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Efficacy of different limestone particle size and 25-hydroxycholecalciferol in broiler diets*

J. Koreleski[1] and S. Świątkiewicz

National Research Institute of Animal Production,
Department of Animal Nutrition and Feed Sciences
32-083 Balice, Poland

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ABSTRACT

In a 6-week experiment on 560 Cobb broilers, the response of chickens to supplementation of diets with particulate grit and 25-hydroxycholecalciferol (25-OH-D_3) was evaluated. Maize- and soyabean meal-based diets contained pulverized limestone or a mixture of 70% pulverized and 30% particulate (1.2-4 mm in diameter) limestone as a source of calcium. Both diets were supplemented with vitamin D_3 (cholecalciferol) or 25-OH-D_3 was substituted in amounts equivalent to 20, 40, 50, 60, 80, or 100% of cholecalciferol.

Incorporation of particulate forms of limestone into the diet positively affected performance and the ash and Ca contents in the tibia. Replacement of cholecalciferol by 25-OH-D_3 at a level of 50 and 60% had a beneficial effect on body weight gain and feed conversion, 100% substitution, on the Ca content of the tibia, whereas 50, 60, 80 and 100% substitution, on Ca and P balance.

KEY WORDS: broilers, cholecalciferol, 25-OH-D_3, limestone form, performance, tibia bones, calcium, phosphorus

INTRODUCTION

Adequate dietary levels of calcium, phosphorus and vitamin D are required for chicken growth and normal bone development. Utilization of dietary calcium carbonate by chickens may depend more on particle size than origin. Guinotte et al. (1991) found that in comparison with ground calcium carbonate (<0.15 mm), coarse particles (>1.18 mm) incorporated into the diet diminished weight gain

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[1] Corresponding author: e-mail: jerzy.koreleski@izoo.krakow.pl

and feed conversion, tibia characteristics and ash content, and decreased calcium retention. In contrast, in a study by Zohravi (2002) the incorporation of coarse particle limestone (1.18-4.75 mm) into the diet significantly improved the feed conversion ratio, tibia ossification and Ca and P retention.

Cholecalciferol is supplemented to practical diets for broiler chickens in large amounts and is transformed in tissues to active metabolites (Collins and Norman, 1991). The occurrence of signs of rickets and dyschondroplasia in chickens fed practical diets rich in vitamin D_3 may suggest that transformation of cholecalciferol in the liver and kidney to 25-OH-D_3 and 1,25-$(OH)_2$-D_3 is not sufficient. Both active metabolites added to the diet were found to be effective sources of vitamin D_3 (Edwards, 1990; Rennie and Whitehead, 1996). Yarger et al. (1995) reported better performance of birds fed 25-OH-D_3 in comparison with those fed vitamin D_3 at a dietary level of 2760 IU $\cdot$ kg^{-1}.

The present study was undertaken to evaluate the responses of broilers to diets containing pulverized limestone or mixed particulate grit and a pulverized form of limestone, and cholecalciferol alone or gradually substituted by 25-OH-D_3.

MATERIAL AND METHODS

A 6-week experiment was carried out on 560 Cobb broiler chickens. Sexed day-old chickens were allotted to 14 groups in 5 replicates, each containing 4 males and 4 females, and kept in cages with wire mesh floors. The diets provided in the first (starter, 1-21 days) and second (grower-finisher, 22-42 days) feeding periods contained pulverized limestone or a mixture of 70% pulverized and 30% particulate (1.2-4 mm mesh) limestone as the calcium source. The starter and grower-finisher diets (Table 1) were supplemented with 2000 and 1500 IU of cholecalciferol $\cdot$ kg^{-1}, respectively (as Lutavit 500, BASF). The diets for groups I and VIII were supplemented only with vitamin D_3; in the diets for the other groups, the 25-OH-derivative replaced 20, 40, 50, 60, 80 or 100% of the cholecalciferol (Table 2). The source of 25-OH-D_3 (Hy·D Beadlet, DSM) contained 12.5 mg 25-OH-D_3 per g, which was equivalent to 500 000 IU of vitamin D_3.

Body weight and feed intake were measured, mortality was registered and body weight gain (BWG) and feed conversion ratio (FCR) were calculated for the first and second periods of feeding and for the whole experiment. On the basis of body weight gain, feed conversion and mortality, the performance efficiency index (EPE-index) was calculated. Between days 15 to 19 of life, feed intake was measured and total collection of excreta from each pen was carried out in groups I-VII fed pulverized limestone. Excreta were stored at -20°C. After thawing, the excreta were dried in an oven at 50°C, left on air for two days,

TABLE 1

Composition of diets, g·kg⁻¹

Item	Starter	Grower/Finisher
Component		
ground maize	533.6	582.5
soyabean meal	385.0	325.0
rapeseed oil	40.0	50.0
limestone	9.0	9.0
dicalcium phosphate	20.0	20.5
NaCl	3.0	3.0
DL-methionine	2.3	2.2
L-lysine HCl	2.1	2.8
vitamin-mineral premix[1]	5.0	5.0
Analysed		
crude protein	215	195
Lys	13.3	12.5
Met	5.6	5.3
Ca	9.0	9.0
total P	7.65	7.65
$MJ \cdot kg^{-1}$ [2]	12.25	12.7

[1] supplied to 1 kg of starter diet: vit. A 13 500 IU; mg: vit. E 45; K_3 3; B_1 3.25; B_2 7.5; B_6 5; B_{12} 0.0325; biotin 0.15; Ca-pantothenate 15; niacin 45; folic acid 1.5; choline-Cl 600; Mn 100; Zn 75; Fe 67.5; Cu 17.5; J 1; Se 0.275 and Co 0.4; to 1 kg of grower diet: vit. A 12 000 IU; mg: vit. E 40; K_3 2.25; B_1 2; B_2 7.25; B_6 4.25; B_{12} 0.03; biotin 0.1; Ca-pantothenate 12; niacin 40; folic acid 1.0; choline-Cl 450; Mn 100; Zn 65; Fe 65; Cu 15; J 0.8; Se 0.25 and Co 0.4; to 1 kg of finisher diet: vit. A 10 500 IU; mg: vit. E 40 ; K_3 2; B_1 1.5; B_2 5.5; B_6 3.25; B_{12} 0.025; biotin 0.1; Ca-pantothenate 12; niacin 35; folic acid 1.0; choline-Cl 400; Mn 80; Zn 50; Fe 60; Cu 8; J 0.8; Se 0.2 and Co 0.4

[2] calculated according to European Table of Energy Values for Poultry Feedstuffs (1989) as a sum of ME content of diet components

weighed and homogenized. In the samples of diets and excreta, the Ca content was determined by flame atomic absorption spectrophotometry using a Philips PU 9100 apparatus and the P content was determined colorimetrically by the molybdo-vanadate method (AOAC, 1990). Lysine and methionine were analysed in the basal diet in acid hydrolysates in a colour reaction with the ninhydrin reagent, using a Beckman-System Gold 126 AA automatic analyser. Methionine was estimated after preoxidation to methionine sulphone.

On day 43 of age, 8 chickens (4 males and 4 females) from each group were slaughtered by decapitation, plucked, eviscerated and the carcasses were cooled. On the next day the carcass and gizzard were weighed, breast muscles (*M. pectoralis maior* and *minor*) from each carcass were excised and weighed. Eight tibia bones per replicate were collected, dried at 100°C for 24 h, weighed, and dry-ashed at 600°C for determination of crude ash and Ca.

TABLE 2

Scheme of the experiment

Group No.	Pulverized limestone %	Particulate limestone grit %	Starter		Grower/Finisher	
			cholecalciferol IU · kg^{-1}	25-OH-D_3 IU · kg^{-1}	cholecalciferol IU · kg^{-1}	25-OH-D_3 IU · kg^{-1}
I			2000	-	1500	-
II			1600	400	1200	300
III	100	-	1200	800	900	600
IV			1000	1000	750	750
V			800	1200	600	900
VI			400	1600	300	1200
VII			-	2000	-	1500
VIII			2000		1500	-
IX			1600	400	1200	300
X			1200	800	900	600
XI	70	30	1000	1000	750	750
XII			800	1200	600	900
XIII			400	1600	300	1200
XIV			-	2000	-	1500

The data were subjected to statistical analysis using two-way analysis of variance, except Ca and P balance data, which were calculated using one-way analysis of variance. The significance of differences between means was determined by Duncan's multiple range test using the Statistica 5.0 PL software package.

RESULTS

In the first period of feeding (1-21 days of age) the performance of chickens was not affected by the kind of calcium source and vitamin D_3 substitution (Table 3).

In the second period of feeding (Table 4), the replacement of pulverized limestone by particulate limestone had a positive effect on BWG ($P \leq 0.001$), feed intake ($P \leq 0.05$) and FCR ($P < 0.01$). Chickens fed the diet supplemented with cholecalciferol had the lowest weight gain and feed intake. Substitution of 25-OH-D_3 for cholecalciferol at a level of 50, 60, 80 and 100% significantly improved BWG and FCR.

For the whole period of feeding (Table 5) the improvement of BWG and FCR was confirmed when the diet contained 30% limestone in particulate form ($P \leq 0.001$). Replacement of cholecalciferol at a level of 50 and 60% also positively

TABLE 3

Performance in the first period of feeding (1-21 days of age)

% of 25-OH-D_3 in whole amount of added vitamin D_3	BWG, g			Feed intake, g			FCR, g feed · g BWG^{-1}		
	% of grit in whole amount of used calcium carbonate								
	0	30	mean	0	30	mean	0	30	mean
0	588	564	576	877	861	869	1.49	1.53	1.51
20	577	547	562	854	828	841	1.49	1.52	1.50
40	549	555	552	824	855	839	1.50	1.54	1.52
50	591	556	573	864	820	842	1.46	1.48	1.47
60	590	590	590	882	853	868	1.50	1.45	1.47
80	573	573	573	858	855	856	1.50	1.49	1.50
100	544	568	556	843	863	852	1.55	1.52	1.53
Mean	573	565		857	848		1.50	1.50	
SEM	4.01			3.95			0.0077		

all differences between means and interactions were not significant

TABLE 4

Performance in the second period of feeding (22-42 days of age)

% of 25-OH-D_3 in whole amount of added vitamin D_3	BWG, g			Feed intake, g			FCR, g feed · g BWG^{-1}		
	% of grit in whole amount of used calcium carbonate								
	0	30	mean	0	30	mean	0	30	mean
0	1603	1706	1655[a]	3032	3037	3035	1.89	1.78	1.84[c]
20	1627	1734	1681[ab]	3003	3191	3097	1.84	1.84	1.84[c]
40	1597	1763	1680[ab]	2947	3147	3047	1.84	1.79	1.81[bc]
50	1720	1793	1756[bc]	3110	3052	3081	1.81	1.70	1.76[a]
60	1720	1782	1751[bc]	3062	3139	3100	1.78	1.76	1.77[ab]
80	1689	1760	1725[bc]	3057	3141	3099	1.81	1.79	1.80[abc]
100	1734	1788	1761[c]	3048	3140	3094	1.76	1.76	1.76[a]
Mean	1670[x]	1761[y]		3037[x]	3121[y]		1.82[x]	1.77[y]	
SEM		11.3			20.3			0.00830	
Effect of:									
Ca source		***			*			**	
vitamin D_3		*			NS			**	
interaction		NS			NS			NS	

[x, y] - values in the rows with different letters differ significantly ($P \leq 0.05$)
[a,b,c] - values in the columns with different letters differ significantly ($P \leq 0.05$)
NS - $P>0.05$; * - $P \leq 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.001$

affected BWG and FCR. Improvement of the performance efficiency index was observed when 30% of the particulate calcium source was incorporated into the diet and 25-OH-D_3 substituted for 50, 60 and 100% of the cholecalciferol added to the diet. Experimental factors had no effect on the results of slaughter yield and on the percent of breast muscle and gizzard in the carcass (Table 6).

The crude ash content in tibia bones (Table 7) differed significantly between chickens fed different forms of limestone, but substitution of 25-OH-D_3 for vitamin D_3 made no difference. The bone ash content in chickens fed pulverized limestone was lower than in groups fed mixed forms of limestone ($P \leq 0.05$). The calcium content in tibia bones (Table 7) was significantly affected by both

TABLE 5

Performance in the whole period of feeding (1-42 days of age)

% of 25-OH-D_3 in whole amount of added vitamin D_3	BWG, g			Feed intake, g			FCR, g feed · g BWG^{-1}			Performance efficiency index		
	% of grit in whole amount of used calcium carbonate											
	0	30	mean	0	30	mean	0	30	mean	0	30	mean
0	2191	2270	2231[a]	3910	3898	3904	1.78	1.72	1.75[c]	296	319	308[a]
20	2204	2280	2242[ab]	3857	4019	3938	1.75	1.76	1.76[c]	304	313	309[a]
40	2146	2318	2232[a]	3770	4002	3886	1.76	1.73	1.74[bc]	295	324	309[a]
50	2311	2349	2329[bc]	3973	3871	3923	1.72	1.65	1.68[a]	324	344	334[b]
60	2310	2373	2341[c]	3944	3992	3968	1.71	1.68	1.69[a]	326	340	333[b]
80	2262	2334	2298[abc]	3914	3996	3955	1.73	1.71	1.72[abc]	316	329	322[ab]
100	2277	2356	2317[abc]	3890	4002	3947	1.71	1.70	1.70[ab]	322	334	328[b]
Mean	2243[x]	2326[y]		3895	3969		1.74[x]	1.71[y]		312[x]	328[y]	
SEM		12.9			21.3			0.00626			2.48	
Effect of:												
Ca source		***			NS			***			***	
vitamin D_3		*			NS			***			***	
interaction		NS			NS			NS			NS	

[x, y] - values in the rows with different letters differ significantly ($P \leq 0.05$)

[a,b,c] - values in the columns with different letters differ significantly ($P \leq 0.05$)

NS - $P > 0.05$; * - $P \leq 0.05$; *** - $P \leq 0.001$

treatment factors. In chickens fed pulverized limestone the Ca content in bones was lower than in groups fed mixed forms of limestone ($P \leq 0.001$). Complete replacement of cholecalciferol by 25-OH-D_3 significantly increased the Ca content as compared with groups fed only vitamin D_3 ($P \leq 0.05$). An interaction was observed between limestone source and D_3 substitution by 25-OH-D_3.

A balance trial was conducted only in groups I-VII of chickens fed pulverized limestone (Table 8) because the excreta from groups fed the particulate limestone contained grit and were not susceptible to homogenization. The daily calcium retention in chickens was lowest when 100% D_3 was added to the diet as cholecalciferol and increased ($P \leq 0.05$) when 25-OH-D_3 substituted for 60-100% of vitamin D_3. Daily amounts of Ca excreted in excrements, and relative Ca retention (in % of Ca ingested) did not differ statistically, however. Nevertheless a

TABLE 6

Results of slaughter analysis

% of 25-OH-D_3 in whole amount of added vitamin D_3	Carcass yield, %			Breast meat yield, % carcass			Relative weight of gizzard, % carcass		
	% of grit in whole amount of used calcium carbonate								
	0	30	mean	0	30	mean	0	30	mean
0	73.1	74.8	73.9	22.7	22.9	22.8	1.88	1.85	1.87
20	73.6	74.6	74.1	23.0	23.4	23.2	1.89	1.82	1.85
40	73.5	74.3	73.9	23.0	24.0	23.5	1.93	1.91	1.92
50	73.9	72.7	73.3	21.3	23.2	22.2	1.83	1.94	1.88
60	73.9	74.5	74.2	22.0	23.3	22.7	1.81	1.85	1.83
80	74.4	74.5	74.4	23.5	22.7	23.1	1.88	1.90	1.89
100	73.7	73.5	73.6	23.2	23.9	23.5	1.97	1.86	1.92
Mean	73.7	74.1		22.7	23.3		1.88	1.87	
SEM	0.135			0.216			0.0199		

all differences between means and interactions were not significant

TABLE 7

Content of crude ash and calcium in fresh tibia bones, %

% of 25-OH-D_3 in whole amount of added vitamin D_3	Crude ash			Ca		
	% of grit in whole amount of used calcium carbonate					
	0	30	mean	0	30	mean
0	21.4	23.1	22.3	9.9	10.6	10.2^{a}
20	22.6	23.0	22.8	10.6	10.0	10.3^{a}
40	22.7	22.3	22.5	10.3	10.7	10.5^{ab}
50	21.7	24.8	23.3	9.8	12.3	11.0^{ab}
60	23.3	22.8	23.1	10.3	11.3	10.8^{ab}
80	23.1	23.2	23.2	10.5	11.6	11.1^{ab}
100	22.9	23.9	23.4	10.4	12.0	11.2^{b}
Mean	22.5^{x}	23.3^{y}		10.3^{x}	11.2^{y}	
SEM		0.189			0.131	
Effect of:						
Ca source		NS			*	
vitamin D_3		*			***	
interaction		NS			**	

$^{x, y}$ - values in the rows with different letters differ significantly (P≤0.05)
^{a,b} - values in the columns with different letters differ significantly (P≤0.05)
NS - P>0.05; * - P≤0.05; ** - P≤0.01; *** - P≤0.001

numerical tendency was observed for 3-8% higher values of relative Ca retention when the 25-OH-D_3 metabolite was supplemented to the diet. Daily retention of phosphorus and relative P retention (in % of P ingested) were the lowest when the diet was supplemented with cholecalciferol (Table 8). Daily P retention

TABLE 8

Retention and excretion of Ca and P, mg per chicken per day

Group	Calcium			Phosphorus		
	excretion mg	retention mg	retention as% of intake	excretion mg	retention mg	retention as % of intake
I	255	341^{a}	57.2	172	222^{a}	56.3^{a}
II	230	375^{abc}	62.0	159	242^{abc}	60.3^{bc}
III	244	358^{ab}	59.4	170	228^{ab}	57.2^{ab}
IV	260	374^{abc}	58.9	163	257^{bcd}	61.0^{bc}
V	263	410^{c}	60.9	164	281^{d}	63.1^{c}
VI	262	405^{bc}	60.7	178	263^{cd}	59.6^{abc}
VII	245	393^{bc}	61.6	173	250^{abcd}	59.1^{abc}
SEM	3.98	6.49	0.526	2.12	4.91	0.559

[a,b,c,d] - values in columns with different letters differ significantly at $P \leq 0.05$

significantly increased ($P \leq 0.05$) when 70-80% vitamin D_3 was replaced by 25-OH-D_3. The relative P retention in chickens (as % of P intake) increased at vitamin D_3 substitution levels of 20, 50 and 60%.

DISCUSSION

The kind of calcium source and range of 25-OH-D_3 substitution for cholecalciferol affected chicken performance only in the second period of feeding and for the whole experiment. Incorporation of 30% of limestone as particulate grit had a positive effect on weight gain, feed intake and the feed conversion ratio. Grit can probably stimulate the digestive tract and nutrient utilization. Compared with diets containing pulverized limestone, a mixture of pulverized and particulate limestone forms increased the percentage ash and calcium contents in the tibia. These findings agree with the results of Zohravi (2002), who used 0.6 to 1.18 mm and 1.18 to 4.75 mm particle sized limestone versus less than 0.15 mm. Guinotte et al. (1991) used coarse particles of limestone (greater than 1.18 mm) as the sole source of Ca added to the diet and found a negative effect on performance and bone quality of chickens. These results and the results of the present study suggest that for good performance, chickens require the presence of limestone in powdered form in the diet and that particulate grit should make up only a part of the limestone.

Introduction of the 25-OH-derivative as a partial or complete (50 to 100%) substitute for cholecalciferol increased body weight gain and improved the feed conversion ratio as compared with chickens fed only vitamin D_3. No positive effect was observed at lower levels of substitution. A significant increase of the

Ca content in the tibia was noted when 25-OH-D_3 completely replaced D_3 in the diet. This may suggest that synthesis of the active form of cholecalciferol in the liver is not sufficient for chicken performance and bone calcification.

Improvement of body weight in chickens fed 25-OH-D_3 as a complete substitute for D_3 was reported by Fritts and Waldroup (2003) for diets with added 125-1000 IU D_3 and by Yarger et al. (1995) for diets with 2760 IU $D_3 \cdot kg^{-1}$. A greater percentage of bone ash and a lower incidence and severity of tibial dyschondroplasia in chickens fed 25-OH-D_3 were reported by Fritts and Waldroup (2003).

The data from the present experiment may suggest that a mixture of pulverized and particulate limestone is a more effective source of calcium than pulverized limestone alone and that addition of the 25-OH vitamin D_3 derivative to the diet better covers chickens' requirements for calcium-vitamin D_3 metabolism than cholecalciferol. Improved performance was noted when chickens were fed a mixture of particulate and pulverized limestone and when 50 and more percent of the added vitamin D_3 was replaced by 25-OH-D_3.

The results of the balance trial were consistent with performance and bone ash and Ca content. Retention of Ca and P in chickens increased when cholecalciferol added to the diet was substituted by 25-OH-D_3 at a level of 60-100 and at 70-80%, respectively. Relative P retention grew significantly at 50-60% of substitution, but daily P excretion in excrements was not changed. Substitution of 25-OH-D_3 for cholecalciferol did not decrease the emission of P into the environment. Fritts and Waldroup (2005) reported that phosphorus utilization was not improved as a result of 25-OH-D_3 incorporation into a diet with a reduced nonphytate P content and relatively high vitamin D_3 level, supplemented with microbial phytase. This could suggest that the efficacy of 25-OH-D_3 is greater at lower levels of dietary vitamin D_3 supplementation. At levels typically used by the poultry industry, e.g., 10 to 20 times above NRC (1994) recommendation, the effect of 25-OH-D_3 substitution for cholecalciferol on P utilization by chickens is negligible (Fritts and Waldroup, 2005).

CONCLUSIONS

Replacement of 30% of pulverized limestone in the diet by particulate grit had a beneficial effect on performance, ash and Ca content in the tibia. Partial or complete substitution of 25-OH-D_3 for the cholecalciferol added to the diet positively affected performance, Ca content in the tibia, and results of Ca and P balance.

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STRESZCZENIE

Efektywność różnych źródeł kredy paszowej i witaminy D_3 w żywieniu kurcząt brojlerów

W 6-tygodniowym doświadczeniu na 560 kurczętach Cobb badano wpływ wprowadzenia do diety żwiru wapiennego oraz 25-hydroksycholekalcyferolu (25-OH-D_3). Mieszanki paszowe jako źródło wapnia zawierały miałką kredę paszową lub mieszaninę 70% kredy i 30% żwiru wapiennego o średnicy cząstek 1.2-4 mm. Witamina D_3 (cholekalcyferol) była stopniowo zastępowana przez 25-OH-D_3: 20, 40, 50, 60, 80 lub 100%.

Wprowadzenie żwiru wapiennego do mieszanki polepszyło wyniki produkcyjne oraz zwiększyło zawartość popiołu surowego i Ca w kościach piszczelowych. Zastąpienie 50 lub 60% cholekalcyferolu przez 25-OH-D_3 spowodowało poprawę przyrostu masy ciała i wykorzystania paszy. Całkowite zastąpienie witaminy D_3 jej pochodną miało dodatni wpływ na zawartość Ca w kościach piszczelowych, natomiast zastąpienie w 50, 60, 80 lub 100% - na bilans wapnia i fosforu.