An Investigation of Calcium Citrate-Malate as a Calcium Source for Young Broiler Chicks[1]

M. H. Henry[2] and G. M. Pesti[3]

Department of Poultry Science, The University of Georgia, Athens, Georgia 30602-2772

ABSTRACT Two experiments were conducted to compare a sample of calcium citrate-malate (CC-M) with a sample of commercial-grade limestone in starting broiler chick diets. In the first experiment, with 0.7 or 0.9% calcium from limestone or CC-M, no differences in bone development (dry fat-free tibia, tibia weight, tibia ash, or tibia calcium) were observed due to calcium source. However, chicks fed the diets based on CC-M had better 0- to 18-d body weight gains and feed conversion ratios than those fed limestone. In the second experiment with 0.50, 0.55, 0.60, 0.65, or 0.70% calcium from limestone or CC-M, chicks again had better body weight gains when fed CC-M compared to those fed limestone. Chicks fed diets based on CC-M and NaP_2PO_4 had very similar bone development and tibial dyschondroplasia pathology to those fed limestone and $Na_2H_2PO_4$. However, a control group of chicks fed 0.70% calcium from limestone and dicalcium phosphate did not grow as well as the others and had lower weights of tibia and tibial bone ash, calcium, and phosphorus compared to the others. It is concluded that CC-M is a good calcium source, comparable in bioavailability to limestone. Although CC-M may improve broiler growth, its action is not through increased bioavailability of calcium.

(*Key words*: calcium citrate-malate, broiler, limestone)

2002 Poultry Science 81:1149–1155

INTRODUCTION

Recent reports have shown that calcium citrate-malate (CC-M), a compound used to fortify food with calcium for human consumption, is 10 times more soluble than calcium citrate, which is more soluble than calcium carbonate (Andon et al., 1996; Heaney et al., 1990; Smith et al., 1987). Results from studies with humans also demonstrate that calcium absorption and retention from CC-M-fortified food is significantly greater than food fortified with calcium carbonate (Andon et al., 1996; Miller et al., 1988). In animal studies, trabecular bone was significantly affected by calcium source and level (Kochanowski, 1990). Rats fed CC-M had 23 to 25% more trabecular bone than those rats fed calcium carbonate at 4 wk, and by 12 wk the difference increased to 44%. Based on these results, it is concluded that calcium in CC-M is more bioavailable than calcium from calcium carbonate. Because of these differences in mammalian species, it is of interest to evaluate CC-M as a source of calcium in poultry diets, especially in early chick development.

In growing chicks, tibial dyschondroplasia (TD) is a major skeletal problem and is associated with calcium and phosphorus deficiency and imbalance. The TD lesion is characterized by a mass of white, opaque, unmineralized, unvascularized cartilage and is found predominantly in the proximal metaphysis of the tibiotarsus. The lesion is a result of the failure of the prehypertropic cartilage cells to undergo normal maturation and vascularization (Riddell, 1975; Poulos et al., 1978; McCaskey et al., 1982). Birds afflicted with severe cases of TD generally have bowed legs. They sit on their hocks and are reluctant to move. Birds with TD spend a significant portion of their time on their breasts, resulting in a higher incidence of breast blisters. In most instances, only a very small percentage of the birds affected by TD show clinical symptoms. Both clinical and subclinical cases can result in economic loss due to trimming and down-grading of carcasses (Burton et al., 1981).

Studies have shown that the expression of TD was influenced by genetics (Leach and Nesheim, 1965; Riddell, 1976; Sheridan et al., 1978). These researchers were able to produce, by genetic selection, lines of chickens with high (80%) and low (2%) incidence of TD. These

Received for publication September 14, 2001.

Accepted for publication February 6, 2002.

[1]Supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations of The University of Georgia and the U.S. Poultry and Egg Association.

[2]Present address: U.S. Food and Drug Administration, Center for Veterinary Medicine, HFV-222, 7500 Standish Place, Rockville, MD 20855.

[3]To whom correspondence should be addressed: gpesti@arches.uga.edu.

Abbreviation Key: CC-M = calcium citrate-malate; TD = tibial dyschondroplasia.

lines were used to study the relationship between calcium and phosphorus and this disease. It was also shown that male chicks were more susceptible to the development of TD (Riddell, 1976; Edwards, 1984). In a study by Leach and Nesheim (1965), it was concluded that calcium and phosphorus had no influence on the etiology of TD. However, Edwards and Veltmann (1983) reported that TD could be induced by diets with a low calcium:phoshorus ratio regardless of the calcium levels. Chicks fed diets of 1.1% calcium and total phosphorus of 0.53% did not exhibit TD, but a 37% incidence was found in birds fed 0.70% calcium and 1.01% phosphorus. These results were later confirmed in studies by several researchers (Lilburn et al., 1983; Edwards, 1984; Hulan et al., 1985; Kling, 1985; Riddell and Pass, 1987; Lilburn et al., 1989) and clearly demonstrate that calcium is important in the prevention of TD. However, the availability of dietary calcium to chicks for proper bone development and prevention of TD are affected by several factors including age, strain, calcium source, 1, 25-dihydroxycholecalciferol level in the gut, and ultraviolet irradiation (Edwards 1992; Cook et al., 1994; Leach and Twal, 1994; Lilburn, 1994).

The objectives of the experiments reported here were to compare a sample of commercial grade limestone with CC-M for growth, feed utilization, bone composition, and the development of TD in young broiler chicks. The diets in Experiment 1 were formulated with corn and soybean meal. In Experiment 2, 10% poultry by-product meal was included in the diets so that higher proportions of total calcium would come from CC-M or limestone. The control diets in Experiment 2 were based only on corn and soybean meal (no poultry by-product meal) as in Experiment 1.

MATERIALS AND METHODS

General Procedures

The CC-M[4] contained 21.5% calcium. In both experiments, eggs from two strains of broiler breeders were obtained from a commercial breeder farm and incubated locally. At hatch, chicks were vent sexed, tagged, and placed in electrically heated Petersime wire-floored battery brooders with eight birds per replicate cage. The chicks were raised with continuous fluorescent lighting (24 h/d). Feed and water were provided ad libitum throughout the 18-d experiment.

Body weights and residual feed were measured at 18 d, and feed conversion ratio was calculated. On Day 18, blood samples from three randomly chosen chicks per pen were drawn via cardiac puncture and centrifuged, and plasma was removed and stored at –20 C for calcium and phosphorus analysis. All chicks were then killed by asphyxiation with carbon dioxide and inspected for the presence and severity of TD (Edwards and Veltmann, 1983). The left tibia was removed and stored at –20 C for the determination of tibia fat-free dry weight, tibia ash, calcium, and phosphorus analyses. Tibia fat-free dry weight and tibia ash were determined by the AOAC method (1990). Calcium and Phosphorus of both plasma and bone ash were measured by flame atomic absorption using a Perkin Elmer 5000 atomic absorption spectroscope.[5]

Experiment 1

The female chicks of two strains were used in this experiment. The eggs for one strain were from Arbor Acres "High Yield" males mated to Arbor Acres "High Yield" females and eggs for the other strain were from Peterson × "Classic" Arbor Acres cross. Five pens of each strain were randomly assigned to each of four dietary treatments. CC-M or limestone[6] was added to a corn and soybean meal basal diet formulated to provide 0.7% calcium (Table 1). To obtain dietary calcium of 0.90%, additional CC-M and limestone[3] were added to their basal diets, respectively. Dicalcium phosphate[7] was the supplemental phosphorus source and it provided 34.5% of the dietary calcium in the basal diets.

Experiment 2

In Experiment 2, one strain of birds was from Arbor Acres "High Yield" males mated to Arbor Acres "High Yield" females and the other strain was from a "High Yield" Arbor Acres male × "Classic" Arbor Acres female cross. Male chicks were used to evaluate the diets in this experiment. Corn and soybean meal rations, similar to those of the first experiment were formulated and fed to chicks for 18 d. In this experiment, the two control diets had calcium levels of 0.70% provided by CC-M or limestone with dicalcium phosphate[8] as the phosphorus source. The amount of dietary calcium provided by CC-M and limestone in the experimental diets was increased by using sodium diphosphate as the phosphate source. CC-M or limestone was used to formulate experimental diets with calcium levels at 0.50, 0.55, 0.60, 0.65, and 0.70% of diet (Table 2). This design resulted in 12 dietary treatments: two control diets and 10 experimental diets. Each diet was assigned to four pens, two of each strain cross, creating a total of 48 pens.

Statistical Analyses

The experimental unit was the pen mean. Bioavailability of the calcium from the calcium sources in Experiment 2 was calculated by using the slope-ratio methodology of Finney (1978). All data were analyzed within experiments by two-way analysis of variance with the general linear models procedure of SAS software (SAS, 1985). When appropriate, mean differences were sepa-

[4]Jost Chemicals, St. Louis, MO.
[5]Perkin Elmer Corp., Norwalk, CT.
[6]Franklin Industrial Minerals, Nashville, TN.
[7](Dynafos) IMC-AGRICO Feed Ingredients, Bannockburn, IL.
[8]Product No. 3820, IT Baker, Phillipsburg, NJ.

TABLE 1. Composition of the basal diets (Experiment 1)

Ingredients and composition	Basal limestone diet	Basal calcium citrate-malate diet
Ground yellow corn (%)	53.241	52.265
Soybean meal (dehulled) (%)	37.537	37.713
Poultry fat (%)	6.019	6.366
Iodized NaCl (%)	0.400	0.400
Dicalcium phosphate (feed grade) (%)	1.640	1.643
Limestone (%)	0.595	. . .
Calcium citrate-malate	. . .	1.047
Vitamin mixture[1] (%)	0.250	0.250
DL-Methionine (%)	0.193	0.191
Mineral mixture[2] (%)	0.075	0.075
Bacitracin (%)	0.050	0.050
Calculated composition[3]		
Protein (%)	23.0	23.0
Calcium (%)	0.70	0.70
Available phosphorus (%)	0.45	0.45
Chlorine (%)	0.28	0.28
Metabolizable energy kcal/g	3.20	3.20
Chemical analysis		
Protein[4]	22.7	22.8
Calcium[5]	0.73	0.72

[1]Vitamin premix provided per kilogram of diet: vitamin A (as retinyl acetate), 9,920 IU; cholecalciferol, 3,300 IU; vitamin E (as dl-α-tocopheryl acetate), 19.8 IU; menadione, 1.8 mg; vitamin B_{12}, 16.5 μg, thiamin, 1.65 mg; riboflavin, 9.9 mg; niacin, 58 mg; pantothenic acid, 16.5 mg; folic acid, 1.06 mg; pyroxidine, 2.88 mg; biotin, 0.08 mg.

[2]Mineral premix provided per kilogram of diet: Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; I, 2.1 mg; Se, 0.1 mg and contained calcium carbonate, ferrous sulfate, magnesium oxide, manganese sulfate, zinc sulfate, cupric sulfate pentahydrate, calcium iodate, and sodium selenite.

[3]Based on NRC (1994) feed composition tables.

[4]Etheridge et al. (1998).

[5]Hill (1955).

rated by Duncan's new multiple-range test. Unless otherwise stated, statements of significance are based on $P < 0.05$. In both experiments, there were no significant differences between the broiler strains in response to dietary treatments, thus the data were pooled by strain.

RESULTS AND DISCUSSION

Broilers fed CC-M grew better than those fed limestone in both experiments (Tables 3 and 4). Feed intake was not affected in either experiment. Feed conversion ratio differences were detected (at $P \leq 0.05$) only in Experiment 1 and then only at the highest calcium level (which was not repeated in Experiment 2).

Two interesting interactions were observed in Experiment 1 (Table 5). Birds fed CC-M compared to limestone had more plasma calcium when fed 0.9% calcium but less when fed 0.7% calcium. Conversely, tibia weight was higher in birds fed CC-M at 0.7% calcium but low when fed CC-M at 0.9% calcium. The tibia weight results were not consistent in Experiment 2 when lower calcium levels were fed and no significant interaction was detected (Table 6; $P = 0.73$).

Bone ash was the most sensitive indicator of calcium level in Experiment 2, with a significant effect of Ca level on bone ash (Table 6; $P \leq 0.047$). Significant differences in tibia weight were mainly due to the small tibia from the chicks fed the limestone and dicalcium phosphate control diet. The diets based on limestone were expected to result in some TD incidence and severity (average score and scores of 3), especially at the lower calcium levels. There was a trend toward TD incidence and severity reductions by increasing dietary calcium from limestone but not from CC-M. The overall result was no significant main effects or interactions.

When the slope-ratio analysis of Finney (1978) was applied to the data, there were no significant slope differences found (Table 7). Therefore, the bioavailabilities of the samples of CC-M and limestone tested here can be assumed to be the same.

These results demonstrate that CC-M is a good source of calcium for broiler chicks. The reasons for the improvement in growth rate of CC-M fed chicks are not clear. The citrate-malate conjugate is two carbon chain moieties that are substrates in the citric acid cycle of the energy production system. The citrate-malate conjugate may be absorbed and used as an energy source to promote broiler growth. We did not include an energy value for the CC-M in balancing the diets. The increases in growth due to CC-M seem large for a small difference in the energy level of the diets due to calcium source but may be related to some specific action of the citrate-malate conjugate on nutrient absorption or energy metabolism.

In Experiment 2, diets formulated with CC-M as the calcium supplement and dicalcium phosphate as the

TABLE 2. Composition of the basal diets (Experiment 2)

	Limestone		Calcium citrate-malate	
Ingredients and composition	Control	Basal	Control	Basal
Ground yellow corn (%)	53.173	61.522	52.185	60.974
Soybean meal (dehulled) (%)	37.576	23.068	37.752	13.172
Poultry by-product meal	. . .	10.000	. . .	10.000
Poultry fat (%)	6.096	3.177	6.443	3.371
Iodized NaCl (%)	0.400	0.240	0.400	0.240
Dicalcium phosphate (feed grade)	1.641	. . .	1.644	. . .
Sodium phosphate monobasic	. . .	0.748	. . .	0.749
Limestone (%)	0.594	0.329	. . .	. . .
Calcium citrate-malate	. . .	. . .	1.055	0.581
Vitamin premix[1]	0.250	0.250	0.250	0.250
Mineral premix[2]	0.075	0.075	0.075	0.075
DL-Methionine (%)	0.190	0.244	0.191	0.245
L-Lysine (%)	. . .	0.274	. . .	0.271
L-Threonine (%)	. . .	0.068	. . .	0.067
Calculated analysis[3]				
Protein (%)	23.00	23.0	23.00	23.0
Calcium (%)	0.70	0.50	0.70	0.50
Available phosphorus (%)	0.45	0.45	0.45	0.45
Chlorine (%)	0.28	0.20	0.28	0.20
Metabolizable energy kcal/g	3.20	3.20	3.20	3.20
Chemical analysis[4]				
Protein[4]	22.9	23.1	23.2	22.7
Calcium[5]	0.74	0.53	0.72	0.52

[1]Vitamin premix provided per kilogram of diet: vitamin A (as retinyl acetate), 9,920 IU; cholecalciferol, 3,300 IU; vitamin E (as dl-α-tocopheryl acetate), 19.8 IU; menadione, 1.8 mg; vitamin B_{12}, 16.5 μg, thiamin, 1.65 mg; riboflavin, 9.9 mg; niacin, 58 mg; pantothenic acid, 16.5 mg; folic acid, 1.06 mg; pyroxidine, 2.88 mg; biotin, 0.08 mg.

[2]Mineral premix provided per kilogram of diet: Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; I, 2.1 mg; Se, 0.1 mg and contained calcium carbonate, ferrous sulfate, magnesium oxide, manganese sulfate, zinc sulfate, cupric sulfate pentahydrate, calcium iodate, and sodium selenite.

[3]Based on NRC (1994) feed composition tables.

[4]Etheridge et al. (1998).

[5]Hill (1955).

available phosphorus source resulted in heavier weights of the dry fat-free tibia, tibia ash, and tibia calcium than similar diets with limestone. The incidence of TD in male chicks was also lower for CC-M—dicalcium phosphate diets than that of limestone-dicalcium phosphate diet (Table 6). These differences seem to be due more to the poor performance of the limestone-dicalcium phosphate-fed chicks than any special response from the CC-M-fed chicks. Chicks fed similar diets in Experiment 1 had better performance, as did those fed the calcium sources with sodium phosphate in Experiment 2. The ability of calcium intake to promote healthy skeleton involves the consideration of bioavailability of calcium from dietary supplemental source (Buchnowski and Miller, 1991; Weaver, 1992) as well as the capacity to absorb the calcium consumed (Heaney, 1988). It has been shown by several reports that calcium from calcium citrate and CC-M are more bioavailable than the com-

TABLE 3. Effect of calcium source and level on growth, feed intake, and feed conversion of young broiler chicks at 18 d (Experiment 1)[1]

Source	Calcium level (%)	Body weight gain (g)	Feed intake (g)	FCR[2] (g/g)
Limestone	0.7	545 ± 11	784 ± 21	1.438 ± 0.020
	0.9	553 ± 10	802 ± 20	1.450 ± 0.030
CC-M[3]	0.7	579 ± 9	815 ± 12	1.407 ± 0.010
	0.9	571 ± 10	790 ± 12	1.383 ± 0.012
		Probability		
ANOVA				
Source		0.014	0.567	0.044
Level		0.999	0.830	0.812
Source × level		0.427	0.198	0.301

[1]Means and standard errors are based on 10 pens of eight chicks per treatment.

[2]FCR = feed conversion ratio (feed intake/body weight gain).

[3]CC-M = calcium citrate-malate.

TABLE 4. The effects of calcium source and level on growth, feed intake, and feed conversion of young broiler chicks (Experiment 2)[1]

Treatment and calcium source	Phosphorus source	n	Level (%)	Body weight gain (g)	Feed intake (g)	FCR[2] (g/g)
Limestone	NaH_2PO_4	4	0.50	550 ± 20^{abc}	723 ± 19^{ab}	1.316 ± 0.023
		4	0.55	565 ± 14^{ab}	735 ± 20^{ab}	1.301 ± 0.009
		4	0.60	549 ± 9^{abc}	699 ± 25^{abc}	1.272 ± 0.038
		4	0.65	559 ± 15^{abc}	725 ± 43^{ab}	1.295 ± 0.049
		4	0.70	536 ± 22^{bc}	676 ± 33^{abc}	1.267 ± 0.011
CC-M[3]	NaH_2PO_4	4	0.50	578 ± 4^{ab}	754 ± 14^{a}	1.304 ± 0.029
		4	0.55	575 ± 22^{ab}	730 ± 23^{ab}	1.272 ± 0.038
		4	0.60	588 ± 5^{a}	738 ± 21^{ab}	1.255 ± 0.042
		4	0.65	568 ± 18^{ab}	742 ± 21^{ab}	1.312 ± 0.065
		4	0.70	556 ± 9^{ab}	701 ± 17^{abc}	1.261 ± 0.025
Limestone	Dicalcium	4	0.70	512 ± 10^{c}	624 ± 6^{c}	1.222 ± 0.021
CC-M	Dicalcium	4	0.70	556 ± 9^{abc}	662 ± 34^{bc}	1.191 ± 0.060
Main effect means						
Limestone	NaH_2PO_4	20		552 ± 7	711 ± 13	1.289 ± 0.013
CC-M	NaH_2PO_4	20		573 ± 6	733 ± 9	1.280 ± 0.018
			df	Probability		
ANOVA						
Source			3	0.022	0.106	0.443
Limestone vs. CC-M			1	0.026	0.185	0.416
Level			4	0.484	0.304	0.646
Source × level			4	0.823	0.918	0.983

[a–c]Values within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Means and standard errors are based on four pens of eight chicks each per treatment.

[2]FCR = feed conversion ratio (feed intake/body weight).

[3]CC-M = calcium citrate-malate.

[4]Dicalcium = dicalcium phosphate.

monly used standard of calcium carbonate or limestone for other species (Miller et al., 1988; Andon et al., 1996). Koshanowski (1990) also showed that CC-M improved skeletal development in young rats.

Surprisingly, the data of our study does not show that CC-M in chicks was significantly more bioavailable for tibia ash and tibia calcium despite the increase in body weight gain. There were no significant slope differences (Table 7). Solubility data of calcium citrate (a precursor for CC-M) in other studies showed that it is 10 times more soluble than calcium carbonate (Smith et al., 1987; Heaney et al., 1990; Andon et al., 1996). Studies with humans also showed that calcium absorption and retention from CC-M fortified food was significantly greater than food fortified with calcium carbonate (Miller et al., 1988; Andon et al., 1996). The results of Experiments 1 and 2 demonstrate that the chick's capacity to absorb calcium was not maximized at 0.5 to 0.6% calcium. The lack of a significant slope difference (Table 7) suggests that the bioavailabilities of these particular samples of limestone and CC-M were very similar.

The differences in the incidence of TD, tibia ash, and tibia calcium of the two experiments may be attributed to the sex of the chicks used. It has been shown that male chicks were more susceptible to the development of TD (Riddell, 1976; Edwards, 1984). The results of this

TABLE 5. Effects of calcium source and level on plasma calcium, dry fat-free tibia weight, tibia ash, and total tibia calcium of two broiler strains (Experiment 1)

	Calcium level	Plasma calcium[1] (mg)	Dry fat-free tibia[2] weight	Tibia ash[2]	Tibia calcium[1]
Limestone	0.7%	5.99 ± 0.18	1.756 ± 0.050	40.09 ± 0.31	254.5 ± 8.6
	0.9%	5.91 ± 0.17	1.871 ± 0.032	40.39 ± 0.18	274.7 ± 5.1
CC-M[3]	0.7%	5.56 ± 0.17	1.909 ± 0.050	39.73 ± 0.60	273.7 ± 10.4
	0.9%	6.14 ± 0.16	1.829 ± 0.046	40.16 ± 0.22	267.5 ± 8.7
		Probability			
ANOVA					
Source		0.640	0.227	0.418	0.480
Level		0.115	0.693	0.320	0.415
Source × level		0.041	0.037	0.853	0.125

[1]Means and standard errors are based on 10 pens of three chicks each per treatment.

[2]Means and standard errors are based on 10 pens of eight chicks each per treatment.

[3]CC-M = calcium citrate-malate.

TABLE 6. Effects of calcium source and level on dry fat-free tibia weight, tibial bone ash, tibia calcium, tibia phosphorus, and the development of tibial dyschondroplasia in young broiler chicks (Experiment 2)[1]

Treatment							Tibial dyschondroplasia		
Calcium source	Phosphorus source	Level (%)	Tibia weight (g)	Tibial bone ash (%)	Tibia calcium (mg)	Tibia phosphorus (mg)	Score (0–3)	Incidence (%)	Number 3 score[2] (%)
Limestone	NaH_2PO_4	0.50	1.781^{ab}	39.1^{ab}	200.1^{ab}	76.4^{bc}	2.29	43.7	31.7
		0.55	1.812^{ab}	40.4^{a}	207.5^{ab}	77.2^{abc}	2.52	31.7	19.2
		0.60	1.780^{ab}	40.5^{a}	210.4^{ab}	76.8^{bc}	3.00	20.8	20.8
		0.65	1.882^{ab}	40.6^{a}	218.7^{ab}	79.6^{ab}	2.94	22.9	19.8
		0.70	1.810^{ab}	40.6^{a}	212.1^{ab}	75.6^{bc}	2.67	17.6	14.4
CC-M[3]	NaH_2PO_4	0.50	1.829^{ab}	37.9^{b}	199.1^{ab}	85.2^{a}	2.04	35.3	18.8
		0.55	1.911^{a}	39.5^{ab}	212.9^{ab}	85.2^{a}	2.40	22.3	12.5
		0.60	1.890^{ab}	40.6^{a}	220.7^{a}	81.1^{ab}	2.23	33.3	16.2
		0.65	1.842^{ab}	40.5^{a}	210.5^{ab}	78.7^{ab}	2.17	33.8	16.1
		0.70	1.838^{ab}	40.3^{a}	210.1^{ab}	78.9^{ab}	2.66	44.9	29.4
Limestone	Dicalcium[4]	0.70	1.489^{c}	37.6^{b}	159.9^{c}	69.7^{c}	2.50	34.8	25.5
CC-M	Dicalcium[4]	0.70	1.705^{b}	39.6^{ab}	192.8^{b}	74.2^{bc}	2.48	27.8	14.0
SEM			0.111	1.52	15.6	5.1	0.47	19.1	18.5
		df	Probability						
ANOVA									
Source		3	0.0002	0.023	0.0001	0.007	0.112	0.677	0.798
Level		4	0.810	0.047	0.272	0.543	0.289	0.659	0.864
Source × level		4	0.738	0.916	0.795	0.329	0.394	0.258	0.635

[a–c]Values within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Means and standard error are based on four pens of eight chicks each per treatment.

[2]Percentage of birds scored as 3 (large mass of cartilage in the proximal end of the tibiotarsus).

[3]CC-M = calcium citrate-malate.

[4]Dicalcium = dicalcium phosphate.

TABLE 7. Bioavailability of calcium citrate-malate compared to that of limestone using slope ratio assay (Experiment 2)

Source of variation	df	Body weight gain	Feed conversion ratio	Tibia weight	Tibia ash	Tibia calcium
		Probability				
Average slope	1	0.252	0.108	0.284	0.012	0.005
Slope difference	1	0.110	0.229	0.884	0.590	0.218
Blank	1	0.279	0.599	0.236	0.236	0.783
Intersection	1	0.716	0.942	0.425	0.778	0.944
Curvature	5	0.824	0.915	0.517	0.912	0.940
Parameter						
Intercept		569.0	1.306	1.819	38.7	199.9
Limestone slope		–135.0	–0.154	0.204	12.19	121.6
Calcium citrate-malate slope		–4.31	–0.458	0.253	17.59	218.4

study also show that male chicks fed marginal levels of calcium will have a significantly higher incidence and severity of TD.

In conclusion, by using all the criteria measured here, it was demonstrated that CC-M is a good source of calcium for young growing chicks. Furthermore, it has been shown that growth rate and feed efficiency are greater for young chicks fed CC-M as a calcium supplement. The specific reason for this increase in growth needs to be investigated. Therefore, the only limiting factor on the use of calcium citrate-malate in young broiler chick diets is the price, which is significantly higher than limestone. Longer-term studies could show if the increases in body weight are worth the higher cost of calcium citrate-malate.

REFERENCES

Andon, M. A., M. Peacock, R. L. Kanerva, and J. A. S. De Castro. 1996. Calcium absorption from apple and orange juice fortified with calcium citrate-malate (CCM). J. Am. Col. Nutr. 15:313–316.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Buchnowski, M. S., and D. D. Miller. 1991. Lactose, calcium source and age affect calcium bioavailability in rats. J. Nutr. 121:1746–1754.

Burton, R. W., A. K. Sheridan, and C. R. Howlett. 1981. The incidence and importance of tibial dyschondroplasia to commercial broiler industry in Australia. Br. Poult. Sci. 222:153–160.

Cook, M. E., Y. Bai, and M. W. Orth. 1994. Factors influencing growth plate cartilage turnover. Poult. Sci. 73:889–896.

Edwards, H. M., Jr., and J. R. Veltmann. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chickens. J. Nutr. 113:1568–1575.
Edwards, H. M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001–1013.
Edwards, H. M., Jr. 1992. Nutritional factors and leg disorders. Pages 167–193 in Bone Biology and Skeletal Disorders in Poultry. C. C. Whitehead, ed. Carfax Publishing Co., Abingdon, UK.
Etheridge, R. D., G. M. Pesti, and E. H. Foster. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. Anim. Feed Sci. Technol. 73:21–28.
Finney, D. J. 1978. Statistical Methods in Biological Assay. 3rd ed. Charles Griffin and Co. Ltd., High Wycombe, Buckinghamshire, UK.
Heaney, R. P. 1988. Nutritional factors in bone health. Pages 297–312 in Osteoporosis: Etiology, Diagnosis, and Management. B. L. Riggs and L. J. Melton, ed. Raven Press, New York.
Heaney, R. P., R. R. Recker, and C. M. Weaver. 1990. Absorbability of calcium sources: The limited role of solubility. Calcif. Tissue Int. 46:300–304.
Hill, J. B. 1955. Automated fluorometric method for determination of serum calcium. Clin. Chem. 2:122–130.
Hulan, H. W., G. Degroote, G. Fontaine, and G. Demunter. 1985. The effect of different totals and ratios of calcium and phosphorus on the performance and incidence of leg abnormalities of male and female broiler chickens. Poult. Sci. 64:1157–1169.
Kling, L. T. 1985. The effects of calcium and phosphorus on the incidence of tibial dyschondroplasia in young chicks. Nutr. Rep. Int. 32:1473–1480.
Kochanowski, B. A. 1990. Effect of calcium citrate-malate on skeletal development in young, growing rats. J. Nutr. 120:876–881.
Leach, R. M., and M. C. Nesheim. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236–244.
Leach, R. M., and W. O. Twal. 1994. Autocrine, paracrine, and hormonal signals involved in growth plate chondrocyte differentiation. Poult. Sci. 73:883–888.
Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897–903.
Lilburn, M. S., T. J. Lauterio, K. N. Rilling, and J. H. Smith. 1989. The relationships among mineral balance in the diet, early growth manipulation, and incidence of tibial dyschondroplasia in different strains of meat type chickens. Poult. Sci. 68:1263–1273.
Lilburn, M. S., K. Nigiani, and J. H. Smith. 1983. The effects of mineral and electrolyte balance on the incidence of leg abnormalities in different strain of commercial broilers. Poult. Sci. 62:1353. (Abstr.)
McCaskey, P. C., G. N. Rowland, R. K. Page, and L. R. Minear. 1982. Focal failure of endochondral ossification in the broiler. Avian Dis. 26:701–717.
Miller, J. Z., D. L. Smith, L. Flora, C. Slemenda, X. Jiang, and C. C. Johnson, Jr. 1988. Calcium absorption from calcium carbonate and a new form of calcium (CCM) in healthy male and female adolescents. Am. J. Clin. Nutr. 48:1291–1294.
Poulos, P. W., S. Reiland, K. Elwinger, and S. E. Olson. 1978. Skeletal lesions in the broiler, with special reference to dyschondroplasia (osteochondrosis). Acta. Radiol. Suppl. 358:229–275.
Riddell, C. 1975. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19:443–462.
Riddell, C. 1976. Selection of broiler chickens for a high and low incidence of tibial dyschondroplasia with observation on spondylolisthesis and twisted legs (perosis). Poult. Sci. 55:145–151.
Riddell, C., and D. A. Pass. 1987. The influence of dietary calcium and phosphorus on tibial dyschondroplasia in broiler chickens. Avian Dis. 31:771–775.
SAS Institute. 1985. SAS User's Guide: Stastistics. Version 5 Edition. SAS Institute Inc., Cary, NC.
Sheridan, A. K., C. R. Howlett, and R. W. Burton. 1978. The inheritance of tibial dyschondroplasia in broilers. Br. Poult. Sci. 19:491–499.
Smith, K. T., R. P. Heaney, L. Flora, and S. M. Hinders. 1987. Calcium absorption form a new calcium delivery system. Calcif. Tissue 41:351–352.
Weaver, C. M. 1992. Calcium bioavailability and its relation to osteoporosis. Proc. Soc. Exp. Biol. Med. 200:157–160.