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RESEARCH ARTICLE

AN INVESTIGATION OF CALCIUM CITRATE MALATE AS A CALCIUM SOURCE FOR YOUNG BROILER CHICKS : A REVIEW.

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Abstract

To evaluate calcium citrate malate as a good source of calcium when compared to commercial grade limestone, two experiments were conducted. In the first experiment, two different diets with calcium concentration 0.7% and 09% were formulated using calcium citrate malate and limestone, which had no difference in bone development (dry fat-free tibia, tibia weight, tibia ash, or tibia calcium), but chicks had better 0-18 days body weight gains and feed conversion ratios with calcium citrate malate diet than limestone diet. In the second experiment, five different diets with calcium concentration 0.50%, 0.55%, 0.60%, 0.65% and 0.70% were formulated using calcium citrate malate and limestone along with sodium diphosphate as a phosphorus source. Chicks fed with these diets had similar bone development and tibial dyschondroplasia pathology. Two control diets were formulated using limestone and calcium citrate malate with dicalcium phosphate as a phosphorus source. Limestone diet did not promote good growth of chicks and had lower weights of tibia, tibial bone ash, calcium, and phosphorus compared to calcium citrate malate diet. It was concluded that calcium citrate malate was a good calcium source than limestone.

Introduction:-

Many foods are being fortified with calcium to treat the deficiency or to maintain the calcium levels in the body. Calcium citrate malate is one of many calcium salts which is used to fortify food with calcium for human consumption. It has 21.5% elemental calcium levels and it is 10 times more soluble than calcium carbonate (Andon et al., 1996; Heaney et al., 1990; Smith et al., 1987). Clinical studies on humans have demonstrated that calcium citrate malate has notable absorption and calcium retention than calcium carbonate fortified foods (Andon et al., 1996; Miller et al., 1988). Calcium source and levels in the body has marked effect on trabecular bone in animal studies (Kochanowski, 1990). Rats which were fed on calcium citrate malate diet has 23% to 25% more trabecular bone than those rats which were fed calcium carbonate diet at 4 and 12 weeks and the difference increased to 44%. Based on these results, it is concluded that calcium citrate malate is more bioavailable than calcium carbonate.

Tibial dyschondroplasia (TD) is a major skeletal disorder which is associated with deficiency or imbalance of calcium and phosphorus. TD lesions are characterized by white, unmineralized, opaque, unvascularized cartilage and found predominantely in the metaphysis of tibiotarsus. The prehypertropic cartilage cells fail to undergo

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maturation and vascularization (Riddell, 1975; Poulos et al., 1978; McCaskey et al., 1982). As a result lesions are formed. Birds which are affected by TD have bowed legs, sit on their hocks, reluctant to move and spend a significant amount of their time on their breasts, resulting in higher incidence of breast blisters. In most cases, only a small percentage of affected chicks show clinical symptoms which can result in economic loss. Initially the researcher Leach and Nesheim, 1965; Sheridian et al, 1978 ; showed that expression of TD was influenced by genetics and were able to produce by genetic selection of lines of chicken with high (80%) and low (2%) incidence of TD. These lines of chicken were then used to study the effect of calcium and phosphorus on the disease. Riddle (1976) and Edward (1984) showed that male chicks were more susceptible to the development of TD. However, Edward and veltmann (1983) showed that TD can be induced by diets with low calcium:phosphorus ratio. Chicks which were fed with diets 1.1% calcium and total phosphorus 0.53% did not develop TD but 37% increase was seen in chicks fed with 0.70% calcium and 1.01% phosphorus. These results were later confirmed 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.

Materials And Methods:-

M. H. Henry and G. M. Pesti (2002) conducted two experiments to evaluate the effect of calcium citrate malate as a calcium source for young broiler chicks from the Department of Poultry Science, The University of Georgia, Athens, Georgia 30602-2772. Eggs from two different strains of chicken were obtained from a commercial breeder and incubated. At hatch, chicks were vent sexed, tagged and placed in electrically heated Petersime wire-floored battery brooders with eight birds per replicate cage. On 18^{th} day body weights and residual feed were measured and feed conversion ratio was calculated. Three random chicks were chosen per pen and blood samples were drawn via cardiac puncture, centrifuged and plasma was removed. It was stored at $-20^{o}C$ for calcium and phosphorus analysis. Calcium and phosphorus levels were measured by flame atomic absorption using Perkin Elmer 5000 atomic absorption spectroscope (Perkin Elmer Corp., Norwalk, CT). All chicks were euthanized by asphyxiation, and inspected for the presence and severity of TD (Edwards and Veltmann, 1983). The left tibia was removed and stored at $-20^{o}C$ for the determination of tibia fat-free dry weight, tibia ash, calcium, and phosphorus analysis. Tibia fat-free dry weight and tibia ash were determined by the AOAC method (1990).

In the experiment 1, female chicks were used which were obtained from two different strains of chicken. The eggs for one strain of chicken were obtained from Arbor Acres 'High Yield' males mated with Arbor Acres 'High Yield' females and eggs for second strain of chicken were obtained from Peterson × 'Classic' Arbor Acres cross. Five pens were randomly selected from each strain and assigned to each of four diets. Calcium citrate malate (Jost Chemical, St. Louis, MO) and limestone (Franklin Industrial Minerals, Nashville, TN) were added separately to corn and soybean basal diets formulated to obtain elemental calcium concentration of 0.7% and 0.9% each (table 1). Dicalcium phosphate (Dynafos; IMC-AGRICO Feed Ingredients, Bannockburn, IL.) was used as phosphorus source and it provides 34.5% elemental calcium in the basal diets. No poultry by product meal was used.

In the experiment 2, male chicks were used which were obtained from two different strains of chicken. The eggs for one strain of chicken were obtained from Arbor Acres 'High Yield' males mated with Arbor Acres 'High Yield' females and eggs for second strain of chicken were obtained from a 'High Yield' Arbor Acres male crossed with 'Classic' Arbor Acres female cross. Basal diets were formulated similarly to those of first experiment using corn and soybean meal, and fed to chicks for 18 days. Two control diets were formulated in this experiment having elemental calcium levels of 0.7% provided by calcium citrate malate and limestone with dicalcium phosphate (Product No. 3820, IT Baker, Phillipsburg, NJ) as phosphorus source. Experimental diets were formulated using calcium citrate malate and limestone separately to obtain elemental calcium levels of 0.50%, 0.55%, 0.60%, 0.65% and 0.70% each (table 4) with sodium diphosphate as phosphorus source. This resulted in twelve dietary treatment sets: two control and 10 experimental diets. Each diet was assigned to four pens, two of each strain, creating a total of 48 pens. 10% poultry by product meal was used in experimental diets so that higher proportion of total calcium would come from calcium citrate malate and limestone. Only corn and soybean meal was used in control diet.

Slope ratio methodology of Finney(1978) was used to calculate the bioavailability of calcium in experiment 2. Two-way analysis of variance was used to analyse the data obtained within the experiments, with general linear models procedure of SAS software (SAS, 1985). When appropriate, mean differences were separated by Duncan's new multiple range test. Unless otherwise stated, statements of significance are based on $P < 0.05$. No significant differences were observed between the strains in both the experiments in response to dietary treatments, thus the data was pooled by strains.

Results And Conclusion:-

In experiment 1, two interesting interactions are observed. Chicks have more plasma concentration when fed with calcium citrate malate diet than limestone diet at 0.9% calcium concentration. Tibial weight in chicks are higher when fed with calcium citrate malate diet at 0.7% calcium concentration but low with 0.9% calcium concentration.

In experiment 2, diets formulated with calcium citrate malate and dicalcium phosphate as calcium and phosphorus source resulted in heavier dry fat-free tibia, tibial calcium and tibial bone ash when compared to limestone with dicalcium phosphate diet. TD incidence is also lower in calcium citrate malate with dicalcium phosphate diet, when compared with limestone with dicalcium phosphate diet. The results obtained from five different calcium concentration of the experimental diet formulated with sodium diphosphate as phosphorus source are identical.
When slope-ratio analysis of Finney (1978) is applied to the data, there are no significant slope differences found (Table 7). Therefore, the bioavailabilities calcium citrate malate and limestone sample tested here is same.

In both the experiments, chicks grow better when fed with calcium citrate malate diet than limestone diet (table 2 and 5). Feed intake is not affected in both the experiment. Only in experiment 1, differences in feed conversion ratio is identified (at $P \leq 0.05$) and at highest calcium level, which is not repeated in experiment 2.

By referring the results obtained from the two experiments, it is concluded that calcium citrate malate is a good source of calcium for growing young chicks. Chicks fed with calcium citrate malate as a calcium supplement have better growth rate and feed efficiency. The price of calcium citrate malate is significantly higher than the limestone, which is the only limiting factor.

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 sodium chloride (%)	0.400	0.400
Dicalcium phosphate (%)	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 (%)	23.00	23.00
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 B12, 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).

Table 2:-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
CCM[3]	0.7	579 ± 10	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]CCM = calcium citrate-malate.

Table 3:-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)

Source	Calcium level (%)	Plasma calcium1[1] (mg)	Tibia calcium[1]	Dry fat free tibia weight[2]	Tibia ash[2]
Limestone	0.7	5.99 ± 0.18	254.5 ± 8.6	1.756 ± 0.050	40.09 ± 0.31
	0.9	5.91 ± 0.17	274.7 ± 5.1	1.871 ± 0.032	40.39 ± 0.18
CCM[3]	0.7	5.56 ± 0.17	273.7 ± 10.4	1.909 ± 0.050	39.73 ± 0.60
	0.9	6.14 ± 0.16	267.5 ± 8.7	1.829 ± 0.046	40.16 ± 0.22
	----------**Probability**----------				
ANOVA					
Source		0.640	0.480	0.227	0.418
Level		0.115	0.415	0.693	0.320
Source × Level		0.041	0.125	0.037	0.853

[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]CCM = calcium citrate-malate.

TABLE 4:-Composition of the basal diets (Experiment 2)

Ingredients and composition	Limestone		Calcium citrate malate	
	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 sodium chloride (%)	0.400	0.240	0.400	0.240
Dicalcium phosphate (%)	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	23.00	23.00	23.00
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 B12, 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).

Table 5:-The effects of calcium source and level on growth, feed intake, and feed conversion of young broiler chicks (Experiment 2)[1]

Treatement and calcium source	**Phosphorus source**	**n**	**Level (%)**	**Body weight gain (g)**	**Feed intake (g)**	**Feed conversion ratio (g/g)**
Limestone	**Sodium diphosphate**	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
CCM[2]	**Sodium diphosphate**	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 phosphate**	4	0.70	512 ± 10^{c}	624 ± 6^{c}	1.222 ± 0.021
Calcium citrate malate	**Dicalcium phosphate**	4	0.70	556 ± 9^{abc}	662 ± 34^{bc}	1.191 ± 0.060
Main effect mean						
Limestone	**Sodium diphosphate**	20		552 ± 7	711 ± 13	1.289 ± 0.013
Calcium	**Sodium**	20		573 ± 6	733 ± 9	1.280 ± 0.018

citrate malate	**diphosphate**					
			df	**----------probability----------**		
ANOVA						
Source			3	0.022	0.106	0.443
Limestone vs. CCM			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]CCM = 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 weight (g)**	**Tibial bone ash (%)**	**Tibia calcium (mg)**	**Tibia phosphorus (mg)**	**Tibial dyschondroplasia**		
Calcium source	**Phosphorus source**	**Level (%)**					**Score (0-3)**	**Incidence (%)**	**Number 3 score[2] (%)**
Limestone	**Sodium diphosphate**	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
Calcium citrate malate	**Sodium diphosphate**	0.50	1.892^{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 phosphate**	0.70	1.489^{c}	37.6^{b}	159.9^{c}	69.7^{c}	2.50	34.8	25.5
Calcium citrate malate	**Dicalcium phosphate**	0.70	1.705^{b}	39.^{6ab}	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 x 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).

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		569.00	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

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