Effects of dietary calcium formate inclusion on broiler performance, skeletal development, and gut maturation

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Primary Audience: Live Production Managers, Nutritionists, Feed Mill Managers, Veterinarians

SUMMARY

Modern broilers grow much faster and more efficiently than their predecessors. Because of the rapid growth rates of broilers, particular attention must be given to the skeletal development of the bird. Calcium formate (CaFo) has been used as a calcium source in animal diets in the European Union for many years. The objective of this study was to evaluate CaFo in broiler diets as an alternative source of calcium. Four experimental groups (0.0, 0.5, 1.0, and 1.5% CaFo) were evaluated, with 10 replicate pens per treatment. Variables measured included BW, FCR, tibia weight, bone ash percentage, tibia breaking strength, and gut morphology. Many of the parameters measured were statistically similar to the calcium carbonate control treatment. However, the 1.0% CaFo treatment significantly increased tibia breaking strength and duodenal villus height when compared with the other treatments at the completion of grow out. Therefore, CaFo can serve as an alternative calcium source in broiler diets and may significantly improve skeletal development.

Key words: calcium formate, performance, broiler, bone

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DESCRIPTION OF PROBLEM

The modern broiler grows much faster and is significantly heavier than its pre-1950s counterpart [1]. The short rearing period and rapid growth rates present challenges with regard to proper bone development for nutritionists, veterinarians, and production personnel. Seemingly minor deficiencies within a nutrient profile can have an immediate effect on the immune response and skeletal development of birds. Once errors are detected, there is no way to correct for these costly mistakes because a critical stage of development has already passed. According to Fleming [2], "Leg and gait disorders have been a considerable problem for the broiler industry, and although recent genetic, management and nutritional approaches have improved the situation, there remains scope for further improvement" (p. 179).

Calcium formate (**CaFo**), a salt of formic acid, is a by-product of varnish and dye man-

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ufacturing [3]. Because of its relatively high concentration of calcium (30.8% by weight), its use as a dietary calcium source has been investigated in various species [4]. Its effectiveness as a calcium supplement in dietary rations has been evaluated in humans in recent years and has been shown to have increased bioavailability when compared with calcium citrate and calcium carbonate [5, 6]. Supplementation of commercial swine diets with CaFo has also led to improved growth rates and FE [7–10].

Calcium formate has been discussed as an alternative to antibiotic growth promoters in animal rations, associated with its properties as a gut acidifier. Partanen and Mroz [11] and Partanen et al. [12] demonstrated the advantages of using formic acid, a product of the hydrolysis of CaFo, as a gut acidifier to alter gut microflora and improve performance in swine. A decrease in the pH of the gut can result in a significant reduction in bacterial loads resulting from the inhibition of pathogenic bacterial growth [13]. This has been exhibited in swine by a significant reduction in *Escherichia coli* and *Enterococcus* populations [14]. Organic acids have previously been shown to have positive effects in broilers, with a reduction in *Salmonella* spp. in both cecal counts and postslaughter carcass recovery [15, 16].

The authors hypothesized that dietary CaFo inclusion would result in broiler performance and bone mineralization equal to, if not improved as compared with, broilers fed a diet containing calcium carbonate as the exclusive source of calcium. The objective of this research was to evaluate CaFo as an alternative source of calcium supplementation in commercial broiler diets. The variables average broiler BW, corrected FCR, tibia weight, tibia breaking strength, bone ash content, and duodenal morphology were studied.

MATERIALS AND METHODS

Experimental Birds and Rearing

The study was conducted in an experimental broiler rearing facility, with the experimental design consisting of 4 treatment groups with 10 replicates per treatment, for a total of 40 pens. Each replicate pen contained 54 broilers at placement, for a total of 2,160 Cobb [17] male by-product broilers. Day-old broilers were obtained from a local hatchery, weighed, wing banded, and assigned to dietary treatments based on chick weight to ensure that all treatments began with statistically similar BW. Birds were provided supplemental heat, an age-appropriate diet (Table 1), and water ad libitum for the duration of the trial. Care was provided in accordance with a Texas A&M University-approved animal use protocol. All birds were euthanized according to the American Veterinary Medical Association and National Chicken Council animal welfare guidelines.

Treatment groups consisted of 4 levels of CaFo inclusion (0.0, 0.5, 1.0, and 1.5%) [18]. These inclusion levels of CaFo would contribute, respectively, 0.0, 0.15, 0.30, and 0.45% of total calcium to each diet, with the difference in calcium provided from calcium carbonate. A 4-phase dietary program was used, which included a starter (d 1 to 15), grower (d 15 to 28), finisher (finisher 1; d 28 to 42), and withdrawal ration (finisher 2; d 42 to 49). Experimental variables were evaluated on each day of dietary change (i.e., on d 15, 28, 42, and 49). On each of these days, average broiler BW and feed consumption were determined per replicate pen. On d 15, 28, and 42, one broiler was removed from each pen and euthanized, and the right tibia and a duodenal intestinal sample were collected. On d 49, at the termination of the study, the sample size was increased to 3 broilers from each pen.

Tibia Analysis

Tibias were removed, cleaned of all adhering material, packaged in plastic bags, and frozen at 20°C for storage. Before analysis, tibias were warmed to room temperature. Breaking strength was determined by using an Instron [19] machine. Instron settings for each day of sampling were as follows: d 15: 50-kg load cell, 10-kg load range, 50 mm/min crosshead speed, and 3-cm span; d 28: 50-kg load cell, 50-kg load range, 50 mm/min crosshead speed, and 3-cm span; d 42: 50-kg load cell, 50-kg load range, 50 mm/min crosshead speed, and a 3-cm span; and d 49: 50-kg load cell, 100-kg load range, 50 mm/min crosshead speed, and a 3-cm span. After breaking strength determination, tibias were

Table 1. Calculated nutrient concentration of the 4-phase treatment diets [with 0.0, 0.5, 1.0, or 1.5% calcium formate (CaFo)] fed to male broilers through 49 d of age

	Starter, d 1 to 15				Grower, d 16 to 28				Finisher 1, d 29 to 42				Finisher 2, d 43 to 49			
Item	0.0%	0.5%	1.0%	1.5%	0.0%	0.5%	1.0%	1.5%	0.0%	0.5%	1.0%	1.5%	0.0%	0.5%	1.0%	1.5%
Ingredient, %																
Corn	56.03	56.13	55.53	55.25	63.12	62.88	62.66	62.46	67.88	67.71	67.65	67.23	67.72	67.53	67.29	67.04
Soybean meal (48% CP)	35.24	35.24	35.31	35.39	28.71	28.74	28.76	28.80	24.19	24.21	24.10	24.29	23.69	23.71	23.75	23.80
Animal-vegetable fat blend	3.89	3.89	4.11	4.22	3.60	3.70	3.80	3.86	3.70	3.75	3.80	3.95	4.60	4.65	4.75	4.85
Monocalcium phosphate	1.85	1.85	1.84	1.84	1.65	1.65	1.65	1.65	1.54	1.54	1.54	1.53	1.40	1.40	1.40	1.40
Limestone	1.58	1.18	0.79	0.39	1.47	1.07	0.68	0.28	1.28	0.88	0.49	0.09	1.31	0.92	0.52	0.13
Sodium chloride	0.51	0.51	0.51	0.51	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.51	0.51	0.51	0.51
DL-Methionine (98%)	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine hydrochloride	0.11	0.11	0.11	0.10	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.10	0.10	0.10	0.10
Choline chloride (60%)	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.06	0.06	0.06	0.06	0.03	0.03	0.03	0.03
Hydrated vitamin D_3	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Copper sulfate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.03
L-Threonine (98%)	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
CaFo	0.00	0.50	1.00	1.50	0.00	0.50	1.00	1.50	0.00	0.50	1.00	1.50	0.00	0.50	1.00	1.50
Calculated nutrient, %																
CP	22.14	22.15	22.13	22.14	19.61	19.60	19.59	19.60	17.81	17.80	17.75	17.79	17.50	17.50	17.49	17.50
ME, kcal/kg	3,078	3,082	3,081	3,083	3,134	3,135	3,136	3,135	3,190	3,189	3,189	3,191	3,245	3,244	3,245	3,246
Methionine	0.61	0.61	0.61	0.61	0.57	0.57	0.57	0.57	0.52	0.52	0.52	0.52	0.51	0.51	0.51	0.51
TSAA	0.97	0.97	0.97	0.97	0.90	0.90	0.90	0.90	0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81
Lysine	1.27	1.27	1.27	1.27	1.12	1.12	1.12	1.12	1.00	1.00	1.00	1.00	0.95	0.95	0.95	0.95
Threonine	0.86	0.86	0.86	0.86	0.76	0.76	0.76	0.76	0.69	0.69	0.69	0.96	0.68	0.68	0.68	0.68
Tryptophan	0.27	0.27	0.27	0.27	0.23	0.23	0.23	0.23	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcium	1.00	1.00	1.00	1.00	0.91	0.91	0.91	0.91	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80
Sodium	0.22	0.22	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22
Total phosphorus	0.76	0.76	0.76	0.76	0.70	0.70	0.70	0.70	0.66	0.66	0.66	0.66	0.63	0.63	0.63	0.63
Available phosphorus	0.51	0.51	0.51	0.51	0.46	0.46	0.46	0.46	0.43	0.43	0.43	0.43	0.40	0.40	0.40	0.40
CF	2.61	2.61	2.60	2.60	2.51	2.50	2.50	2.50	2.44	2.43	2.43	2.43	2.41	2.41	2.41	2.40
ADF	3.47	3.47	3.46	3.46	3.32	3.31	3.31	3.30	3.21	3.20	3.20	3.19	3.18	3.17	3.17	3.16
Crude fat	6.35	6.35	6.55	6.64	6.26	6.35	6.45	6.49	6.50	6.54	6.59	6.72	7.38	7.43	7.52	7.61

[1]Vitamin premix added at this rate per kilogram of diet yielded the following: 11,023 IU of vitamin A, 3,858 IU of vitamin D_3, 46 IU of vitamin E, 0.0165 mg of B_{12}, 5.845 mg of riboflavin, 45.93 mg of niacin, 20.21 mg of D-pantothenic acid, 477.67 mg of choline, 1.47 mg of menadione, 1.75 mg of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamine, 0.55 mg of biotin. The carrier was ground rice hulls.

[2]Trace mineral premix added at this rate per kilogram of diet yielded the following: 149.6 mg of manganese, 125.1 mg of zinc, 16.5 mg of iron, 1.7 mg of copper, 1.05 mg of iodine, 0.25 mg of selenium, a minimum of 6.27 mg of calcium, and a maximum of 8.69 mg of calcium. The carrier was calcium carbonate, and the premix contained less than 1% mineral oil.

dried for 24 h at 105°C and ashed at 600°C for 24 h to calculate ash content [20].

Gut Morphology

Intestinal samples consisted of approximately 2.5 cm of the ascending duodenum of each bird sampled. Samples were removed and flushed with ice-cold saline. The samples were then stored in 50 mL of 10% neutral buffered formalin in a plastic tissue sample container for at least 3 d at room temperature. The samples were prepared for morphological evaluation by cutting approximately 5 mm of each sample with a razor blade and placing the tissue in a cassette for paraffin embedding. The cassettes were then stored in 10% neutral buffered formalin and shipped to a private laboratory [21] for embedding and hematoxylin and eosin staining.

Sample slides were scanned into Adobe Photoshop CS4 Extended [22] with an Epson Perfection 4990 photo scanner [23] at 4,800 dots/in. (pixels/in.). The Photoshop measurement function was used to determine the number of pixels constituting the height and width of the villi. Pixels were converted to millimeters by using the dots per inch from the original scan. Villus height was measured from the top of the villus to the top of the lamina propria [24]. Villus width was measured at the base of the villus [24]. Surface area was calculated using the formula: (2Π) × (villus width/2) × (villus height) [24, 25].

Statistical Analysis

Data for all variables were analyzed via one-way ANOVA using SPSS [26]. Means were deemed significant at $P \leq 0.05$ and were separated using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Broiler Performance

A significant difference ($P \leq 0.05$) was observed for d 15 BW, with the 1.0% CaFo inclusion rate resulting in the lowest BW compared with all other treatments (Table 2). On d 28, the inclusion rate of 0.5% dietary CaFo increased ($P \leq 0.05$) the BW of broilers compared with those fed CaFo at inclusion rates of 1.0 and 1.5%; however, all CaFo inclusion rates resulted in similar BW when compared with the control. After d 28, no differences in BW were observed between any of the experimental treatments for the remainder of the study (Table 2).

When comparing mortality-corrected FCR of the different dietary phases, differences ($P \leq 0.05$) were observed in the starter phase of the diet, with the 1.0% CaFo inclusion rate resulting in an increased ($P \leq 0.05$) FCR compared with all other treatments (Table 2). When the FCR were compared cumulatively for the entire growing period, significant differences were observed from d 0 to 28 between the 0.5 and 1.5% CaFo treatments. The 0.5% CaFo treatment resulted in a lower cumulative FCR than the 1.5% treatment ($P \leq 0.05$). No differences were observed when percentage mortality was compared among treatment groups for the duration of the trial (data not shown).

Although significant differences in FCR and BW data were observed at some of the CaFo treatment sampling points, the CaFo treatment performance data were statistically similar to the controls at the completion of the study. Conversely, in previous studies, it has been demonstrated that organic acids may be suitable as an alternative to antibiotic growth promoters in swine [12]. It is important to note that these birds were reared in a research facility and that differences in performance may have been observed if these birds had been reared in a commercial facility that may have had a less rigorous cleaning and disinfection program. Although organic acids have been shown to affect performance positively, the benefits are typically more variable than those from antibiotics [27]. The variability in bird performance that we observed with CaFo inclusion was also reported previously [16, 28].

Tibia Analysis

No significant differences in tibia weight relative to bird weight were observed (data not shown); therefore, only tibia weights are reported. Tibia sampling did not result in any observed differences in weight, strength, or ash percentage through 42 d of age (Table 3). However, the authors hypothesized that an increased sample size could potentially result in significant differences. Therefore, the sample size was tripled for the

Table 2. Average BW and corrected periodic and cumulative FCR for male broilers through 49 d of age

	CaFo[1]				
Item	0.0% (control)	0.5%	1.0%	1.5%	SEM
BW, kg					
d 0	0.042	0.042	0.042	0.042	0.000
d 15	0.51[a]	0.51[a]	0.49[b]	0.51[a]	0.002
d 28	1.48[ab]	1.50[a]	1.46[b]	1.45[b]	0.006
d 42	2.63	2.62	2.61	2.57	0.016
d 49	3.31	3.30	3.29	3.25	0.019
FCR by phase					
Starter	1.31[b]	1.31[b]	1.35[a]	1.32[b]	0.006
Grower	1.65	1.65	1.66	1.68	0.007
Finisher 1	2.26	2.29	2.23	2.25	0.022
Finisher 2	2.23	2.13	2.16	2.08	0.027
Cumulative FCR					
d 0 to 28	1.54[ab]	1.53[b]	1.55[ab]	1.56[a]	0.004
d 0 to 42	1.84	1.84	1.84	1.85	0.006
d 0 to 49	1.91	1.90	1.90	1.89	0.006

[a,b]Means in columns with different superscripts differ significantly at $P \leq 0.05$.

[1]CaFo = calcium formate.

final sample day at the termination of the trial. The increase in sample size resulted in observed increases ($P \leq 0.05$) in tibia breaking strength in the 1.0% CaFo treatment on d 49 when compared with the control group and the 1.5% CaFo treatment (Table 3). Calcium formate has been demonstrated to be a superior calcium source for women when compared with calcium citrate and calcium carbonate [7]. Researchers determined that the calcium from CaFo was more bioavailable, resulting in increased levels of calcium in the bloodstream. This observation in humans may explain the increased bone strength we observed in broilers fed a 1.0% dietary CaFo diet.

Table 3. Tibia weight, tibia breaking strength, and ash percentage collected from male broilers from 15 through 49 d of age

Item	d 15	d 28	d 42	d 49
Tibia weight, g				
0.0% CaFo[1] (control)	3.05	8.60	15.21	18.58
0.5% CaFo	2.97	8.48	14.62	18.79
1.0% CaFo	2.85	8.14	15.74	19.08
1.5% CaFo	2.89	8.26	15.00	18.59
SEM	0.043	0.145	0.248	0.262
Tibia breaking strength, kg				
0.0% CaFo (control)	13.10	28.58	36.45	46.05[b]
0.5% CaFo	12.79	26.68	33.44	51.25[ab]
1.0% CaFo	12.36	29.28	41.61	53.64[a]
1.5% CaFo	12.34	28.95	34.19	46.89[b]
SEM	0.300	1.102	1.535	1.728
Ash, %				
0.0% CaFo (control)	49.52	48.71	44.75	42.19
0.5% CaFo	49.47	48.01	43.86	42.26
1.0% CaFo	50.07	48.92	45.38	42.52
1.5% CaFo	49.46	48.53	43.29	42.15
SEM	0.006	0.027	0.004	0.002

[a,b]Means in columns with different superscripts differ significantly at $P \leq 0.05$.

[1]CaFo = calcium formate.

Table 4. Duodenal villus height, width, and surface area calculations for intestinal samples collected at 15, 28, 42, and 49 d of age from male broiler chickens

Item	d 15	d 28	d 42	d 49
Duodenal villus height, mm				
0.0% CaFo[1] (control)	2.13	2.41	2.47	2.48[b]
0.5% CaFo	2.20	2.39	2.30	2.53[b]
1.0% CaFo	2.09	2.52	2.36	2.81[a]
1.5% CaFo	2.13	2.42	2.46	2.56[b]
SEM	0.414	0.098	0.056	0.044
Duodenal villus width, mm				
0.0% CaFo (control)	0.24	0.26	0.27	0.29
0.5% CaFo	0.25	0.26	0.28	0.30
1.0% CaFo	0.24	0.27	0.30	0.30
1.5% CaFo	0.25	0.27	0.28	0.28
SEM	0.006	0.006	0.009	0.008
Duodenal villus surface area, mm^2				
0.0% CaFo (control)	1.12	1.31	1.41	1.56
0.5% CaFo	1.18	1.35	1.37	1.64
1.0% CaFo	1.09	1.44	1.49	1.83
1.5% CaFo	1.13	1.39	1.45	1.54
SEM	0.040	0.060	0.054	0.063

[a,b]Means in columns with different superscripts differ significantly at $P \leq 0.05$.

[1]CaFo = calcium formate.

Gut Morphology

Comparisons among treatment groups with regard to duodenal villus width and surface area yielded no significant differences (Table 4). Villus height was increased ($P \leq 0.05$) on d 49 in the 1.0% CaFo group when compared with villus heights in all other treatments (Table 4). Although the mechanism for the increase in villus height was not determined in this study, it is known that organic acids do affect the gut microflora, which has a direct effect on the gut mucosa [12, 29]. Calcium formate is known to control diarrhea in weaning pigs, possibly by preventing bacterial translocation and the resulting colonization by pathogens [13, 29]. Organic acids are also known to affect gut pH, gastrin production, acid secretion, epithelial cell proliferation, and nutrient absorption, and they are bacteriostatic, which could have a possible effect on gut morphology as well [12, 17, 29–31].

CONCLUSIONS AND APPLICATIONS

1. Dietary inclusion of 1.0% CaFo increased bone strength and duodenal villus height at the termination of grow out when compared with broilers receiving calcium carbonate exclusively as a calcium source.
2. Calcium formate can be used effectively as a calcium supplement in broiler diets.
3. The authors hypothesize that calcium formate may have a greater effect in layers, broiler breeders, and turkeys because of their longer rearing periods and increased calcium requirements.
4. Future studies are needed to evaluate calcium formate further as a gut acidifier in poultry.

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