Influence of Calsporin on Commercial Leghorns

S. S. Sohail, M. M. Bryant, R. A. Voitle, and D. A. Roland, Sr.[1]

Department of Poultry Science and Alabama Agricultural Experiment Station, Auburn University, Alabama 36849

Primary Audience: Nutritionists, Feed Manufacturers, Egg Producers

SUMMARY

A study was conducted to determine if Calsporin (*Bacillus subtilis* C-3102), a direct-fed microbial (probiotic), improved performance of commercial Leghorns and eggshell quality. Hyline W36 hens (n = 1,440; age = 65 wk) in 90 groups (16 hens/group) were randomly allocated to three dietary protein levels (17.3, 16.1, and 15.0%), and three Calsporin levels (0.0, 0.003 and 0.006%) in a 3 × 3 factorial for 10 wk. A Calsporin × protein interaction was observed on egg specific gravity (SG; $P < 0.01$). Egg SG increased more in hens fed the higher Calsporin level than in hens fed the lower level when dietary protein was 15%. This Calsporin level effect was reversed as dietary protein was increased to 16.1%. Trends similar to that of SG were also observed on eggshell thickness. Increasing dietary protein had a beneficial effect ($P < 0.001$) on egg production, feed consumption, and egg weight. Addition of Calsporin to hen diets had no significant influence on feed consumption, egg production, egg weight, or body weight of hens. More research is required to optimize any potential benefit of Calsporin with respect to dietary protein on eggshell quality.

Key words: Calsporin, eggshell quality, Leghorns, probiotic

2002 J. Appl. Poult. Res. 11:379–387

DESCRIPTION OF PROBLEM

In spite of the thorough and detailed information available on factors influencing eggshell quality [1, 2, 3, 4], egg producers are losing millions of dollars annually due to losses associated with poor shell quality [5]. Eggshell quality has always challenged egg producers and layer nutritionists because of its economic importance [6] and its roles in containing egg contents and retaining internal egg quality. A decline in eggshell quality increases the number of undergrades causing losses to the egg processors and producers. Previous research has focused on physiological [7, 8, 9], nutritional [3, 10, 11], mechanical [12], and environmental [13, 14] effects to improve eggshell quality. Also, several dietary supplements have been tested in commercial layers to improve shell quality [15, 16]. Trials have also been conducted to study the internal environment of the gut and determine mechanisms involved in the absorption of nutrients in maintaining shell quality [17, 18, 19].

Research has shown that under healthy and nonstressful conditions, beneficial microflora colonize gut surfaces in a symbiotic relationship with the host and suppress undesirable microbes that may be pathogenic [20]. Direct-fed microbials, commonly known as probiotics, have been found to establish a balance between beneficial and pathogenic bacterial

[1] To whom correspondence should be addressed: droland@acesag.auburn.edu.

TABLE 1. Ingredient and nutrient composition of experimental diets

Ingredient	Diet 1	Diet 2	Diet 3
	(%)		
Corn (8.6% CP)	61.52	65.65	68.99
Soybean meal (48% CP)	25.10	21.70	18.94
Limestone[A]	7.04	7.05	7.05
Hardshell[A]	2.00	2.00	2.00
Poultry oil	1.59	0.87	0.29
Dicalcium phosphate (Ca 21.7%; P 18.5%)	1.63	1.64	1.66
NaCl	0.46	0.46	0.46
Vitamin premix[B]	0.25	0.25	0.25
Mineral premix[C]	0.25	0.25	0.25
DL-Methionine	0.17	0.13	0.11
Calsporin[D]	0	0	0
Calculated analysis			
Crude protein[E] (%)	17.34	16.06	15.02
ME (kcal/kg)	2,823	2,823	2,823
Met + Cys (%)	0.74	0.67	0.62
Lys (%)	0.92	0.83	0.75
Ca (%)[E]	4.00	4.00	4.00
Total P, (%)[E]	0.61	0.60	0.59
Nonphytate P[E] (%)	0.40	0.40	0.40

[A]Calcium carbonate was supplied as limestone (16 × 120 US mesh, 1.19 to 0.125 mm) and hardshell (4 × 8 US mesh, 4.75 to 2.36 mm).
[B]Provided per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 IU; vitamin E (as dl-α tocopheryl acetate), 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; D-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamin mononitrate, 1 mg; pyridoxine, 2.2 mg; d-biotin, 0.05 mg; menadione sodium bisulfite complex, 2 mg.
[C]Provided per kilogram of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg.
[D]Calpis Co., Ltd., Kanagawa, Japan. Calsporin levels of 0.003 and 0.006% were supplemented to these diets to create the nine treatments.
[E]Dietary protein, Ca, total P, and nonphytate P values were determined by chemical analysis.

populations in the intestine [20, 21, 22, 23]. Previous research has indicated beneficial effects of direct-fed microbials on egg production (EP), feed conversion, and yolk cholesterol [23]. *Bacillus subtilis*, a direct-fed microbial that produces amylases and proteases, has been shown to withstand high temperatures of pelleting [22] and may prove beneficial to the feed industry.

Calsporin, a commercially available *Bacillus subtilis*-originated direct-fed microbial has been reported to improve live performance and microbiological status in broilers [20, 24]. Research on the influence of Calsporin on the performance of commercial layers is not yet available. Induced molting is a popular management technique used in the commercial egg industry [25] to extend the period of production [26] and hen performance. Little research is available on the nutrient requirement of molted hens [27]. Therefore, the objective of this study was to determine the influence of Calsporin on performance and eggshell quality of commercial Leghorns after induced molting.

MATERIALS AND METHODS

A 10-wk study was conducted on Hyline W36 hens in their second cycle (molted hens) to determine if *Bacillus subtilis* C-3102, commercially available as Calsporin, has a beneficial effect on hen performance, particularly eggshell quality. This experiment used three dietary protein levels (17.3, 16.1, and 15.0%) and three Calsporin levels [0.0 (control), 0.003, and 0.006%], and data were analyzed as a 3 × 3 factorial. All nine dietary treatments were

TABLE 2. Influence of Calsporin on egg specific gravity of Hy-Line W36 hens (second cycle, 69 to 79 wk of age)

		Egg specific gravity (unit)					
		Week					
Treatment		2	4	6	8	10	Mean
Protein (%)							
17.3		1.0798[b]	1.0818[b]	1.0813[b]	1.0808[a]	1.0801[a]	1.0808[b]
16.1		1.0816[a]	1.0826[a]	1.0819[ab]	1.0814[a]	1.0805[a]	1.0816[a]
15.0		1.0805[b]	1.0825[a]	1.0822[a]	1.0811[a]	1.0805[a]	1.0814[a]
SEM		0.0004	0.0002	0.0003	0.0003	0.0002	0.0002
Calsporin (%)							
0		1.0803	1.0821	1.0819	1.0809	1.0804	1.0811
0.003		1.0809	1.0825	1.0816	1.0812	1.0805	1.0813
0.006		1.0808	1.0822	1.0819	1.0812	1.0802	1.0813
SEM		0.0004	0.0002	0.0003	0.0003	0.0002	0.0002
Protein (%) ×	Calsporin (%)						
17.3							
	0	1.0794[a]	1.0815[a]	1.0810[a]	1.0801[a]	1.0798[a]	1.0804[a]
	0.003	1.0806[a]	1.0822[a]	1.0815[a]	1.0811[a]	1.0802[a]	1.0811[a]
	0.006	1.0794[a]	1.0815[a]	1.0813[a]	1.0812[a]	1.0803[a]	1.0808[a]
16.1							
	0	1.0811[a]	1.0827[ab]	1.0822[a]	1.0814[ab]	1.0806[ab]	1.0806[b]
	0.003	1.0824[a]	1.0832[a]	1.0818[a]	1.0823[a]	1.0811[a]	1.0822[a]
	0.006	1.0814[a]	1.0818[b]	1.0816[a]	1.0806[b]	1.0797[b]	1.0810[ab]
15.0							
	0	1.0803[a]	1.0820[a]	1.0824[a]	1.0812[a]	1.0808[a]	1.0813[ab]
	0.003	1.0798[a]	1.0822[a]	1.0814[a]	1.0803[a]	1.0801[a]	1.0807[b]
	0.006	1.0815[a]	1.0833[a]	1.0828[a]	1.0818[a]	1.0804[a]	1.0820[a]
SEM		0.0006	0.0004	0.0004	0.0005	0.0004	0.0003
Significance							
Protein		**	*	*	NS	NS	**
Calsporin		NS[a]	NS	NS	NS	NS	NS
Protein × Calsporin		NS	*	NS	**	NS	**

[a,b]Means within a column and treatment section having different superscripts are significantly different ($P < 0.05$).
[A]Not significant at $P > 0.05$.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

isocaloric (Table 1). Experimental hens (n = 1,440) were divided into 90 replicates (groups) of 16 hens each, such that hens from each replicate had no access to feed or water of other replicates. Each replicate had four adjacent cages housing four birds per cage (31 × 41 cm). A cage was left vacant between the replicates to avoid cross-contamination through feed and water. Replicates were also equally divided between the two cage tiers.

Molting of hens was initiated at 65 wk of age, and the experiment was run from 69 wk of age until 79 wk of age. Prior to commencement of the study, postmolt feed was supplemented for 2 wk with one of three Calsporin levels to acclimate hens to Calsporin levels. All 90 replicates were equally and randomly assigned to the prestudy treatments and were fed for 2 wk. To avoid contamination between levels, diets with the same Calsporin level were assigned a color-marked scoop for feeding the hens. In addition, feeder troughs were also marked with color (similar to scoops) to avoid any accidental contamination among treatments. At commencement of the study, 10 replicates within each Calsporin level were randomly allocated to each protein level (Table 1).

Feed and excreta samples were analyzed periodically to monitor cross-contamination between Calsporin levels, maintain an accurate

TABLE 3. Influence of Calsporin on eggshell thickness and body weight of Hy-Line W36 hens (second cycle, 69 to 79 wk of age)

Treatment		Eggshell thickness[A] (mm)	Body weight[B] (kg)
Protein (%)			
17.3		0.31	1.632
16.1		0.32	1.618
15.0		0.32	1.568
SEM		0.005	0.023
Calsporin (%)			
0		0.31	1.586
0.003		0.32	1.627
0.006		0.31	1.605
SEM		0.005	0.023
Protein (%) ×	Calsporin (%)		
17.3			
	0	0.31	1.536
	0.003	0.32	1.682
	0.006	0.31	1.677
16.1			
	0	0.31	1.641
	0.003	0.34	1.627
	0.006	0.31	1.586
15.0			
	0	0.31	1.582
	0.003	0.32	1.568
	0.006	0.32	1.555
SEM		0.009	0.041
Significance			
Protein		NS[C]	NS
Calsporin		NS	NS
Protein × Calsporin		NS	NS

[A]Egg samples were taken at the tenth week of the experiment.
[B]Body weight was determined at the termination of study.
[C]Not significant at $P > 0.05$.

measure of Calsporin concentration in the diets, monitor the effect of Calsporin level on intestinal microflora, and determine the respective release of microbes in feces from each Calsporin level [28]. This study was conducted in an environmentally controlled facility where daily temperatures were maintained at approximately 25.5°C except for a few days when unusually high summer temperatures occurred. To determine hen performance, mortality was recorded daily, feed consumption (FC) and EP were measured weekly, and egg weight (EW) and egg specific gravity (SG) [29] were determined biweekly from a 2-d collection of eggs during the week. Eggshell thickness [30] and body weight measured at the termination of study. All data were analyzed statistically [31] to determine the effect of Calsporin and protein level on hen performance.

RESULTS AND DISCUSSION

Egg SG

Egg SG is significantly related to percentage of cracks and is useful in estimating eggshell quality [29]. Calsporin level had an interaction ($P < 0.01$) with protein level on SG during the 10-wk study (Table 2). The SG increased more in hens fed the higher Calsporin level (0.006%) than in hens fed the lower level (0.003%) when dietary protein was 15%. This effect of Calsporin level was reversed as di-

TABLE 4. Influence of Calsporin on feed consumption of Hy-Line W36 hens (second cycle, 69 to 79 wk of age)

		Feed consumption (g)										
		Week										
Treatment		1	2	3	4	5	6	7	8	9	10	Mean
Protein (%)												
17.3		70.2[a]	77.3[a]	87.1[a]	91.3[a]	91.9[a]	92.0[a]	91.2[a]	88.8[a]	93.7[a]	87.4[a]	87.0[a]
16.1		69.2[a]	74.9[a]	83.7[b]	88.2[b]	88.7[b]	89.9[ab]	88.9[ab]	86.6[ab]	90.9[b]	85.9[ab]	84.7[b]
15.0		68.4[a]	74.8[a]	82.4[b]	86.4[b]	87.2[b]	88.3[b]	87.6[b]	84.8[b]	90.0[b]	84.4[b]	83.4[b]
SEM		0.60	0.76	0.73	0.71	0.77	0.81	0.81	0.75	0.62	0.63	0.56
Calsporin (%)												
0		69.5	74.8	84.7	88.5	88.9	89.4	88.5	86.0	91.1	85.1	84.6
0.003		68.3	75.1	84.0	88.3	89.1	90.1	89.3	86.5	91.1	85.8	84.8
0.006		70.0	77.1	84.5	89.0	89.8	90.7	90.0	87.2	92.4	86.8	85.7
SEM		0.60	0.76	0.73	0.71	0.77	0.81	0.81	0.75	0.62	0.63	0.56
Protein (%) ×	Calsporin (%)											
17.3												
	0	71.5	77.1	88.0	91.0	90.8	90.5	90.0	87.1	93.2	86.1	86.5
	0.003	69.2	77.3	86.9	91.9	92.3	92.2	92.2	89.2	93.8	88.5	87.4
	0.006	69.9	77.5	86.4	90.9	92.5	93.3	91.3	88.8	94.2	87.5	87.2
16.1												
	0	67.8	72.8	83.9	88	88.7	89.8	88.7	86.8	91.2	86.2	84.4
	0.003	68.4	74.7	82.8	87.2	88.5	89.6	89.1	86.5	90.6	85.9	84.3
	0.006	71.4	77.3	84.4	89.3	89.0	90.2	88.9	86.5	90.8	85.6	85.3
15.0												
	0	69.1	74.5	82.2	86.5	87.1	87.9	86.8	84.1	88.9	83.0	83.0
	0.003	67.4	73.4	82.3	85.8	86.4	88.4	86.5	83.9	89.0	82.9	82.6
	0.006	68.6	76.4	82.8	86.9	88.1	88.7	89.6	86.3	92.1	87.4	84.7
SEM		1.04	1.31	1.26	1.23	1.33	1.40	1.40	1.29	1.07	1.08	0.98
Significance												
Protein		NS[A]	NS	***	***	***	**	**	**	***	**	***
Calsporin		NS	0.08	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Calsporin		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.06	NS

[a,b]Means within a column and treatment section having different superscripts are significantly different ($P < 0.05$).
[A]Not significant at $P > 0.05$.
**$P < 0.01$.
***$P < 0.001$.

etary protein was increased to 16.1%, and SG increased more in hens fed the lower level (0.003%) than fed the higher level (0.006%). The Calsporin level effect on SG was lost when dietary protein was further increased to 17.3%. This finding indicated that dietary protein level influenced the effect of Calsporin on SG. Increasing dietary protein from 16.1 to 17.3% had an adverse effect on SG of hens ($P < 0.01$), which was expected due to increase in EW. On average, SG decreased from 1.0816 to 1.0808 as protein level increased from 16.1 to 17.3%.

Eggshell Thickness

Dietary Calsporin or protein had no significant effect ($P > 0.05$) on eggshell thickness (Table 3). However, trends similar to that of SG were observed when eggshell thickness was measured.

Body Weight

Calsporin level or dietary protein had no significant effect ($P > 0.05$) on body weight of hens, and there were no Calsporin × protein interactions (Table 3).

TABLE 5. Influence of Calsporin on egg production of Hy-Line W36 hens (second cycle, 69 to 79 wk of age)

		Egg production (%)										
		week										
Treatment		1	2	3	4	5	6	7	8	9	10	Mean
Protein (%)												
17.3		10.8[a]	39.0[a]	54.9[a]	70.5[a]	80.4[a]	82.9[a]	82.3[a]	82.1[a]	81.7[a]	82.7[a]	66.7[a]
16.1		7.8[b]	33.9[b]	49.6[b]	67.5[a]	76.6[b]	80.1[b]	82.5[a]	82.0[a]	80.7[a]	81.2[a]	64.2[b]
15.0		7.1[b]	31.2[b]	46.7[b]	62.4[b]	72.1[c]	76.6[c]	79.1[b]	79.2[a]	78.3[b]	78.7[b]	61.2[c]
SEM		0.94	1.54	1.52	1.25	1.06	0.85	0.78	0.94	0.82	0.82	0.65
Calsporin (%)												
0		7.7	33.7	48.9	67.9	76.9	79.4	81.6	81.4	80.1	82.0	64.0
0.003		9.2	33.6	49.8	66.4	76.0	80.6	81.8	81.5	91.4	81.8	64.2
0.006		8.6	36.3	52.1	66.0	75.9	79.3	80.5	80.4	79.2	78.8	63.7
SEM		0.94	1.53	1.51	1.25	1.05	0.85	0.78	0.94	0.81	0.81	0.65
Protein (%) ×	Calsporin (%)											
17.3												
	0	8.9	39.0	55.1[a]	71.0	80.9	82.8	82.1	83.5	82.8	84.2	67.0
	0.003	12.4	37.8	55.0[a]	71.2	82.3	85.2	84.1	83.6	83.3	83.7	67.9
	0.006	11.3	40.5	54.5[a]	69.0	77.4	80.1	80.3	78.3	78.5	79.5	64.9
16.1												
	0	6.3	30.7	44.8[b]	68.4	77.4	79.5	83.2	82.5	80.5	83.3	63.7
	0.003	8.2	33.2	49.7[ab]	66.0	74.2	78.2	81.2	80.4	80.8	81.0	63.3
	0.006	8.7	37.0	53.5[a]	68.1	78.0	82.0	83.0	82.9	80.9	79.7	65.4
15.0												
	0	8.0	31.4	46.9[a]	64.2	72.6	75.9	79.5	78.1	77.0	78.6	61.2
	0.003	7.1	29.9	44.6[a]	62.1	71.6	78.5	80.1	80.4	80.1	80.5	61.5
	0.006	6.4	32.1	48.7[a]	61.0	72.1	75.5	77.7	79.1	77.8	77.0	60.7
SEM		0.63	2.64	2.61	2.17	1.82	1.46	1.34	1.63	1.41	1.40	1.11
Significance												
Protein		**	***	***	***	***	***	**	*	**	**	***
Calsporin		NS[A]	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
Protein × Calsporin		NS	NS	NS	NS	NS	*	NS	0.07	NS	NS	NS

[a,b,c]Means within a column and treatment section having different superscripts are significantly different ($P < 0.05$).
[A]Not significant at $P > 0.05$.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

FC

Within 2 wk, dietary protein had a linear effect ($P < 0.05$) on FC of hens (Table 4). As protein level increased from 15.0 to 17.3%, average FC linearly increased ($P < 0.001$) from 83 to 87 g/hen per d. Reports on the effect of dietary protein on FC of hens are inconsistent [34, 35, 36, 37]. Some report an increase in FC due to increase in the amount of dietary protein [37], whereas others report no effect [36, 38]. Calsporin had no effect ($P > 0.05$) on FC of hens. However, a trend indicating increased FC with increasing dietary Calsporin level was observed within 2 wk.

EP

Consistent with previous research [35, 36, 37] EP linearly increased ($P < 0.01$) within 1 wk as dietary protein was increased (Table 5). Increasing protein from 15.0 to 17.3% linearly increased ($P < 0.001$) average EP from 61.2 to 66.7%, a net increase of 5.5%. Dietary Calsporin, however, had no effect on EP ($P > 0.05$), and there were no interactions.

EW

Increasing dietary protein had a linear effect ($P < 0.01$) on EW within 2 wk (Table 6). This finding was in agreement with previous

TABLE 6. Influence of Calsporin on egg weight of Hy-Line W36 hens (second cycle, 69 to 79 wk of age)

		Egg weight (g)					
		Week					
Treatment		2	4	6	8	10	Mean
Protein (%)							
17.3		62.6[a]	63.7[a]	63.7[a]	63.0[a]	63.0[a]	63.2[a]
16.1		61.4[b]	62.4[b]	62.4[b]	62.4[ab]	61.9[b]	62.1[b]
15.0		60.6[b]	61.9[b]	62.2[b]	61.9[b]	62.0[b]	61.7[b]
SEM		0.38	0.24	0.25	0.23	0.26	0.21
Calsporin (%)							
0		61.9	62.9	63.1	62.7	62.3	62.6
0.003		61.3	62.2	62.3	62.2	62.2	62.1
0.006		61.5	62.8	62.8	62.4	62.4	62.4
SEM		0.38	0.24	0.25	0.23	0.26	0.21
Protein (%) ×	Calsporin (%)						
17.3							
	0	62.8	63.9	63.8	63.0	62.7	63.3
	0.003	62.6	63.6	63.2	62.9	63.2	63.1
	0.006	62.4	63.6	64.0	63.0	62.9	63.2
16.1							
	0	61.7	62.5	62.9	62.6	62.0	62.4
	0.003	60.6	61.8	62.0	62.1	61.9	61.7
	0.006	61.8	62.8	62.1	62.3	61.8	62.2
15.0							
	0	61.1	62.3	62.4	62.4	62.0	62.0
	0.003	60.6	61.3	61.8	61.6	61.6	61.4
	0.006	60.2	62.2	62.4	61.8	62.4	61.8
SEM		0.66	0.41	0.43	0.39	0.45	0.36
Significance							
Protein		**	***	***	**	**	***
Calsporin		NS	0.07	NS	NS	NS	NS
Protein × Calsporin		NS	NS	NS	NS	NS	NS

[a,b]Means within a column and treatment section having different superscripts are significantly different ($P < 0.05$).
[A]Not significant at $P > 0.05$.
**$P < 0.01$.
***$P < 0.001$.

research that increasing dietary protein increases EW [36, 37, 39]. Increasing protein level from 15 to 17.3% linearly increased average ($P < 0.001$) EW from 61.7 to 63.2 g. Calsporin supplementation in the diet had no influence ($P > 0.05$) on EW, and there were no Calsporin × protein interactions.

Fecal Bacterial Count and Hen Mortality

Fecal bacteria were counted to determine the level of bacteria excreted relative to the Calsporin level fed and to determine the accuracy of feeding Calsporin levels within each treatment. Increasing each dietary Calsporin increased ($P < 0.001$) the fecal bacterial count (Table 7). This increase was observed at the beginning and toward the end of the experiment. The amount of Calsporin fed was directly related to the amount of intestinal microflora

TABLE 7. Influence of dietary Calsporin on fecal bacterial count

	Fecal bacterial count Mean (log cfu/g feces)		
Calsporin (%)	Begin	End	Average
0.000	2.66[c]	2.71[c]	2.65[c]
0.003	5.12[b]	4.91[b]	4.98[b]
0.006	5.45[a]	5.25[a]	5.35[a]
SEM	0.07	0.08	0.05

[a–c]Means within a column having different superscripts are significantly different ($P < 0.05$).

excreted. This result suggests that Calsporin had no adverse effect on the performance of hens. These results also indicate that Calsporin levels were correctly fed during the course of this study, and measures taken to avoid contamination among the treatments were successful. There were no Calsporin × protein interactions (not shown) nor was an effect of Calsporin or dietary protein on mortality of hens observed.

CONCLUSIONS AND APPLICATIONS

1. Calsporin (*Bacillus subtilis* C-3102) level had a significant interaction with dietary protein level on egg SG.
2. Further research is needed to optimize any potential benefit of using Calsporin relative to dietary protein in commercial hen diets to improve hen performance and eggshell quality.

REFERENCES AND NOTES

1. 1. Roland, D. A., Sr. 1979. Factors influencing shell quality of aging hens. Poult. Sci. 58:774–777.

2. Hamilton, R. M. G. 1982. Methods and factors that affect the measurement of egg shell quality. Poult. Sci. 61:2022–2039.

3. Roland, D. A., Sr. 1987. Egg shell quality III: Calcium and phosphorus requirements of commercial Leghorns. World's Poult. Sci. J. 42:154–165.

4. Strong, C. F., Jr. 1989. Relationship between several measures of shell quality and egg-breakage in a commercial processing plant. Poult. Sci. 68:1730–1733.

5. Madison, M. E., and A. M. Perez. 1994. US Egg and Poultry Statistical Series. 1960–1992. USDA Stat. Bull. 872. USDA, Washington, DC.

6. Roland, D. A., Sr. 1988. Eggshell breakage. Incidence and economic impact. Poult. Sci. 67:1801–1803.

7. Castaldo, D. J., and D. V. Maurice. 1990. Shell gland adenosine triphosphatase in hens producing strong and weak egg shells. Br. Poult. Sci. 31:225–229.

8. Keshavarz, K., and R. E. Austic. 1990. Effects of dietary minerals on acid-base balance and eggshell quality in chickens. J. Nutr. 120:1360–1369.

9. Berry, W. D., and J. Brake. 1991. Induced molt increases eggshell quality and calbindin-D28k content of eggshell gland and duodenum of aging hens. Poult. Sci. 70:655–657.

10. Sohail, S. S., and D. A. Roland, Sr. 2000. Influence of phytase on calcium utilization in commercial layers. J. Appl. Poult. Res. 9:81–87.

11. Zapata, L. F., and A. G. Gernat. 1995. The effect of four levels of ascorbic acid and two levels of calcium on eggshell quality of forced-molted White Leghorn hens. Poult. Sci. 74:1049–1052.

12. Fajardo, T. A., R. C. Anantheswaran, and V. M. Puri. 1996. Effect of cooling on crack development and mechanical behavior of eggshells. Appl. Eng. Agric. 12:49–55.

13. Sauveur, B., and M. Picard. 1987. Environmental effects on egg quality. Pages 219–234 in Poultry Science Symposium, Kent, UK. Butterworths, London, UK.

14. Peakall, D. B., and J. L. Lincer. 1996. Do PCBs cause eggshell thinning? Environ. Pollut. 91:127–129.

15. Grimes, J. L., D. V. Maurice, S. F. Lightsey, and J. G. Lopez. 1997. The effect of dietary Fermacto on layer hen performance. J. Appl. Poult. Res. 6:399–403.

16. Biswas, M. A. H., Y. Miyazaki, K. Nomura, and M. Wakita. 2000. Influences of long-term feeding of Japanese green tea powder on laying performance and egg quality in hens. Asian-Australas. J. Anim. Sci. 13:980–985.

17. Brodin, E., J. Alumets, R. Hakanson, S. Leander, and F. Sundler. 1981. Immunoreactive substance P in the chicken gut:distribution, development and possible functional significance. Cell Tissue Res. 216:455–469.

18. Schneitz, C., T. Kiiskinen, V. Toivonen, and M. Nasi. 1998. Effect of BROILACT on the physicochemical conditions and nutrient digestibility in the gastrointestinal tract of broilers. Poult. Sci. 77:426–432.

19. Wasserman, R. H. 1981. Intestinal absorption of calcium and phosphorus. Pages 68–72 in Proceedings of The Federation of American Societies for Experimental Biology, Bethesda, MD. FASEB, Bethesda, MD.

20. Maruta, K., H. Miyazaki, S. Masuda, M. Takahashi, T. Marubashi, Y. Tadano, and H. Takahashi. 1995. Exclusion of intestinal pathogens by continuous feeding with *Bacillus subtilis* C-3102 and its influence on the intestinal microflora in broilers. Anim. Sci. Technol. 67:273–280.

21. Jin, L. Z., Y. Y. Ho, N. Abdullah, and S. Jalaludin. 1997. Probiotics in poultry: modes of action. World's Poult. Sci. J. 53:351–368.

22. Ziprin, R. L., and J. R. Deloach. 1993. Comparison of probiotics maintained by in vivo passage through laying hens and broilers. Poult. Sci. 72:628–635.

23. Abdulrahim, S. M., M. S. Y. Haddadin, E. A. R. Hashlamoun, and R. K. Robinson. 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. Br. Poult. Sci. 37:341–346.

24. Fritts, C. A., J. H. Kersey, M. A. Motl, E. C. Kroger, F. Yan, J. Si, Q. Jiang, M. M. Campos, A. L. Waldroup, and P. W. Waldroup. 2000. *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. J. Appl. Poult. Res. 9:149–155.

25. Koelkebeck, K. W., C. M. Parsons, R. W. Leeper, S. Jin, and M. W. Douglas. 1999. Early postmolt performance of laying hens fed a low-protein corn molt diet supplemented with corn gluten meal, feather meal, methionine, and lysine. Poult. Sci. 78:1132–1137.

26. Zimmerman, N. G., and D. K. Andrews. 1987. Comparison of several induced molting methods on subsequent performance of single comb White Leghorn hens. Poult. Sci. 66:408–417.

27. National Research Council. 1994. Nutrient Requirements for Poultry. 9th rev. ed. National Academy Press, Washington, DC.

28. One or two grams of fresh fecal samples was mixed with anaerobic diluent A to make fecal dilutions. Anaerobic diluent A comprised KH_2PO_4 (monopotassium phosphate), 4.5 g; Na_2HPO_4 (disodium phosphate), 6.0 g; L-cysteine, 0.5 g; Tween 80, 0.5 g; agar, 1.0 g; and distilled water, 1 L. The diluent was sterilized after substitution of CO_2 for O to detect anaerobic bacteria. Tenfold dilutions of fecal samples were then incubated at 65°C for 35 min. The diluted and heated fecal solutions were then inoculated (0.1 or 0.2 mL) onto trypticase soy agar media and incubated at 37°C for 20 h. The number of colonies peculiar to *Bacillus subtilis* C-3102 were determined and calculated for each level of Calsporin used in the diet.

29. Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked and percent shell. Poult. Sci. 58:250–251.

30. Egg shell thickness was determined using an Ames thickness guage 25 M-5, B. C. Ames Company, Waltham, MA.

31. Data were subjected to ANOVA [32] using the general linear models procedure of the SAS statistical package [33]. Student-Newman-Keuls' test was used to separate the means.

32. Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Inc., New York, NY.

33. SAS Institute. 1988. SAS/STAT User's Guide. Release 6.03 Edition. SAS Institute Inc. Cary, NC.

34. Deaton, J. W., and J. H. Quisenberry. 1965. Effect of dietary protein level on performance of four commercial egg production stocks. Poult. Sci. 44:936–942.

35. Brake, J. D., and E. D. Peebles. 1992. Laying hen performance as affected by diet and caging density. Poult. Sci. 71:945–950.

36. Keshavarz, K., and S. Nakajima. 1995. The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. Poult. Sci. 74:50–61.

37. Parsons, C. M., K. W. Koelkebeck, Y. Zhang, X. Wang, and W. Leeper. 1993. Effect of dietary protein and added fat levels on performance of young laying hens. J. Appl. Poult. Res. 2:214–220.

38. Keshavarz, K. 1998. The effect of light regimen, floor space, and energy and protein levels during the growing period on body weight and early egg size. Poult. Sci. 77:1266–1279.

39. Calderon, V. M., and L. S. Jensen. 1990. The requirement for sulfur amino acid by laying hens as influenced by the protein concentration. Poult. Sci. 69:934–944.