم ارتفعت بصورة طفيفة نتيجة لاستبدال كبريتات الكالسيوم و سترات الكالسيوم بكربونات الكالسيوم في العليقة.

نسبة الكالسيوم المحتجز كانت 54.1٪ و 63٪ للدجاجات المغذاة على سترات الكالسيوم بنسبة 0.3٪ و 0.6٪ على التوالي وهذه القيم أعلى من القيمة المتحصل عليها من مجموعة الكنترول (49 ٪).

جودة قشرة البيضة تحسنت بدرجة طفيفة٪ نتيجة لإضافة الكالسيوم في صورة كالسيوم سترات بمستوي0.3 ٪ و 0.6٪ بينما المستوي العالي من كبريتات الكالسيوم أدى لتدهور طفيف في مواصفات جودة القشرة.