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Efficacy of different levels of a cholecalciferol 25-OH-derivative in diets with two limestone forms in laying hen nutrition*

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ABSTRACT

An experiment was conducted on 180 Hy-Line Brown hens (from 26 to 70 weeks of age) in a 5 × 2 factorial arrangement with five levels of vitamin D_3 and 25-OH-D_3 derivative rations and two forms of limestone in the diet. Diets were supplemented with 1500 IU of vitamin D_3; vitamin 25-OH-D_3 derivative was substituted for cholecalciferol in amounts equivalent to 0, 25, 50, 75 and 100%. Limestone (8.5% in the diet) was supplied in fine pulverized (LF, 0.1-0.4 mm) or mixed: fine + grit (LG, 2-4 mm) form as a 1:1 mixture of LF:LG. Eggshell quality, tibia bone breaking strength and performance indicators were studied.

Supplementation of the diet with LF + LG limestone decreased feed intake and, at 70 weeks of age, increased eggshell breaking strength and tibia bone strength in comparison with group LF. At 66 weeks of age, hens fed 25% of vitamin D_3 as 25-OH-D_3 laid eggs with thicker and denser shells. At 66 and 70 weeks of age, partial or complete substitution of cholecalciferol with 25-OH-D_3 increased eggshell breaking strength. The effect of 25-OH-D_3 on bone breaking strength was not confirmed statistically. Under the conditions of this experiment, simultaneous use of LF + LG and 25-OH-D_3 in the diet for older layers appears to be the most efficient combination for achieving high resistance of eggshells and tibia bones.

KEY WORDS: eggshell, cholecalciferol, 25-OH-D_3, limestone, tibia bone

INTRODUCTION

Vitamin feed premixes for laying hens are relatively rich in cholecalciferol, but above-requirement levels of vitamin D_3 generally have no beneficial effect

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on eggshell calcification (Garlich and Wyatt, 1971; Keshavarz, 2003). In older hens the ability to produce the active metabolite, 1,25-$(OH)_2$-D_3, is reduced (Bar and Hurvitz, 1987), renal 25-hydroxylase enzyme activity is decreased, and the rate of cracked eggshells increased (Abe et al., 1982). McLoughlin and Soares (1976) observed a positive response of eggshell and bone mineralization in older hens after adding 25-OH-D_3 to the diet, while Keshavarz (2003) did not find any advantages of complete substitution of vitamin D_3 in the diet with the 25-OH-D_3 derivative, either in terms of shell quality or laying performance of hens.

The positive effect of coarse particle limestone in the diet on covering calcium requirements of laying hens has been reported many times, especially in hens kept under lighting schedules with a long dark period of no feed intake (Scott et al., 1971; Roland and Harms, 1973; Guinotte and Nys, 1991; Zhang and Coon, 1997; Richter et al., 1999). A positive effect of moderate substitution of limestone grit for fine particles in the diet on increased eggshell calcium deposition and breaking strength and, in some cases, also on tibia bone breaking strength, was confirmed in an earlier paper (Koreleski and Świątkiewicz, 2004).

In this study, the 25-OH-D_3 derivative was gradually substituted for vitamin D_3 in diets supplemented with calcium carbonate in the form of fine pulverized or mixed (pulverized plus ground) limestone, and its effect on laying performance, eggshell quality and bone breaking strength was studied.

MATERIAL AND METHODS

One hundred eighty Hy-Line Brown hens, 26 weeks old, were assigned to a 5×2 factorial arrangement with five vitamin D_3 to 25-OH-D_3 ratios and two limestone forms in the diet. In the pre-experimental period, at 25 weeks of age, eggs were evaluated for shell breaking strength and groups of hens were equalized for this parameter at the start of the experiment. Ten isocaloric and isonitrogenous cereal-soyabean diets (Table 1) were supplemented with 1500 IU D_3 and contained 8.5% of limestone. Cholecalciferol was used as the source of vitamin D_3 or was supplemented by its 25-OH-derivative at a rate of 25, 50, 75 and 100%. In successive groups, 25-OH-D_3 was added to the diet at a rate from 9.35 to 37.5 $\mu g \cdot kg^{-1}$. The source of monohydroxy-cholecaliferol [Hy·D Beadlet (Roche)] contained 12.5 µg 25-OH-D_3 per g, which was equivalent to 500 000 IU vitamin D_3. Lutavit 500 (BASF) contained 500 000 IU of cholecalciferol·g^{-1}. As calcium supplements, fine pulverized limestone of 0.1-0.4 mm in square sieve size (LF) or particulate limestone grit of 2-4 mm (LG) mixed with LF at a ratio of 1:1 (w/w) were used (Table 2).

TABLE 1

Composition of basal diet, $g \cdot kg^{-1}$

Item	Content
Ground maize	340.0
Ground wheat	255.0
Soyabean meal	192.0
Rapeseed meal	40.0
Grass meal	30.0
Rapeseed oil	32.0
Limestone	85.0
Dicalcium phosphate	17.0
NaCl	3.0
DL-Methionine	1.0
Vitamin-mineral premix [1]	5.0
Metabolizable energy, MJ/kg[2]	11.3
Analysed	
crude protein	170
Lys	8.2
Met	3.8
Ca	36.9
total P	6.4

[1] lutamix BASF, supplied to 1 kg of diet: vit. A 10 000 IU; mg: vit. E 15; vit. K_3 2; vit. B_1 1; vit. B_2 4; vit. B_6 1.5; vit. B_{12} 0.01; Ca-pantotenate 8; PP 25; folic acid 0.5; choline-Cl 250; Mn 100; Zn 50; Fe 50; Cu 8; J 0.8; Se 0.2, Co 0.2

[2] calculated according to European Table (1989) as a sum of ME content of components

TABLE 2

Scheme of experiment (additives per 1 kg of the diet)

Group[1]	Pulverised limestone g	Limestone (grit) g	Cholecalciferol IU	25-OH-D_3 µg
I	85	-	1500	-
II	85	-	1125	9.35
III	85	-	750	18.75
IV	85	-	375	28.1
V	85	-	-	37.5
VI	42.5	42.5	1500	-
VII	42.5	42.5	1125	9.35
VIII	42.5	42.5	750	18.75
IX	42.5	42.5	375	28.1
X	42.5	42.5	-	37.5

[1] explanation - see Material and methods

Hens belonging to one treatment (18 layers) were kept individually in cages (40 cm wide×40 cm deep) with individual egg collection. During the 44 weeks of the experiment (up to 70 weeks of age) the hens were offered water and feed *ad libitum*. Layers were exposed to a 14 L:10 D lighting schedule, with the dark period at night.

Feed intake, number and weight of laid eggs were recorded for all periods of the experiment. For shell weight, thickness and density assessment, one egg from each hen was individually collected from hens, at 44, 48, 53, 57, 62, 66 and 70 weeks of age. Because in eggs from earlier collections (44-53 weeks of age) no differences in eggshell quality were found, these results are omitted in this paper. Egg Quality Measurement (Micro version 3.2, 1990) was used for egg testing, shell density was calculated from egg and shell weight, and shell thickness measured equatorially in washed and air-dried shells by an electronic micrometer. At the start of the 44, 48, 53, 57, 62, 66 and 70 week of age, in the morning (after the dark period at night) one egg per hen was collected and tested for shell breaking strength (in Newtons - N) with an Instron Table model 5542 apparatus at a head speed of 10 mm per min. At the end of the experiment (70 weeks of age), 5 representative hens were chosen from each group and decapitated. Tibia bones were prepared and breaking strength was estimated using an Instron 5542 apparatus (distance between supports - 50 mm).

The chemical composition of feed components and basal diet was analysed according to AOAC (1990) methods. The metabolizable energy content of the basal diet was calculated from the content of basic nutrients using calculation factors from European Tables (1989). The basal diet was mineralized (AOAC, 1990) and analysed using a Philips PU 9100 atomic absorption spectrometer. The phosphorus content in the basal diet was assayed by a colorimetric method (AOAC, 1990). Amino acids in acid hydrolysates (methionine after peroxidation to methionine sulphone) were estimated in a colour reaction with the ninhydrin reagent using a Beckman-System Gold 126 AA automatic analyser.

Data were subjected to statistical evaluation using two-way factorial analysis of variance. The significance of differences between means was determined by Duncan's multiple range test. All procedures were performed using Statistica 5.0 PL software.

RESULTS

Performance. The average laying rate in the experiment was 88%, feed conversion per kilogram of eggs, 2.14 kg and per egg, 135 g. Laying rate, egg weight and feed conversion were statistically not affected by the source of vitamin

TABLE 3

Average production results in whole laying period (26-70 weeks of age)

% of 25-OH-D_3 in whole amount of added vitamin D_3	Laying rate %			Daily mass of eggs g per hen			Daily feed intake g per hen			Feed kg per 1 kg of eggs			Feed g per 1 egg		
	% of grit in whole amount of used calcium carbonate														
	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean
0	92.3	84.9	88.6	56.2	53.7	55.0	120	118	119	2.13	2.21	2.17	130	140	135
25	88.2	85.6	86.9	57.5	54.2	55.9	121	117	119	2.11	2.15	2.13	138	136	137
50	88.8	85.0	86.9	55.4	53.7	54.5	120	116	118	2.14	2.14	2.14	133	137	135
75	88.5	89.6	89.0	56.0	56.9	56.5	120	118	119	2.13	2.07	2.10	135	132	134
100	88.9	88.8	88.8	56.1	56.3	56.2	122	120	121	2.18	2.14	2.16	138	134	136
Mean	89.3	86.8		56.2	55.0		121[b]	118[a]		2.14	2.14		135	136	
SEM		0.66			0.26			0.62			0.014			1.04	
Effect of:															
Ca source		NS			NS			*			NS			NS	
vitamin D_3		NS			*			NS			NS			NS	
interaction		NS			NS			NS			NS			NS	

[a,b] values in columns with different letters differ significantly at P≤0.05
* P≤0.05, NS - P>0.05

TABLE 4

Eggshell percent (57-70 weeks of age)

% of 25-OH-D_3 in whole amount of added vitamin D_3	57 weeks of age			62 weeks of age			66 weeks of age			70 weeks of age			Mean		
	% of grit in whole amount of used calcium carbonate														
	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean
0	10.12	9.81	9.96	9.51	9.57	9.54	9.52	9.11	9.32	9.54	9.52	9.53	9.67	9.50	9.59
25	9.51	9.43	9.47	9.40	9.69	9.55	9.80	9.70	9.74	9.47	9.70	9.58	9.55	9.62	9.59
50	9.48	9.68	9.58	9.65	9.59	9.62	9.47	9.71	9.59	9.26	9.69	9.48	9.47	9.67	9.57
75	9.72	9.93	9.85	9.47	9.59	9.53	9.62	9.65	9.63	9.47	9.36	9.42	9.58	9.64	9.61
100	9.52	9.52	9.52	9.46	9.30	9.38	9.58	9.39	9.48	9.53	9.73	9.63	9.52	9.49	9.50
Mean	9.68	9.68		9.50	9.55		9.59	9.51		9.46	9.60		9.56	9.58	
SEM		0.07			0.06			0.07			0.06			0.04	
Effect of:															
Ca source		NS			NS			NS			NS			NS	
vitamin D_3		NS			NS			NS			NS			NS	
interaction		NS			NS			NS			NS			NS	

NS - P>0.05

TABLE 5

Egg shell thickness (57-70 weeks of age), µm

% of 25-OH-D_3 in whole amount of added vitamin D_3	57 weeks of age			62 weeks of age			66 weeks of age			70 weeks of age			Mean		
	% of grit in whole amount of used calcium carbonate														
	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean
0	369	389	379	373	371	372	358	354	356[a]	366	369	368	367	371	369
25	367	367	367	375	380	377	382	377	379[b]	357	375	366	370	375	372
50	365	371	368	379	378	379	368	381	374[ab]	360	368	364	368	375	371
75	380	378	378	373	381	377	374	374	374[ab]	371	364	367	375	374	374
100	374	364	369	367	368	367	374	360	367[ab]	370	370	370	371	366	368
Mean	371	374		373	376		371	369		365	369		370	372	
SEM		2.71			2.34			2.90			2.83			1.61	
Effect of:															
Ca source		NS			NS			NS			NS			NS	
vitamin D_3		NS			NS			*			NS			NS	
interaction		NS			NS			NS			NS			NS	

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

TABLE 6

Eggshell density (57-70 weeks of age), mg · square cm^{-1}

% of 25-OH-D_3 in whole amount of added vitamin D_3	57 weeks of age			62 weeks of age			66 weeks of age			70 weeks of age			Mean		
	% of grit in whole amount of used calcium carbonate														
	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean
0	84.4	83.8	84.1	80.1	80.4	80.2	79.7	77.3	78.4[a]	80.2	79.9	80.1	81.1	80.3	80.7
25	80.7	79.1	79.9	81.3	82.3	81.8	85.0	82.7	83.8[b]	79.8	80.8	80.3	81.7	81.2	81.5
50	78.7	80.7	80.7	81.6	82.2	81.9	79.9	81.9	80.9[sb]	78.6	82.7	80.6	79.7	82.4	81.0
75	82.5	84.4	83.4	80.6	81.6	82.1	81.8	82.2	82.0[ab]	80.5	80.1	80.3	81.3	82.1	81.7
100	81.5	80.9	81.2	80.1	79.4	79.7	81.8	76.3	79.1[a]	81.2	82.5	81.9	81.2	79.8	80.5
Mean	81.6	82.2		80.7	81.2		81.6	80.1		80.1	81.2		81.0	81.2	
SEM		0.646			0.512			0.702			0.505			0.345	
Effect of:															
Ca source		NS			NS			NS			NS			NS	
vitamin D_3		NS			NS			*			NS			NS	
interaction		NS			NS			NS			NS			NS	

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

TABLE 7

Eggshell breaking strength (44-70 weeks of age), N (Newtons)

% of 25-OH-D_3 in whole amount of added vitamin D_3	44 weeks of age			48 weeks of age			53 weeks of age			57 weeks of age			62 weeks of age			66 weeks of age			70 weeks of age			Mean		
	% of grit in whole amount of used calcium carbonate																							
	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean	0	50	mean
0	37.5	38.2	37.9	38.1	38.5	38.3	36.4	37.0	36.7	37.7	37.9	37.8	38.0	37.0	37.5	32.9	35.6	34.3^{a}	31.1	33.2	32.1^{a}	35.9	36.8	36.4
25	39.2	38.6	38.9	37.0	40.1	38.5	37.5	39.6	38.5	37.3	39.7	38.5	37.7	39.3	38.5	36.7	38.8	37.7^{b}	37.6	36.4	35.0^{b}	37.0	38.9	37.9
50	37.3	42.2	39.7	38.6	37.6	38.1	36.6	38.1	37.3	36.3	39.7	38.3	37.7	37.9	37.8	35.5	38.9	37.2^{b}	36.0	37.5	36.8^{b}	36.9	38.9	37.9
75	35.8	38.5	37.2	36.5	38.1	37.2	36.4	37.4	36.9	37.5	40.4	38.1	37.5	38.5	38.0	36.2	39.0	37.6^{b}	36.0	37.3	36.6^{b}	36.6	38.4	37.5
100	39.9	36.1	38.0	38.0	36.8	37.4	38.7	38.6	38.6	38.7	37.8	38.2	39.1	37.9	38.5	38.8	36.6	37.7^{b}	37.0	37.1	37.1^{b}	38.6	37.3	37.9
Mean	37.9	38.7		37.6	38.2		37.1	38.1		37.5	38.9		38.0	38.1		36.0	37.8		34.7^{a}	36.3^{b}		37.0^{a}	38.1^{b}	
SEM		0.473			0.418			0.359			0.407			0.407			0.464			0.375			0.256	
Effect of:																								
Ca source		NS			NS			NS			NS			NS			NS			*			*	
vitamin D_3		NS			NS			NS			NS			NS			*			**			NS	
interaction		*			NS			NS			NS			NS			NS			NS			NS	

^{a,b} values in columns with different letters differ significantly at $P \leq 0.05$

** $P \leq 0.01$, * $P \leq 0.05$, NS - $P > 0.05$

D_3 or the kind of limestone in the diet (Table 3). The feed intake of hens on diets with LF limestone was higher ($P \leq 0.05$) than that of hens fed diets with the LF+LG mixture. The hens thus consumed 4.45 and 4.35 g Ca daily, respectively.

Eggshell quality. The percent of shell in egg weight (Table 4) was not affected by the form of calcium and kind of vitamin D_3 supplemented to the diet. In respect to eggshell thickness (Table 5) and egg shell density (Table 6) differences were only found ($P \leq 0.05$) in eggs laid at 66 weeks of age and between the hens fed the control 100% D_3 diet and the diet containing 25% of 25-OH-D_3 (9.35 $\mu g \cdot kg^{-1}$). Experimental factors did not affect eggshell breaking strength in eggs collected between 44 and 62 weeks of age (Table 7). Statistically confirmed effects were observed for calcium form in eggs collected at 66 and 70 weeks of age and for source of vitamin D_3 at 70 weeks of age. In total, the average eggshell resistance of 7 egg collections was positively affected in diets supplemented with LG ($P \leq 0.05$).

Bone breaking strength. In bones taken from hens at the end of the experiment a tendency was observed for a positive influence of substituting 25-OH-D_3 for cholecalciferol, but the differences in relation to the control group did not reach statistical significance (Table 8). A positive effect of adding LG was, however, confirmed at $P \leq 0.01$.

TABLE 8

Tibia bones breaking strength at 70 weeks of age, Newtons

% of 25-OH-D_3 in whole amount of added vitamin D_3	% of grit in whole amount of used calcium carbonate		
	0	50	Mean
0	175	192	184
25	179	193	186
50	196	199	197
75	176	213	196
100	182	223	203
Mean	182[a]	204[b]	
SEM		0.659	
Effect of:			
Ca source		**	
vitamin D_3		NS	
interaction		NS	

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

** $P \leq 0.01$, NS - $P > 0.05$

DISCUSSION

In the experiment, supplementation of the diet with a mixture of LF+LG limestone decreased feed intake as compared with the fine (LF) source of calcium. This finding confirms the data reported by Vogt (1983) and, in part, a tendency that was not confirmed statistically, shown in a previous report (Koreleski and Świątkiewicz, 2004). The kind of limestone used did not change laying performance, feed conversion for egg production or egg weight.

The amounts of 1500 IU vitamin D_3 (and equivalent 25-OH-D_3) supplemented to 1 kg of experimental diets in the experiment were higher than the 250 IU minimum requirement for hens fed 120 g diet daily according to NRC (1994) and lower than the level exceeding 2000 IU used in commercial diets.

The source of vitamin D_3 and limestone form used did not modify the relative shell weight, the latter result confirming earlier reports from Koreleski and Świątkiewicz (2004). Eggs of hens fed the diet supplemented with 25% D_3 as 25-OH-cholecalciferol had greater shell thickness and density than the control group, but only at 66 weeks of age.

The breaking strength of eggshells from hens fed 100% D_3 as cholecalciferol and LF or LF+LG as a source of calcium in the late period of laying showed a clear tendency to decrease. This result agrees with the lower eggshell calcification ability in older hens (Abe et al., 1982; Bar and Hurvitz, 1987).

Incorporation of the 25-OH-D_3 derivative to the diet in the late period of laying counteracted the decrease of shell resistance with advanced age of hens. The positive effect on eggshell breaking strength was confirmed at 66 and 70 weeks, which is consistent with the results of McLoughlin and Soares (1976). The positive effect was also observed even in the case of 100% substitution of vitamin D_3 with its OH-derivative. This finding contrasts with the study of Keshavarz (2003), who did not find a positive effect when using D_3-rich diets containing in 2760 IU cholecalciferol per kg or an equivalent amount of 25-OH-D_3.

At 70 weeks of age, a beneficial effect on eggshell strength was also confirmed in with LF+LG as the calcium source as compared with LF, which agrees with the result of a previous study (Koreleski and Świątkiewicz, 2004). This suggests that simultaneous use of grit limestone together with the OH-D_3 derivative in the diet is most efficient and counteracts the natural process of eggshell strength reduction in older hens. Grit limestone may minimize the mobilization of bone calcium reserve with simultaneous release of phosphorus and prevent its excess in blood, which suppresses oxidation of 25-OH-D_3 to the active form, 1,25-$(OH)_2$-D_3. Introduction of the first OH-D_3 metabolite as a feed additive may help activate dihydroxy-D_3 metabolite production in the kidneys, the biosynthesis of Ca-binding protein, and active Ca transport (Wasserman and Taylor, 1968).

As regards tibia bone breaking strength, the use of LF+LG forms of calcium had a positive effect on bone strength, which is consistent with the result of our previous studies (Koreleski and Świątkiewicz, 2004). A tendency for tibia bone breaking strength improvement after partial or total substitution of vitamin D_3 deriverate.

Under the conditions of this experiment, simultaneous use of LF + LG and 25-OH-D_3 in the diet for older laying hens appears to be the most efficient combination for achieving high resistance of eggshells and tibia bones.

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STRESZCZENIE

Efektywność różnych poziomów aktywnej pochodnej witaminy D_3 (25-OH-D_3) w diecie zawierającej dwie formy węglanu wapnia w żywieniu kur nieśnych

Celem doświadczenia było określenie wpływu stopniowego zastępowania dodatku witaminy D_3 (cholekalcyferolu) przez jej aktywną pochodną (25-OH-D_3) w paszy dla niosek,zawierającej węglan wapnia w postaci drobno zmielonej lub mieszanej (miałka kreda LF + żwirek wapienny LG), na wydajność nieśną oraz jakość skorupy jaj i kośćca.

Doświadczenie w układzie 5 × 2 czynnikowym wykonano na 180 kurach Hy-Line Brown w wieku od 26 do 70 tygodnia życia. Nioski podzielono na 10 grup doświadczalnych i utrzymywano w indywidualnych klatkach na podłodze z siatki. Mieszanka podstawowa zasobna w składniki pokarmowe (z wyjątkiem witaminy D_3, ponieważ stosowano premiks bez tej witaminy) zawierała węglan wapnia (8,5%) w postaci LF lub mieszaniny LF + LG w stosunku 1:1. Obydwa rodzaje mieszanek uzupełniano dodatkiem 1500 j.m. witaminy D_3, którą zastępowano stopniowo w 0, 25, 50, 75 i 100% jej aktywną pochodną (25-OH-D_3).

Stosowanie mieszanej formy $CaCO_3$ w paszy wpłynęło korzystnie na wytrzymałość skorupy jaj i kości piszczelowych. W 66 tygodniu życia u kur żywionych mieszanką, w której 25% witaminy D_3 zastąpiono jej aktywną pochodną, stwierdzono zwiększenie grubości i gęstości skorupy jaj. W 66 i 70 tygodniu życia częściowe lub całkowite zastąpienie cholekalcyferolu przez 25-OH-D_3 spowodowało zwiększenie wytrzymałości mechanicznej skorup. Zastosowanie dodatku 25-OH-D_3 do paszy nie wpłynęło statystycznie istotnie na wytrzymałość kości piszczelowych na złamanie.

Wyniki doświadczenia wskazują na korzystny wpływ równoczesnego dodatku do paszy dla kur nieśnych mieszaniny LF + LG oraz 25-OH-D_3 na wytrzymałość skorupy jaj i kości piszczelowych.