

## **EFFECT OF ADDING 25-HYDROXYCHOLECALCIFEROL IN PLANT DIETS WITH AND WITHOUT FISH MEAL ON REARING RESULTS AND BONES OF BROILER CHICKENS**

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### **Abstract**

The objective of the research was to determine an impact of 25-hydroxycholecalciferol addition to mixes containing either fish meal or plant-based mixes, on rearing results and thigh and tibial bone physico-chemical characteristics of broiler chickens. A feeding experiment was conducted on 192 Ross 308 broiler chickens arranged in a two-factor design, the factors being a type of feed raw materials used to formulate mixtures and an addition of 25-OH-D<sub>3</sub>. The chickens were divided into 2 groups (main factor) which were further divided into two subgroups (side factor). Within each subgroup, there were 6 replications consisting of 8 birds. The chickens were reared for 42 days in metal cages with a mesh floor, under standard microclimatic conditions and with an unlimited access to feed and water. They were fed starter (from 1 to 21 days of age) and grower mixtures (from 22 to 42 days of age). Two mixtures were analysed: K1 (including fish meal) and K2 (based on plant material only). The mixtures were supplemented with 62.5 µg 25-OH-D<sub>3</sub>. Over the first period of rearing (1 to 21 days of age), the most beneficial production results were recorded for birds of the group offered the mixture containing fish meal and 25-hydroxycholecalciferol. The addition of vitamin D<sub>3</sub> metabolite significantly ( $P \leq 0.05$ ) increased body weight (by 9%) as well as feed and nutrient utilization (by 6%) compared with the group fed the plant material-based mixture. The application of the mixture supplemented with 25-hydroxycholecalciferol significantly ( $P \leq 0.05$ ) reduced the weight of both thigh and tibia bones and increased ( $P \leq 0.01$ ) crude ash, calcium and phosphorus contents in the above bones of broiler chickens.

**Key words:** 25-hydroxycholecalciferol, rearing results, bone, broiler chickens

Breeding progress and the introduction of rapidly growing bird lines make breeders verify chicken needs in respect of some vitamins. At present poor broiler bone quality is a significant problem which negatively affects economic results of chicken rearing and welfare. It has been found that bones in fast growing broiler chickens are characterized by a low mineralization level and high porosity, which results in them

being more prone to injuries (Williams et al., 2000; Kwiecień, 2003 a). Nutritional factors, kinds of materials used in feeds in the first place (plant materials, fish meal, mineral supplements), condition the appropriate development of bones as they influence the appropriate supply to the body of vitamin D<sub>3</sub> which is responsible for metabolism of Ca and, indirectly P (Rath et al., 2000; Świątkiewicz et al., 2006; Kwiecień and Winiarska-Mieczan, 2008). Impaired calcium and phosphorus metabolism in the body is probably a result of disturbances in the process of cholecalciferol hydroxylation and production of active derivatives of vitamin D<sub>3</sub>, that is temporary form 25-OH-D<sub>3</sub> and active form 1.25-(OH)2-D<sub>3</sub> (Reichel et al., 1989; Soares et al., 1995; Whitehead et al., 2004). Studies carried out on the application of particularly active vitamin D<sub>3</sub> metabolites – 25-hydroxycholecalciferol and 1.25-dihydroxycholecalciferol – showed their positive effects on health, increased bird body weight and egg shell thickness, and decreased risk of bone disorders (Rennie and Whitehead, 1996; Fritts and Waldroup, 2003; Koreleski and Świątkiewicz, 2004).

The objective of this research was to evaluate the effect of adding 25-OH-D<sub>3</sub> to mixtures containing plant components only, or plant materials and fish meal on rearing results as well as physical and chemical properties of thigh (femur) and tibial bones in broiler chickens.

## Material and methods

A feeding experiment was conducted on 192 Ross 308 broiler chickens arranged in a two-factor design. Experimental factors were as follows: kind of feed raw materials applied in mixtures and supplementation or not with 25-OH-D<sub>3</sub>. All the chickens were divided into 2 groups (main factor) and each group was further divided into two subgroups (side factor). Within the subgroups there were established 6 replications, each with 8 birds. The chickens were reared for 42 days in metal cages with a mesh floor, under standard microclimatic conditions and with an unlimited access to feed and water. The birds were fed *ad libitum* and offered friable starter mixtures from 1 to 21 days of age, and, from 22 to 42 days of age, balanced grower mixtures formulated following the Nutritional Recommendations and Nutritive Value of Feeds, Poultry Nutrition Standards (2005) so as to make sure they are isoenergetic and isoprotein diets. The composition and nutritive value of the feed mixtures are presented in Table 1. The nutritive value of 1 kg of mixtures was calculated on the basis of the content of metabolizable energy, nutrients and macroelements (Ca, P, Na) in individual raw materials cited above in the Nutritional Recommendations.

The layout of the experiment was as follows:

- group 1, feed mixes K1,
- group 2, feed mixes K2,
- group 3, feed mixes K1 plus 62.5 µg · kg<sup>-1</sup> 25-OH-D<sub>3</sub>,
- group 4, feed mixes K2 plus 62.5 µg · kg<sup>-1</sup> 25-OH-D<sub>3</sub>.

Table 1. Composition (%) and nutritive value of mixtures

Raw materials	Starter		Grower	
	K1	K2	K1	K2
Ground maize	56.10	56.00	60.00	59.90
Soybean meal	30.00	35.00	25.00	31.10
Fish meal	5.00		6.00	
Soybean oil	5.00	5.00	5.00	5.00
Limestone	0.90	0.90	0.95	0.95
Dicalcium phosphate	2.00	2.00	2.05	2.00
Fodder salt NaCl	0.35	0.35	0.35	0.35
Premix Rovimix S 0.5%*	0.50	0.50		
Premix Rovimix G 0.5%**			0.50	0.50
DL – methionine 99%	0.15	0.20	0.15	0.20
L-lysine 99%		0.05		0.10
Total	100	100	100	100
Nutritive value per kg of mixture:				
metabolizable energy (MJ)	12.81	12.75	13.01	12.97
crude protein (g)	219	216	205	203
crude fibre (g)	36.07	39.34	33.90	37.83
lysine (g)	12.10	11.96	11.48	11.51
methionine (g)	5.28	5.33	5.19	5.15
methionine + cystine (g)	9.15	9.19	9.00	9.03
total Ca (g)	10.11	10.18	9.98	9.87
available phosphorus (g)	4.18	4.19	4.08	4.10
Na (g)	1.62	1.62	1.63	1.62

Manufacturer-DSM Nutritional Products Poland.

\*per kg: Vit. A (2 500 000 IU), D<sub>3</sub> (600 000 IU), E (10 000 mg), K (600 mg), B<sub>1</sub> (500 mg), B<sub>2</sub> (1 600 mg), B<sub>6</sub> (1 000 mg), B<sub>12</sub> (4 mg), Niacin (9 000 mg), Pantothenic acid (3 000 mg), Folic acid (300 mg), Biotin (40 mg), Choline (80 000 mg), Fe (10 000 mg), Mn (16 000 mg), Zn (12 000 mg), Cu (1 800 mg), J (150 mg), Co (60 mg), Se (50 mg), antioxidant and coccidiostat.

\*\*per kg: Vit. A (2 200 000 IU), D<sub>3</sub> (500 000 IU), E (8 000 mg), K (500 mg), B<sub>1</sub> (400 mg), B<sub>2</sub> (1 400 mg), B<sub>6</sub> (800 mg), B<sub>12</sub> (4 mg), Niacin (8 000 mg), Pantothenic acid (2 500 mg), Folic acid (200 mg), Biotin (30 mg), Choline (60 000 mg), Fe (9 000 mg), Mn (14 000 mg), Zn (11 000 mg), Cu (1 500 mg), J (120 mg), Co (50 mg), Se (40 mg), antioxidant and coccidiostat.

Mixtures K1 and K2 contained 3000 IU · kg<sup>-1</sup> (starter) and 2500 IU kg<sup>-1</sup> (grower) vitamin D<sub>3</sub> Preparation Hy•D<sup>®</sup> Beadlet 1.25%, DSM Nutritional Products (at a dose of 0.5g · kg<sup>-1</sup>), whose 1 g corresponds to 5000 IU of vitamin D<sub>3</sub>, used as a source of 25-OH-cholecalciferol. The following conversion was applied: 1 IU of cholecalciferol = 0.025 µg 25-OH-D<sub>3</sub>. During the experiment chicken body weight was recorded (1, 21 and 42 days) in addition to feed consumption, health status and potential incidences of bird mortality. At rearing termination 10 birds whose body weight approximated the group mean were selected from each feeding group (5 males and 5 females). They were slaughtered and the carcasses were dissected according to the

methodology described by Ziółcki and Doruchowski (1989). The data obtained were used to calculate slaughter performance. Thigh bones and tibial bones with a calf bone were prepared and their weight and length were determined according to the methodology by Antoniewicz et al. (1992). In the feed mixtures chemical determination was conducted of the content of essential nutrients according to AOAC (2000) as well as of calcium and total phosphorus. The phosphorus level was determined by the colourimetric method using eikonogen as a developer (PN-76/R-64781), and calcium content by means of an absorption method with the air-acetylene flame, on the basis of a standard curve and applying a Carl Zeiss Jena AAS-30 spectrometer. The results obtained throughout the study were statistically analysed according to the model for two-factor variance analysis of an orthogonal design. Significance of differences between group means was checked by Tukey test (STATSOFT, 2001).

## Results

Type of feed raw materials used in mixtures (plant-derived only or plant-derived containing fish meal) influenced broiler chicken body weight obtained after the period of feeding starter mixtures as well as at rearing termination (Table 2). Following the first period of rearing, the chickens offered mixtures containing fish meal were on average by 25 g heavier, and on 42 days of age by 125 g heavier ( $P \leq 0.05$ ) compared with the birds fed mixtures based on plant materials. In contrast, an introduction to the mixtures of 25-hydroxycholecalciferol in the amount of  $62.5 \mu\text{g} \cdot \text{kg}^{-1}$  increased chicken body weight by 9% on 21 days of age, the difference being statistically significant ( $P \leq 0.05$ ). Higher body weight results for group 3 birds were reflected in the amount of feed consumed and nutrients taken in per one unit of gain which, for the first period of rearing, was by about 6% lower ( $P \leq 0.05$ ). Over the whole 6-week rearing period, chickens offered mixtures including fish meal (K1) on average consumed over 3% less feed ( $P \geq 0.05$ ) per unit of gain than the birds fed plant material-based mixtures (K2). Crude protein and metabolizable energy intake was similar to feed consumption because the mixtures for individual groups had the same feeding value.

An analysis of results of post-slaughter assessment showed no statistically significant differences between groups. The average slaughter performance of birds in the experiment was 74%. No interaction between the examined factors (kind of mixture  $\times$  25-OH- $\text{D}_3$ ) was confirmed for any of the characteristics analysed (Table 2).

Table 3 presents results of physical characteristics of broiler chicken thigh and tibial bones. An addition of 25-hydroxycholecalciferol to feed mixtures reduced the relative weight of both thigh bone (from 3.74 to  $3.45 \text{ g} \cdot \text{kg}^{-1}$ ) and tibial bone (from 5.37 to  $5.03 \text{ g} \cdot \text{kg}^{-1}$ ) of chickens. The difference was highly significant ( $P \leq 0.01$ ) and significant ( $P \leq 0.05$ ), respectively. Type of feed raw materials used to formulate the mixtures (plant-derived only or plant-derived and supplemented with fish meal) and an addition of 25-OH- $\text{D}_3$  did not have a significant impact on the length of bird bones assessed. However, there was observed a small reduction (of about 2%) in bone length of group 3 birds fed mixtures containing fish meal and vitamin  $\text{D}_3$  metabolite, although the differences were not statistically confirmed. Similarly to the re-

sults of broiler chicken rearing, there was found no interaction between factors under investigation (kind of mixture  $\times$  25-OH-D<sub>3</sub>) for any of the characteristics analysed (Table 3).

Table 2. Results of rearing broiler chickens

Item	25-OH-D <sub>3</sub>	Mixture K1	Mixture K2	Mean	SEM	The influence of analysed factors		
						25-OH-D <sub>3</sub>	mixture	interaction
Body weight (g):								
initial	-	37.7	37.7	37.7		NS	NS	NS
	+	37.0	38.0	37.5	0.23			
	$\bar{x}$	37.3	37.8					
on day 21	-	528	516	522 a				
	+	600	563	581 b	7.29	*	*	NS
	$\bar{x}$	564 b	539 a	552				
on day 42	-	2402	2246	2324				
	+	2447	2352	2400	29.24	NS	*	NS
	$\bar{x}$	2424 b	2299 a					
Conversion per kg of gain:								
1–21 days:								
feed (kg)	-	1.72	1.73	1.72 b				
	+	1.53	1.69	1.61 a	0,03	*	*	NS
	$\bar{x}$	1.61 a	1.71 b					
crude protein (g)	-	360	359	359 a				
	+	325	363	344 b	6.82	*	*	NS
	$\bar{x}$	343 a	361b					
metabolizable energy (MJ)	-	21.01	20.64	20.82 b				
	+	18.98	20.93	19.95 a	0.38	*	*	NS
	$\bar{x}$	19.99 a	20.78 b					
1–42 days:								
feed (kg)	-	1.80	1.82	1.81				
	+	1.74	1.79	1.77	0.02	NS	NS	NS
	$\bar{x}$	1.77	1.80					
crude protein (g)	-	359	359	359				
	+	350	352	351	2.04	NS	NS	NS
	$\bar{x}$	355	356	355				
metabolizable energy (MJ)	-	22.09	22.19	22.14				
	+	21.70	21.82	21.76	0.11	NS	NS	NS
	$\bar{x}$	21.90	21.95	21.95				
Dressing percentage	-	74.30	73.81	73.81				
	+	74.54	74.84	74.84	0.21	NS	NS	NS
	$\bar{x}$	74.42	74.32	74.32				

The Table presents  $F_{\text{emp}}$  value and level of significance \*\*  $P \leq 0.01$ ; \*  $P \leq 0.05$ ; NS – non-significant differences. The data in the Table are arithmetic means  $\pm$  standard deviation; A, B – significant difference for the analysed traits ( $P \leq 0.01$ ); a, b – significant difference for the analysed traits ( $P \leq 0.05$ ).

Table 3. Weight and length of bones

Item	25-OH-D <sub>3</sub>	Mixture K1	Mixture K2	Mean	SEM	The influence of analysed factors		
						25-OH-D <sub>3</sub>	Mixture	Interaction
Relative weight (g · kg <sup>-1</sup> of body weight):								
femur	-	3.71	3.77	3.74 B	0.06	**	NS	NS
	+	3.50	3.41	3.45 A				
	$\bar{x}$	3.61	3.59					
tibia	-	5.49	5.24	5.37 b	0.10	*	NS	NS
	+	5.01	5.05	5.03 a				
	$\bar{x}$	5.25	5.14					
Length (mm):								
femur	-	79.3	76.2	77.7	0.62	NS	NS	NS
	+	77.7	76.6	77.1				
	$\bar{x}$	78.5	76.4					
tibia	-	108.3	104.3	106.3	1.07	NS	NS	NS
	+	106.2	103.2	104.7				
	$\bar{x}$	107.2	103.7					

The Table presents  $F_{emp}$  value and level of significance \*\*  $P \leq 0.01$ ; \*  $P \leq 0.05$ ; NS – non-significant differences. The data in the Table are arithmetic means  $\pm$  standard deviation; A, B – significant difference for the analysed traits ( $P \leq 0.01$ ); a, b – significant difference for the analysed traits ( $P \leq 0.05$ ).

The application of vitamin D<sub>3</sub> metabolite caused changes in chemical composition of broiler chicken bones (Table 4). Crude ash content increased in both thigh bone (from 520.8 g to 530.4 g · kg<sup>-1</sup>) and tibial bone (from 529.9 to 538.7 g · kg<sup>-1</sup>) of chickens fed diets supplemented with 25-hydroxycholecalciferol irrespective of the types of raw materials used in the mixtures, the differences being highly significant ( $P \leq 0.01$ ). Both bones had significantly higher ( $P \leq 0.01$ ) calcium contents (by 3% for thigh and by 2% for tibial bone) and phosphorus contents (by 2% for both bone types), which indicates that vitamin D<sub>3</sub> metabolite beneficially affected the bone chemical composition. There was found an interaction between the factors examined (kind of mixture  $\times$  25-OH-D<sub>3</sub>) for dry matter and phosphorus (thigh bone), as well as calcium (tibial bone).

Table 4. Chemical composition of bones

Item	25-OH-D <sub>3</sub>	Mixture K1	Mixture K2	Mean	SEM	The influence of analysed factors		
						25-OH-D <sub>3</sub>	Mixture	Interaction
1	2	3	4	5	6	7	8	9
Femur								
Dry matter (g · kg <sup>-1</sup> )	-	909.2	905.0	907.1	0.38	NS	**	**
	+	908.2	908.1	908.1				
	$\bar{x}$	908.7 B	906.6 A					
Crude ash (g · kg <sup>-1</sup> )	-	520.5	521.1	520.8 A	1.65	**	NS	NS
	+	534.7	526.1	530.4 B				
	$\bar{x}$	527.6	523.6					

Table 4 – contd.

1	2	3	4	5	6	7	8	9
Ca	-	182.4	179.1	180.7 A				
(g · kg <sup>-1</sup> )	+	188.4	184.4	186.4 B	0.72	**	**	NS
	$\bar{x}$	185.4 B	181.7 A					
P	-	90.0	94.4	92.2 A				
(g · kg <sup>-1</sup> )	+	94.4	92.6	93.5 B	0.41	**	**	**
	$\bar{x}$	92.2 A	93.5 B					
Tibia								
Dry matter	-	907.7	910.5	907.1				
(g · kg <sup>-1</sup> )	+	910.6	907.0	908.1	0.34	NS	NS	NS
	$\bar{x}$	909.2	908.7					
Crude ash	-	527.9	531.9	529.9 A	1.28			
(g · kg <sup>-1</sup> )	+	540.0	537.5	538.7 B		**	NS	NS
	$\bar{x}$	533.9	534.7					
Ca	-	180.5	183.5	182.0 a				
(g · kg <sup>-1</sup> )	+	185.1	183.1	184.1 b	0.62	*	NS	*
	$\bar{x}$	182.8	183.3					
P	-	98.5	99.6	99.0 A				
(g · kg <sup>-1</sup> )	+	101.0	101.0	101.0 B	0.37	**	NS	NS
	$\bar{x}$	99.7	100.3					

The Table presents  $F_{\text{emp}}$  value and level of significance \*\*  $P \leq 0.01$ ; \*  $P \leq 0.05$ ; NS – non-significant differences. The data in the Table are arithmetic means  $\pm$  standard deviation; A, B – significant difference for the analysed traits ( $P \leq 0.01$ ); a, b – significant difference for the analysed traits ( $P \leq 0.05$ ).

## Discussion

A beneficial effect of 25-OH-D<sub>3</sub> added to the starter mixture on chicken growth and feed utilization may result from the fact that, over the examined period, the enzymatic system of not all chickens is fully efficient, which is necessary for vitamin D<sub>3</sub> hydroxylation in the liver (Soares et al., 1995). After the end of rearing there was a tendency towards an improvement in production results following the addition of 25-hydroxycholecalciferol to mixtures containing either plant-derived materials (K2) only or the plant-derived feed containing fish meal (K1). The results obtained correspond with the results of other authors (Świątkiewicz et al., 2006) who observed a slight improvement of broiler rearing indicators following an addition of 25-OH-D<sub>3</sub> to mixtures whose calcium and phosphorus levels complied with the Nutritional Recommendations and Nutritive Value of Feeds, Poultry Nutrition Standards (2005). A more beneficial influence of 25-hydroxycholecalciferol on chicken productivity was recorded after cholecalciferol was completely substituted with this metabolite (Fritts and Waldroup, 2003; Yarger et al., 2005).

Slaughter performance results obtained in the present study fully corresponded with the findings of Janocha et al. (2004). The authors showed that neither the protein kind applied in mixtures (plant, animal) nor the addition of premix Hy•D<sup>®</sup> signifi-

cantly influenced slaughter performance which averaged 74.2%.

There is a wealth of data in the available literature on the impact of feeding on physical and mechanical characteristics of broiler chicken bones. Results that were similar to the outcome of the discussed study were obtained by Kwiecień (2003 a) who assessed the tibial bone weight and length of chickens representing different breeding lines. The author found that the highest bone weight was in birds fed mixtures whose levels of minerals complied with the Nutritional Recommendations and Nutritive Value of Feeds, Poultry Nutrition Standards (2005). In turn, doubling the Ca rate caused a slower increase in bone weight. Świątkiewicz et al. (2006) applied 25-OH-D<sub>3</sub>-supplemented mixtures and found 14% lower relative thigh bone weight compared with the weight obtained in the present work. However, the above authors showed that combining vitamin D<sub>3</sub> metabolite and phytase gave even better results, which indicated that the feed supplements complemented each other. Similarly, Puzio et al. (2004) reported that a simultaneous introduction of 1.25(OH)<sub>2</sub>D<sub>3</sub> and phytase clearly improved physical and resistance characteristics of thigh bones of broiler chickens.

Macroelement content obtained in the bones analysed indicated that there was a positive effect of adding 25-OH-D<sub>3</sub> to mixtures containing plant materials only or fish meal on chemical properties of thigh and tibial bones. Values for tibial bone fully corresponded with the values reported by Kwiecień (2003 b) in respect of calcium, but they were much lower as far as phosphorus was concerned. They also differed from the values obtained by Pisarski and Kwiecień (2003). It is difficult to confirm the comparability of the results as the authors examined slightly different experimental factors influencing the chemical composition of broiler chicken bones.

The rearing results obtained in the discussed study indicate that an application of vitamin D<sub>3</sub> metabolite (25-OH-D<sub>3</sub>) is recommended especially in the first period of broiler chicken life. The application of mixtures containing 25-hydroxycholecalciferol was followed by reduced relative weight of thigh and tibial bone, as well as increased crude ash, calcium and phosphorus contents in the above bones.

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Accepted for printing 3 XI 2009

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### **Wpływ dodatku 25-hydroksycholekalcyferolu do diet zawierających surowce roślinne lub mączkę rybną na wyniki odchowu oraz kości kurcząt brojlerów**

#### STRESZCZENIE

Celem przeprowadzonych badań było określenie wpływu dodatku 25-hydroksycholekalcyferolu do mieszanek zawierających mączkę rybną lub wyłącznie surowce roślinne na wyniki odchowu oraz cechy fizykochemiczne kości udowej i piszczelowej kurcząt brojlerów. Doświadczenie żywieniowe wykonano na 192 kurczętach brojlerach Ross 308 w układzie dwuczynnikowym. Czynniki doświadczalnymi był rodzaj zastosowanych surowców paszowych w mieszankach oraz dodatek 25-OH-D<sub>3</sub>. Wszystkie kurczęta zostały podzielone na 2 grupy (czynnik główny) z dwiema podgrupami każda (czynnik uboczny), w obrębie podgrupy wyodrębniono 6 powtórzeń po 8 ptaków. Kurczęta odchowywano przez 42 dni w metalowych klatkach z siatkową podłogą, w standardowych warunkach mikroklimatycznych, ze stałym dostępem do paszy i wody, żywiąc je mieszankami starter (od 1. do 21. dnia życia) i grower (od 22. do 42. dnia). Przedmiotem badań były mieszanki K1 (z mączką rybną) i K2 (wyłącznie surowce roślinne). Do mieszanek doświadczalnych wprowadzono 62,5 µg 25-OH-D<sub>3</sub>. W pierwszym okresie odchowu (1.–21. dzień życia) najkorzystniejsze wyniki produkcyjne uzyskano dla ptaków grupy otrzymującej mieszankę z udziałem mączki rybnej i 25-hydroksycholekalcyferolu. Dodatek metabolitu witaminy D<sub>3</sub> pozwolił na istotne ( $P \leq 0,05$ ) zwiększenie o 9% masy ciała i o 6% wykorzystanie paszy i składników pokarmowych w porównaniu do grupy otrzymującej mieszankę sporządzoną w oparciu o surowce roślinne. Stosując mieszanki uzupełnione dodatkiem 25-hydroksycholekalcyferolu odnotowano istotne ( $P \leq 0,05$ ) zmniejszenie względnej masy kości udowej i piszczelowej oraz zwiększenie ( $P \leq 0,01$ ) zawartości popiołu surowego, wapnia i fosforu w tych kościach u kurcząt brojlerów.