THE SYNERGIC EFFECT OF VITAMIN D_3 AND 25-HYDROXY-CHOLECALCIFEROL/CALCIDIOL IN BROILER DIET

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The aim of the present study was to test partial replacement of vitamin D_3 in broiler nutrition through a newly developed dietary supplement of an active metabolite of vitamin D_3 (calcidiol) and confirmation of an effect of such replacement for an improvement of growth and calcium and phosphorus metabolism in the organism. The EU Commission Regulation No 1443/2006 is authorising the use of 25-OH D_3 in feeds for chickens, fattening hens, laying hens, and turkeys. The trial was carried out in field conditions and the following parameters were studied: live weight, feed conversion, selected hematological and biochemical parameters and the quality of bones. The synergic effect of an addition of 25-hydroxycholecalciferol (hereinafter 25-OH D_3) and vitamin D_3 was tested in the trial on ROSS 308 broiler chicks. The 25-OH D_3 is manufactured by the DSM Nutritional Products Ltd. under the trade name Hy-D. Statistically significant differences between the control and experimental group were ascertained in live weight ($p < 0.01$). At an age of 37 days, the live weights of the control and experimental groups were 2164 g and 2274 g, respectively. As for calcium content in fat-free dry matter of bones, a statistically significant difference was ascertained ($p < 0.05$) between the control (200 $mg.g^{-1}$) and experimental (208.7 $mg.g^{-1}$) group. Phosphorus content in the same sample was also statistically significant between the control (99.2 $mg.g^{-1}$) and experimental (102.4 $mg.g^{-1}$) groups. Other parameters of bone quality, feed conversion, selected hematological and biochemical parameters were not statistically significant.

broiler; vitamin D_3 ; 25-OH D_3; blood; bone; Ca; P

INTRODUCTION

Vitamin D originates from provitamins ergosterol and 7-dehydrocholesterol based on activity of sun radiation. In poultry, vitamin D_2 possesses only one tenth of the biological activity of vitamin D_3 (Papešová et al., 1992).

Vitamin D is added to diets in its crystallic form as cholecalciferol (vitamin D_3). This metabolite is converted to 25-hydroxycholecalciferol (calcidiol, 25-OH D_3) in the liver, and further it is converted in kidneys to the active metabolite 1.25-dihydroxycholecalciferol (1.25-$(OH)_2$ D_3). 1.25-dihydroxycholecalciferol (calcitriol) supports calcium and phosphorus absorption in the intestine, affects bone calcification, co-participates in calcium and phosphorus metabolism in the organism. Receptors for 1.25-dihydroxycholecalciferol are present in many tissues, including blood elements (Fritts et al., 2004). A lack of vitamin D in youngsters causes a skeletal disease called rickets (rachitis), whereas in adults it induces decalcification of bones (osteoporosis). Vitamin D_3 in the form of calcitriol has a direct impact on the bone, improves mineralization of bones in young chicks, maintains balanced calcium and phosphorus levels in the organism, reduces an incidence of disorders of extremities and tibial dyschondroplasia (Whitehead, 1992).

The dietary supplement 25-OH D_3 for poultry provides a higher source of calcidiol for the organism to produce active metabolite calcitriol, enhancing thus a supply of the functional metabolite of vitamin D to organism. At present, calcidiol is commercially available for poultry industry. This product does not exhibit toxicity even when a 10-fold value of the recommended ratio of 69 µg/kg is used on a short-term basis (Yarger et al., 1995). Values of long-term toxicity of 25-OH D_3 are presented very similarly as in vitamin D_3. Results of three month toxicity of vitamin D_3 that amount to even 4 fold multiple of the usual nutritional dose is completely safe on very long term feeding (Papešová , 2000).

The absorption of vitamin D in the intestine is a two-stage process, with its first part consisting in a quick vitamin D supply to mucous membranes and its slow transfer to lymphatic vessels. The first quick stage requires the presence of gall acids, while at the second slow stage it is bound to chylomicrons formed. The absorption takes place in the upper small intestine where the highest amount of fats is absorbed (Broulík , 2002).

At the recommended dose of 4000–5000 IU of vitamin D3/kg diet, a combination of 2000–2500 IU of vitamin D3/kg diet and 50–62.5 µg of calcidiol (HyD Use Based on Regulation No 1443/2006) is presented as the comparable activity. The recommended dose of calcidiol in a kilogram of diet is 50–70 µg. This range represents a physiological and, at the same time, economic optimum.

MATERIAL AND METHODS

The synergic effect of an addition of calcidiol (25-OH D_3) and vitamin D_3 was tested in a trial on ROSS broiler chicks.

Trials were carried out based on the scheme as in Table 1.

Table 1. Experimental scheme

Group	Control	Trial
Number of birds	13 900	13 900
Vitamin D_3 dose in UI .kg^{-1} diet	5 000	2 500
25-OH D_3 dose in µg .kg^{-1} diet	0	50
Duration of the trial (length of fattening)*	37 days	37 days

Test substances

Calcidiol contained in the dietary supplement: ROVIMIX® Hy-D® 1.25 % (manufactured by DSM Nutritional Products) and vitamin D_3 contained in the dietary supplement ROVIMIX D_3 500.

Experimental animals

An impact of a 25-OH D_3 addition to a diet was tested on ROSS 308 meat broilers from Xavergen a.s., hatchery Habry. The mean live weight of broilers at all-in was 44 g.

Housing, drinking, way of feeding

Broilers were housed in two air-conditioned halls with controlled heat and light regimen according to the technological process, water supply and feeding from Big-Dutchmann. On days 20 and 37, broilers were weighed and 6–8 animals from each groups was selected according to their mean mass, killed via decapitation and subsequently, their blood was sampled.

Experimental diet

Broilers were fed granulated diets BR 1 starter, BR 1A (crushed), BR 2A (small granules), BR 2B and BR 3 (large granules). The composition and content of nutrients are given in Table 2. Diets were isoenergic and isonitrogenic. They were supplied by the company ZZN Pardubice. Premixes were manufactured and supplied by the company Biofaktory Praha, s.r.o. Anticoccidial agents were used in BR 1 starter, BR 1A and BR 2A – Maxiban (Narazin and Nikarbazin) and in BR 2B – Monenzin**.** The substances tested were administered into the diet through premixes manufactured separately for the experimental and control group.

Parameters studied

The following production parameters were studied: live weight (in grams) through weighing on days 20 and 37 of fattening of chicks and feed conversion (kg feed/kg live weight) on days 1–37 of chick fattening.

Always the same chickens were weighed. The health state is expressed by a number of dead animals (%) and they were collected twice daily. Values of red blood cell count (Er – the number of erythrocytes, Hk – the value of hematocrite, Hb – the value of hemoglobin, MCV – mean

Table 2. Composition of diets

Components in %	BR Starter	BR 1	BR 2A	BR 2B	BR 3
Monocalcium phosphate	1.1	1	0.85	0.7	0.55
Fodder wheat meal	–	–	2	5	5
Wheat	15.41	22.88	26.07	22.76	20.73
Triticale	–	–	5	7	10
Sojax	7.1	4	3.2	4.7	6.4
Soya A	31	30.5	26.2	21	13
Maize	38	30	21	20	22
Peas	2.5	3	3	4	5
Expellers	–	2	4.5	5	7
Vitex – fodder yeast	2	2	1.5	3	4
L-lysine	0.33	0.24	0.18	0.16	0.09
DL-methionine	0.33	0.3	0.23	0.23	0.16
L-threonine	0.07	0.06	0.03	0	–
Oil	–	1.9	4.3	4.5	4.2
Alimet – liquid methionine hydroxyl analog	0.03	0.05	0.04	0.05	0.05
Limestone	1.25	1.3	1.2	1.2	1.15
Salt	0.13	0.17	0.18	0.15	0.17
Soda	0.45	0.3	0.25	0.25	0.2
Aminovitan-vitamin premix BR1 Maxiban*	0.3	0.3	0.27	–	–
Aminovitan BR2 vitamin premix Maxiban*	–	–	–	0.3	–
Aminovitan vitamin premix BR3	–	–	–	–	0.3

* (Trade name) it is a combination of an ionofore and chemical anticoccidial

cell volume) were determined using a MEK 5208K hematological analyzer. Concentrations of Ca and P in blood plasma were determined by means of RANDOX Laboratories, s.r.o., kits. The fresh bone was prepared, cleaned, transferred to refrigerator at 4°C and transferred to the laboratory for processing. Determinations of the quality of bones (ash, Ca, and P contained in tibia – shank bone) were carried out by the company Analytické laboratoře Plzeň a.s.

Statistical methods

Basic statistical characteristics were determined for all parameters studied by the two-sample *t*-test.

RESULTS AND DISCUSSION

The influence of an addition of 25-hydroxycholecalciferol and vitamin D_3 administered to broilers was tested. The trial lasted for 37 days and two samplings were made. The first was made at an age of 20 days, the second – at an age of 37 days.

Results of the trial are presented in Tables 3, 4, 5 and 6.

The recommended dosage is 50–75 micrograms of 25-OH D_3/1kg of diet in combination with 2500 I.U. of vitamin D_3.

62.5 micrograms/1 kg of diet and 2500 I.U. of vitamin D_3 are considered by the manufacturer the most optimum combination from the point of view of efficacy, stability and the economic optimum. According to the experiments mentioned below, 50 micrograms per 1 kg of diet seem to be also a very efficient level. This dose was also tested in our experiment.

25-OH D_3, in comparison with identical levels of vitamin D_3, exhibits an improvement of body weight, feed conversion, bone ash and breast muscle yield and reduces an incidence of tibial dyschondroplasia and rickets (P a r k i n s o n , C r a n s b e r g , 2004) concludes that when calcidiol is added into diets for broiler chicks suffering from malabsorption syndrome, the concentration of 25-OH D_3 increases in plasma, contrast to groups fed a diet containing only vitamin D_3. He also recorded an enhancement of the mean daily weight gain, a reduction of feed conversion, an improvement of the quality of bones and a decrease of mortality.

An improving effect on the development of breast muscles, the yield and the quality of meat was observed by K o r v e r and B l a d e s (2006) when calcidiol was used. They reported an improvement of mineralization and solidity of bones. Calcidiol added in parental egg-laying flocks resulted in an improved immune response to pathogenic bacteria in broilers.

Results of the trial performed correspond to a study by F r i t t s and W a l d r o u p (2003) who recorded a statistically significant elevation of the final weight of chicks and a significantly lower feed conversion than in groups fed a diet containing vitamin D_3. When evaluating the red

Table 3. Production parameters and health status

Group	Control		Trial	
Dose of vitamin D_3 in UI .kg^{-1} diet	5000		2500	
Dose of 25-OH D_3 in µg .kg^{-1} diet	0		50	
	mean	*s*	mean	*s*
Mean weight on day 1 of age (g)**	44		44	
Mean weight on day 20 of age (g)**	788	13	813*	16.9
Mean weight on day 37 of age (g)**	2164	63	2274*	65
Feed conversion on days 1–37	1.77		1.67	
Index (%)	100		94.5	
Mortality on days 1–37 (%)	3.02		2.89	

* $P < 0.01$ – the difference is statistically significant in comparison with the control
** The mean mass was obtained by weighing of 100 birds from both groups
s – standard deviation
n – number of birds

Table 4. Values of red blood cell picture

Group	Control on day 20		Trial on day 20		Control on day 37		Trial on day 37	
n	7		7		7		7	
	mean	*s*	mean	*s*	mean	*s*	mean	*s*
Er (T.l^{-1})	2.92	0.16	2.88	0.16	3.13	0.21	3.33	0.34
Hk (%)	43	2.33	43	2.58	43	3.25	43	4.75
Hb (g.100 ml^{-1})	13.34	0.7	13.01	0.71	14.81	0.85	15.25	1.3
MCV (fl)	149	2.54	150	4.16	140	5.03	130	16.78

The values between groups were not statistically significant

Table 5. Concentrations of Ca and P in blood plasma

Group	Control on day 20		Trial on day 20		Control on day 37		Trial on day 37	
n	8		8		7		7	
	mean	*s*	mean	*s*	mean	*s*	mean	*s*
Ca (mmol.l^{-1})	3.1	0.4	2.88	0.27	2.91	0.24	2.75	0.3
P (mmol.l^{-1})	2.03	0.31	1.87	0.26	1.72	0.5	2.08	0.1

The values between groups were not statistically significant

Table 6. The quality of bones

Group		Control		Trial	
n		6		7	
		mean	*s*	mean	*s*
Specific weight	g.cm^{-3}	2.41	0.15	2.66	0.37
Dry matter	g.kg^{-1}	662.58	19.23	683.93	17.46
FF DM	g.kg^{-1}	535.5	19.94	530.16	12.54
Ash in bones	g.kg^{-1}	295.01	14.81	294.58	9.68
Ash in FF DM	g.kg^{-1}	550.83	15.46	555.86	20.28
Ash in cm^3	g.cm^{-3}	0.712	0.073	0.782	0.107
Ca in g of bone	mg.g^{-1}	107.13	5.99	110.64	3.87
P in g of bone	mg.g^{-1}	53.11	2.37	54.3	1.82
Ca in g of FF DM	mg.g^{-1}	200.00	6.16	208.72*	6.07
P in g of FF DM	mg.g^{-1}	99.17	1.83	102.43*	2.64
Ca in cm^3	mg.cm^{-3}	258.73	27.42	293.34	37.64
P in cm^3	mg.cm^{-3}	128.17	11.79	144.04	19.03

* $P < 0.05$ – the difference is statistically significant in comparison with the control
FF DM – fat-free dry matter

blood cell picture, no significant differences were observed. A similar conclusion was reached by Fritts et al. (2004) in their study. Evaluations of calcium and phosphorus supply to the organism considered levels of the two elements in blood. No statistically significant differences were recorded between groups in the trial.

When analyzing bone mineralization, an improvement of all parameters was recorded, particularly as regards calcium and phosphorus contents in fat-free dry matter. Bones were more compact, what could has indicated a lower incidence of leg disorders , f.e. tibial dyschondroplasia, rickets. Similar results were obtained by Rennie and Whitehead (1996), or by Bar (2003). Tibial dyschondroplasia is one of the most important leg disorder in modern high intensity growth rate broilers. There are typical lesions like enlarged metaphysis, accumulation of a vascular cartilage, tibial bowing and osteomyelitis. Typical etiology is high P, low Ca diet, vitamin D3 deficiency and others– e.g. genotype, $Na^+ : Cl^-$ imbalance, heat stress and mycotoxins (Riddel, 1981, 1992). Administration of vitamin D3 and 25-OH D3 altogether proved significant drop of incidence of leg abnormalities (Thorp , 1992).

CONCLUSION

The addition of 25-OH D_3 /25-hydroxycholecalciferol/ or calcidiol respectively, in combination with vitamin D_3 in a diet for ROSS 308 meat broilers was tested. Statistically significant differences between the control and experimental group were ascertained in live weight ($p < 0.01$). At an age of 37 days, the live weight of the control group was 2164 g, whereas that of the experimental group was 2274 g. As for the calcium content in the fat-free dry matter of the bone, a statistically significant difference was ascertained ($p < 0.05$) between the control (200 mg.g^{-1}) and the experimental (208.7 mg.g^{-1}) group. The content of phosphorus in the same sample was also statistically significant between the control (99.2 mg.g^{-1}) and experimental (102.4 mg.g^{-1}) group. Other parameters of bone quality, feed conversion, selected hematological and biochemical parameters were not statistically significant. The parameters studied confirm a positive influence of an addition of 25-OH D_3 into the ROSS 308 broiler diet. The EU Commission Regulation No 1443/2006 is authorizing the use of 25-OH-D_3 in feeds for chickens, fattening and laying hens and turkeys.

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Synergický účinek vitaminu D_3 a 25-hydroxycholekalciferolu (kalcidiolu) ve výkrmu brojlerů.

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Synergický účinek přídavku 25-OH D_3 a vitaminu D_3 byl ověřen v pokuse na brojlerových kuřatech typu ROSS 308 (doba výkrmu 37 dní). Pokusy proběhly v provozních podmínkách a byla sledována živá hmotnost, konverze krmiva, vybrané hematologické a biochemické ukazatele a kvalita kostí. Statisticky významné rozdíly ($p < 0,01$) mezi kontrolní a pokusnou skupinou byly zjištěny u živé hmotnosti. Živá hmotnost kuřat v kontrolní skupině byla 2 164 g, zatímco v pokusné skupině byla 2 274 g. Statisticky významné rozdíly ($p < 0,05$) mezi kontrolní a pokusnou skupinou byly také zjištěny u obsahu vápníku (200 $mg.g^{-1}$ a 208,7 $mg.g^{-1}$) a fosforu (99,2 $mg.g^{-1}$ a 102,4 $mg.g^{-1}$) v tukuprosté sušině kostí. Ostatní sledované parametry byly statisticky nevýznamné. Výsledky potvrdily pozitivní vliv přídavku 25-OH D_3 do krmných směsí pro brojlery. Předpis Evropské komise No. 1443/2006 povoluje použití kalcidiolu v krmných směsích pro kuřata, výkrm brojlerů a pro nosnice a krůty.

brojler; vitamin D_3 ; 25-OH D_3; krev; kost; Ca; P

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