ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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E-mail: editorijps@gmail.com

International Journal of Poultry Science 10 (4): 295-299, 2011
ISSN 1682-8356

Evaluation of a Fermentation Source of 25-hydroxycholecalciferol in Broiler Diets[1]

S.D. Goodgame, F.J. Mussini, C. Lu, C.D. Bradley, S.E. Watkins and P.W. Waldroup[2]
Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

Abstract: After a seven day depletion period of vitamin D supplementation beginning on day of hatch, male chicks of a commercial broiler strain were placed on diets supplemented with either a commercial source of 25-hydroxycholecalciferol (25-OH-D_3) or a new source derived from fermentation. Levels of 0, 2.5, 5, 10, 20, 40, 60 and 80 µg/kg of each source were added to a common basal diet that was considered as marginal in calcium and phosphorus content. Each diet was fed to six pens of five birds each. Birds were then grown to 21 d of age at which time body weight and feed consumption were determined. All birds were euthanized by CO_2 inhalation and all toes were removed and ashed. The right tibia was subjected to bone ash determination while the left tibia was subjected to break force analysis. Analysis of the data indicated no significant differences in performance between chicks fed the two sources of 25-OH-D_3 ($p \leq 0.05$) although numerical differences in weight gain and feed conversion were observed that neared statistical significance ($p = 0.06$ and 0.08, respectively). Estimates of the amount of 25-OH-D_3 needed by the bird were approximately 10 µg/kg for tibia ash and 20 µg/kg for body weight and bone breaking force.

Key words: Broilers, 25-hydroxycholecalciferol, bone strength, fermentation product

INTRODUCTION

Poultry diets require supplementation with vitamin D in order to implement absorption of calcium and phosphorus (Bethke *et al*., 1928; Hart *et al*., 1930; Wilgus, 1931; Waldroup *et al*., 1963; 1965). Chickens require cholecalciferol (vitamin D_3) as the primary source of vitamin D as they utilize ergosterol (vitamin D_2) very poorly. Therefore, any product that is intended for use in poultry diets should be evaluated to insure that it has proper activity. Biological tests are necessary, as studies have shown that many commercial products demonstrated adequate chemical activity but lacked biological activity (Yang *et al*., 1973).

Cholecalciferol must undergo conversion in the liver to 25-hydroxy-cholecalciferol (25-OH-D_3) before it can be utilized in the body (Collins and Norman, 1991). It is thought that under certain circumstances inhibition of this conversion may take place leading to vitamin D deficiencies (Edwards *et al*., 2002). In 1995, 25-OH-D_3 was given "Generally Recognized as Safe (GRAS) status for use in poultry feeds (Ward, 1995). Numerous studies have demonstrated that 25-OH-D_3 is superior to cholecalciferol as a source of vitamin D for chicks (Sunde, 1975; McNaughton *et al*., 1977; Cantor and Bacon, 1978; Soares *et al*., 1978; Yarger *et al*., 1995; Mitchell and Edwards, 1997; Fritts and Waldroup, 2003; Fritts *et al*., 2004; Yan and Waldroup, 2006). This study was conducted to evaluate a new 25-OH-D_3 product produced by fermentation compared to a commercially available 25-OH-D_3 product.

MATERIALS AND METHODS

A simple corn-soybean meal diet nutritionally adequate in all respects except for vitamin D activity was used as the reference diet (Table 1). Marginal levels of calcium and phosphorus were employed to enhance the response to the vitamin D sources. A commercial sample of 25-OH-D_3 (Hy-D, DSM, Parsippany NJ) and the test product (Bio D, Walco International, Westlake TX) were included in the reference diet at levels calculated to provide 0, 2.5, 5, 10, 20, 40, 60 and 80 µg/kg. This resulted in a total of 15 test diets.

Male chicks of a commercial broiler strain (Cobb 500, Cobb-Vantress, Siloam Springs AR) were placed in electrically heated battery brooders with wire floors and maintained in a windowless room. Birds were fed the unsupplemented diet from day of hatch to 7 d of age to deplete initial body stores. At day 7 post hatch, chicks were individually weighed and divided into subclasses of equal weight. Birds with extremely low or high weights were discarded and the remaining birds randomly assigned to compartments in the battery brooders. Five chicks were placed in each pen. Six replicate pens were assigned to each dietary treatment, stratified across the six tiers of the battery brooder. The chicks were offered the test diets and tap water for ad libitum consumption from 7 to 21 d post hatch.

At 21 d post hatch, surviving birds were weighed and euthanized by CO_2 inhalation. The right tibia was removed, cleaned of adhering tissue and ashed following lipid extraction as outlined by AOAC (1990)

Corresponding Author: P.W. Waldroup, Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

Table 1: Composition and calculated analysis of basal diet

Ingredient	%
Ground yellow corn	54.68
Soybean meal	39.80
Poultry oil	2.89
Dicalcium phosphate	1.14
Ground limestone	0.54
Vitamin premix[1]	0.25
Trace mineral mix[2]	0.10
Iodized salt	0.44
MHA-84[3]	0.16
Total	100.00
Crude protein (%)	23.00
Calcium (%)	0.70
Total P. (%)	0.67
Nonphytate P (%)	0.35
Met (%)	0.53
TSAA (%)	0.90
Lys (%)	1.29
Thr (%)	0.89

[1]Provides per kg of diet: 16,500 IU vitamin A, 44 IU vitamin E, 66 mg niacin, 22 mg pantothenic acid, 13.2 mg riboflavin, 3.3 mg menadione, 4.4 mg thiamin, 6.6 mg pyridoxine, 2.2 mg folic acid, 0.28 mg biotin, 0.03 mg vitamin B_{12}, 966 mg choline.

[2]Provides per kg of diet: Mn (from $MnSO_4 \cdot H_2O$) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H_2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$) 10 mg; I from Ca $(IO_3)_2 \cdot H_2O$), 1 mg.

[3]Methionine hydroxy analogue calcium salt. Novus International, St. Louis MO

protocol for determination of vitamin D in poultry diets. The left tibia was removed, cleaned of adhering tissue and subject to bone breaking strength. Bone breaking strength was measured using an Instron 4502 (Instron Inc., Norwood, MA) with a 100 kg load cell with a crosshead speed of 20 mm/min collecting 10 data points per second; bone was supported on a 30 mm span. Toes of all birds were removed and ashed by pen as described by Yan *et al.* (2005).

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA as a factorial arrangement of treatments with vitamin source and level as the main effects with the interaction of source and level using the General Linear Models procedure of SAS (SAS Institute, 1991). When significant differences among treatments were found, means were separated using repeated t-tests using the LSMEANS option of the GLM procedure. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers. Statements of significance are based on $p \leq 0.05$. Following the ANOVA analysis, nonlinear regression analysis was conducted using the PROC LIN procedure of SAS (SAS Institute, 1991) and incorporating the SAS macro of Robbins (1986). The requirement was established as the inflection point of the one-slope regression model (Robbins *et al.*, 1979; Yu and Morris, 1999; Waldroup *et al.*, 2000).

Table 2: Effect of different sources of 25-hydroxycholecalcifereol on live performance of broilers

		7-21 d gain (kg)	7-21 d FCR	7-21 d FI (kg)	7-21 d Mort. (%)
Source					
Hy-D		0.590	1.46	1.077	3.71
Bio D		0.570	1.50	1.079	3.33
Level (µg/kg)					
2.5		0.493^{d}	1.483	0.950^{d}	6.67
5.0		0.563^{c}	1.488	1.062^{c}	1.67
10		0.565^{bc}	1.507	1.075^{bc}	3.33
20		0.613^{a}	1.452	1.111^{abc}	5.00
40		0.603^{ab}	1.477	1.107^{abc}	6.00
60		0.622^{a}	1.486	1.132^{a}	2.00
80		0.607^{a}	1.483	1.123^{ab}	0.00
Source	**Level**				
Hy-D	2.5	0.504	1.416	0.926	6.66
	5.0	0.568	1.475	1.056	3.33
	10	0.579	1.490	1.082	0.00
	20	0.650	1.423	1.126	0.00
	40	0.611	1.457	1.092	12.00
	60	0.632	1.513	1.155	4.00
	80	0.613	1.469	1.122	0.00
Bio D	2.5	0.482	1.550	0.973	6.66
	5.0	0.560	1.502	1.068	0.00
	10	0.552	1.524	1.069	6.66
	20	0.586	1.481	1.096	10.00
	40	0.596	1.497	1.119	0.00
	60	0.613	1.459	1.110	0.00
	80	0.601	1.497	1.124	0.00
Analysis of variance		---------- Probability > F ----------			
Source of 25-OH		0.06	0.08	0.99	0.93
Level of 25-OH		<0.001	0.91	<0.001	0.53
Source x Level		0.91	0.47	0.77	0.12
CV		6.47	6.52	6.65	4.20

[abc]Means in column with no common superscripts differ significantly ($p<0.05$). Mort. = Mortality

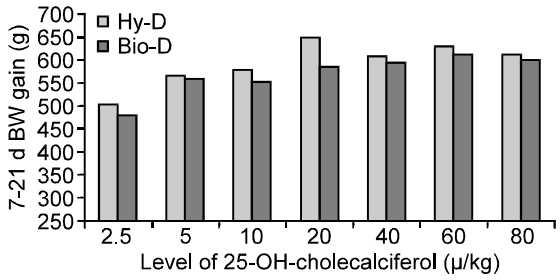

Fig. 1: Effect of different sources and levels of 25-OH-cholecalciferol on 7 to 21 d body weight gain of male chicks

RESULTS AND DISCUSSION

There were no significant differences ($p \leq 0.05$) between live performance of chicks fed the two 25-OH-D_3 products (Hy-D and Bio D) fed on a pound-for-pound basis (Table 2) although numerical differences in weight gain and feed conversion were observed that neared statistical significance ($p = 0.06$ and 0.08, respectively). As seen in Fig. 1, weight gains were similar for chicks fed the two sources for most levels of supplementation with a larger difference at the 20 µg/kg level.

Table 3: Effect of different sources of 25-hydroxycholecalcifereol on bone parameters of broilers

		Tibia diameter (mm)	Break force (kg)	21 d toe Ash %	21 d tibia ash %
Source					
Hy-D		5.22	15.01	12.30	37.55
Bio D		5.36	15.24	12.19	37.33
Level (µg/kg)					
2.5		5.12	10.08^{c}	11.28^{b}	32.54^{d}
5.0		5.31	13.10^{b}	11.93^{a}	34.25^{c}
10		5.35	13.62^{ab}	11.98^{a}	36.31^{b}
20		5.33	14.83^{a}	12.24^{a}	36.07^{b}
40		5.14	13.19^{ab}	11.82^{a}	35.64^{b}
60		5.36	14.73^{ab}	12.09^{a}	36.87^{ab}
80		5.15	14.04^{ab}	12.02^{a}	37.97^{a}
Source	**Level**				
Hy-D	2.5	5.23	10.98^{bc}	11.79	32.96^{fg}
	5.0	5.27	14.88^{a}	12.31	36.04^{cde}
	10	5.33	16.43^{a}	12.67	37.62^{bcd}
	20	5.27	16.59^{a}	12.41	39.12^{ab}
	40	5.15	15.12^{a}	12.49	38.59^{abc}
	60	5.23	16.06^{a}	12.25	38.64^{abc}
	80	4.95	14.96^{a}	12.17	39.90^{a}
Bio D	2.5	5.00	9.57^{bc}	11.12	32.54^{fg}
	5.0	5.47	14.66^{a}	12.12	35.45^{de}
	10	5.32	15.02^{bc}	12.37	38.33^{abc}
	20	5.67	17.46^{a}	12.52	36.42^{cd}
	40	5.25	16.22^{a}	12.34	39.29^{ab}
	60	5.49	16.83^{a}	12.65	39.13^{ab}
	80	5.30	16.87^{a}	12.16	40.11^{a}
Analysis of variance		---------- Probability > F ----------			
Source of 25-OH		0.11	0.73	0.34	0.28
Level of 25-OH		0.30	<0.001	<0.001	<0.001
Source x Level		0.34	0.63	0.35	0.41
CV		10.62	24.31	4.45	6.58

^{abc}Means in column with no common superscripts differ significantly ($p<0.05$)

As would be expected, the overall level of supplementation had a significant effect on body weight gain and feed intake. However, feed conversion ratio was not significantly affected by the level of supplementation of the two products. There were no significant interactions between source and level of supplementation for body weight, feed conversion, feed intake, or mortality. There were no significant differences ($p \leq 0.05$) between chicks fed the two sources of 25-OH-D_3 for tibia diameter, tibia break force, toe ash, or tibia ash at 21 d of age (Table 3). The level of 25-OH-D_3 supplementation had no significant effect on tibia diameter but significantly affected tibia break force, toe ash and tibia ash, as would be expected.

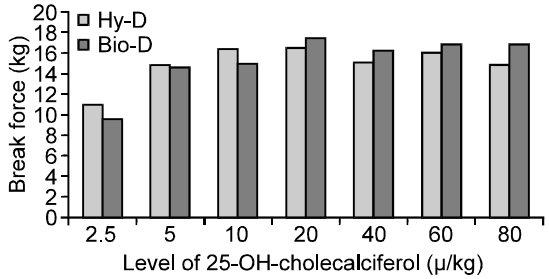

Fig. 2: Effect of different sources and levels of 25-OH-cholecalciferol on breaking force of tibiae from 21 d male chicks

There were significant interactions of source of 25-OH-D_3 and level of supplementation for both tibia break force and tibia ash (Table 3). As shown in Figure 2 for break force and Fig. 3 for tibia ash, the two sources varied in their relative superiority over different levels of supplementation; however there was no consistent trend in these differences. Therefore, for the critical measurement of bone development both of the products appeared to be equal when supplied on a pound-for-pound basis.

Estimating the amount of 25-OH-D_3 required for optimum performance in this study is subject to considerable variation, perhaps related to the limited numbers of observations. Results of the regression analysis are shown in Table 4. The requirement for 25-OH-D_3 for body weight, defined as the breakpoint of the response to the dosage level, was estimated to be 19.18±8.25 µg/kg. Results of the LSMEANS comparison (Table 2) and visual evaluation of the results in Fig. 1 support the estimated needs at approximately 20 µg/kg of diet for optimum body weight. Regression analysis for feed conversion, tibia break force and toe ash failed to show convergence, indicating that no definitive inflection point was established. For tibia ash, the regression analysis indicated an inflection point at 10.33±4.62 µg/kg. This is supported by the LSMEANS comparison and visual evaluation of the data in Fig. 3. For break force of the tibia, there was no estimate available from the

Table 4: Results of nonlinear regression analysis to estimate the needs of the broiler chick for levels of 25-hydroxycholecalciferol

Measurement	Value at inflection	Inflection point[1]	Asymptotic standard error	Asymptotic 95 % confidence interval
7-21 d BW gain	0.610	19.18	8.25	7.08-45.44
7-21 d Feed intake	1.090	6.93	1.20	3.11-10.75
7-21 d FCR	Non convergence			
Tibia break force	Non convergence			
Toe ash	Non convergence			
Tibia ash	35.79	10.33	4.62	4.37-25.03

[1]Defined as the break point of dietary 25-hydroxycholecalciferol concentration as a function of the selected variables according to a nonlinear least squares analysis (Robbins, 1986; SAS Institute, 1991; Yu and Morris, 1999; Yan and Waldroup, 2006)

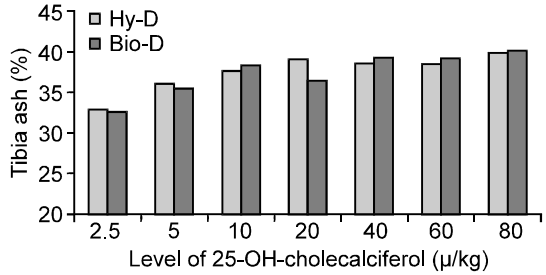

Fig. 3: Effect of different sources and levels of 25-OH-cholecalciferol on tibia ash from 21 d male chicks

regression analysis, but results from the LSMEANS comparison (Table 3) and visual evaluation of the data (Fig. 2) show a peak break force at 20 µg/kg of diet.

In conclusion, a new source of 25-OH-D_3 produced by fermentation was compared to a commercially available product produced by chemical synthesis. For bone development as measured by tibia ash, toe ash, or bone breaking strength the products appeared to be equal when supplied on a pound-for-pound basis. No significant differences were observed between chicks fed the two sources for body weight gain and feed conversion but further studies are needed to evaluate on larger scale production as these differences neared statistical significance. It would appear that 20 µg/kg supplementation with 25-OH-D_3 would provide for optimum performance during this age period.

ACKNOWLEDGEMENTS

This study was supported by a grant in aid from Walco International, Westlake TX.

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[2]To whom correspondence should be addressed: Waldroup@uark.edu.