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EFFECTIVENESS OF VITAMIN D_3 AND CALCIDIOL (25-OH-D_3) APPLICATION IN FEEDING BROILER CHICKENS – PRODUCTION PERFORMANCE AND MEAT QUALITY

Monika Michalczuk[1], Dorota Pietrzak[2], Jan Niemiec[1], Jan Mroczek[2]

[1]Faculty of Animal Science, [2]Faculty of Food Technology; Warsaw University of Life Sciences, Warsaw, Poland

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The experiment was carried out on 720 Cobb 500 broiler chickens, reared to 42 days. Various forms of vitamin D_3 were the group-differentiating factors (cholecalciferol and calcidiol), given in starter, grower and finisher feed mixtures. The control group (group I) received the feed mixture which contained 4000 IU of vitamin D_3 (cholecalciferol), group II received 2500 IU of vitamin D_3 and 1500 IU of calcidiol (25-OH-D_3) and group III – 1240 IU of vitamin D_3 and 2760 IU of calcidiol (25-OH-D_3). The aim of the study was to determine the effect of different forms of vitamin D_3 (cholecalciferol and calcidiol) in feed on production yield and meat quality.

The results showed the usefulness of partial replacement of vitamin D_3 with calcidiol. With the application of 1500 IU (25-OH-D_3) in the diet of broiler chickens, higher body weight at the end of the rearing period was obtained in comparison to the birds from the control group. The chickens from group III, receiving 2760 IU of calcidiol in their diet, had lower body weight on the 42nd day in comparison to the chickens from group II; but also a lower mortality and the lowest feed conversion per one kg of body weight gain was observed in that group. The partial replacement of vitamin D_3 with calcidiol in chickens' nutrition did not have any effect on dressing percentage of males and females; differences were only found in the percentage of offals, especially in the increased heart mass in males from group III.

Improvement was observed in the physicochemical properties (higher water absorption and lower drip after thermal treatment) and in the chemical composition of meat in the group of chickens fed the diet with the addition of calcidiol. Leg muscles from the chickens from group II had higher protein content. Fat in leg muscles and abdominal fat of the chickens from the experimental groups (group II and III) included a significantly higher quantity of monounsaturated fatty acids and a lower content of polyunsaturated acids, especially from the *n*-6 family, as compared to the control group. Moreover, the addition of calcidiol in the mixtures caused a decrease in the rate of oxidation of lipids in abdominal fat of the chickens in comparison to the control group.

INTRODUCTION

Selection conducted on broiler chickens resulted in a higher increase in the mass of breast muscles than of leg muscles and of skeleton [Świerczewska *et al*., 2002]. These disproportions are the reason for the observed bone problems in fast growing lines of meat poultry [Havenstein *et al*., 1988a,b]. Insufficient mineralization of skeleton bones reduces stability of leg bones in fast growing chickens [Nicholson, 1998; Williams *et al*., 2000]. Leg disorders deteriorate the quality of meat from leg and are the reason for economic losses in processing. Vitamin D_3 is one of the nutritional factors which is important for the correct development of the skeleton. Under conditions of commercial intensive production, synthesis of vitamin D_3 from 7-dehydrocholesterol in the skin is very low due to the missing UV radiation [Jones *et al*., 1998]. Industrial vitamin preparations contain vitamin D_3 in a form of cholecalciferol which has to be transformed in the organism into the biologically-active form of 25-hydroxycholecalciferol. Cholecalciferol is transported in the organism *via* blood, under co-participation of specific protein fraction (DBP). Cholecalciferol reaches liver in the bound form. In the liver, an OH group is added in position "25" by the action of the enzyme 25-hydroxylase [Świątkiewicz *et al*., 2006]. The first metabolically-active form, calcidiol (25-OH-D_3), is generated which is transported to kidneys and is transformed (hydroxylation in position "1") into calcitriol (1, 25-$(OH)_2$-D3) [Soares *et al*., 1995]. Biological synthesis of calcitriol in kidneys is regulated by parathormone. The biological activity of calcitriol is many times higher than that of 25-OH-cholecalciferol [Aslam *et al*., 1998]. Utilization of calcidiol in poultry nutrition is of special significance in cases of pathological states of liver, its degeneration and malfunction [Yarger *et al*., 1995; Edwards, 2000; Bar *et al*., 2003; Fritts & Waldroup, 2003].

The aim of the present study was to determine the effect of vitamin D_3 form (cholecalciferol and calcidiol) in feed mixtures on production yield, carcass dressing percentage, proximate chemical composition and technological properties of chicken meat and the composition of fatty acids in their intramuscular and abdominal fat.

MATERIALS AND METHODS

The studies were conducted in the experimental farm of Warsaw University of Life Sciences (SGGW, Warsaw, Poland) on 720 broiler chickens COBB 500, divided at random

Author's address for correspondence: Monika Michalczuk, Faculty of Animal Science, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland; tel.: (48 22) 593 65 55; fax (48 22) 853 09 14; e-mail: monika_michalczuk@sggw.pl

into three feeding groups, each in five repetitions. The group-differentiating factor included the content of various forms of vitamin D_3 (cholecalciferol and calcidiol), and addition of starter, grower and finisher type feed mixtures. The chickens from the first group (control) were administered a feed mixture containing 4000 IU of vitamin D_3 (cholecalciferol), the chickens from group II received 2500 IU of vitamin D_3 and 1500 IU of calcidiol (25-OH-D_3) and the chickens from group III – 1240 IU of vitamin D_3 and 2760 IU of calcidiol (25-OH-D_3). The chickens were reared in accordance with the standards of broiler management, recommended by the COBB company.

During the rearing period, the chickens from each group were administered feed mixtures with composition and nutritive value as shown in Table 1.

In the course of the experiment, on the 21st, 35th and 42nd day of rearing, body weight of all chickens was determined individually; feed consumption per bird and mortality ratio were calculated in each group. On the 42nd day six males and six females of average body weight were chosen from each experimental group. The carcasses (of the chicken) were chilled by the air method at a temperature of 4°C for 24 h and then, dressing percentage was determined and the part of the muscles of breasts and legs, of abdominal fat and offals in the carcass was calculated.

Six samples were prepared for analyses from each of the feeding groups (from 6 carcasses without gender classification); the samples were averaged by mixing equal quantities of disintegrated meat (breast muscles and leg muscles separately). After 48 h from slaughtering, the following determinations were performed in breast and leg muscles: pH – according to the Polish Standard [PN-ISO 2917: 2001], water absorption – by centrifugation method according to Wierbicki *et al*. [1962], quantity of thermal drip (30 g of disintegrated meat were heated in a beaker covered with the self-sticking polyethylene foil in a water bath at 72°C for 30 min); also, proximate chemical composition (the content of water, protein, fat and ash) was determined with standard methods [AOAC, 1995]. In order to determine the oxidative changes in abdominal fat, TBA index [Shahidi, 1990] was determined after 72 h from slaughtering the chickens, and after 7 days of storing the samples in refrigeration conditions (4-6°C) and after 56 days of storage in a frozen state (-18°C). Moreover, a profile of fatty acids was determined in intramuscular and abdominal fat of the chicken according to Polish Standards [PN-ISO 5509:1978 (E); PN-ISO 5508:1990(E)]. The results obtained were evaluated with a variance analysis, calculated with the least square method in a statistical software SPSS 14.0 PL for Windows.

TABLE 1. Composition and nutritional value of feed mixtures (%).

Components	Starter (1-25 day)	Grower (26-35 day)	Finisher (36-42 day)
Wheat	34.0	44.0	44.1
Maize	23.0	17.4	20.0
Soybean meal 46%	31.0	29.0	26.4
Soybean oil	3.6	5.0	5.3
Limestone	1.4	0.6	0.2
Wheat middlings	1.0	-	-
Premix	6.0	4.0	4.0
Analysis			
ME (MJ/kg)	12.62	13.17	13.39
Crude protein	20.22	19.33	18.35
Lysine	1.22	1.22	0.97
Methionine	0.63	0.54	0.28
Methionine + cystine	0.65	0.64	0.61
Threonine	0.88	0.80	0.67
Tryptophan	0.25	0.24	0.23
Fat	5.65	6.79	7.15
Crude fibre	3.69	3.60	3.50
Ca	0.64	0.35	0.19
P	0.11	0.12	0.11

RESULTS

The males from the control group and from the second group, fed with addition of 1500 IU of calcidiol, obtained a higher body weight at the age of 35 days as compared to the chickens, receiving the addition of 2760 IU of calcidiol in their diet (group II, Table 2). The recorded differences were statistically significant ($p \leq 0.05$). At the end of rearing (42nd day of life of the chickens) statistically significant differences were no longer found in body weight values. Somewhat higher body weight of the chickens from group III was found in a comparison to these of groups I and III. The males were heavier by 69 g and the females by 33 g in a comparison to the birds from group III (addition of 2760 IU of calcidiol) and by 16 g and 37 g, respectively, in comparison to the control group. The chicken from group III had the lowest feed intake – 1.79 kg and the lowest mortality rate – 2.6%.

TABLE 2. Effects of vitamin D_3 and calcidiol on body weight of broiler chickens (g).

Specification	Feeding group					
	I		II		III	
	LSM	SEM	LSM	SEM	LSM	SEM
Body weight, 21 days	1021	21.4	1047	21.6	995	21.3
Body weight of male broiler, 35 days	2352[a]	23.7	2326[ab]	22.4	2278[b]	23.3
Body weight of female broiler, 35 days	2038	18.2	2036	18.5	1998	16.9
Body weight of male broiler, 42 days	3080	28.6	3096	30.5	3027	31.1
Body weight of female broiler, 42 days	2560	22.3	2597	21.9	2564	19.9
Feed conversion ratio (kg) per 1 kg of body weight gain	1.81		1.83		1.79	
Mortality rate (%)	5.0		3.6		2.6	

[a,b] – Means with different superscripts differ significantly at $p \leq 0.05$.

Dressing percentage of the chickens (from 75.95 to 76.89%), the contribution of breast muscles in the carcass (from 28.59 to 30.14%) and of leg muscles (from 18.92 to 20.20%) were similar and did not differ significantly (statistically), and were found to depend on the addition of vitamin D_3 form to the diet (Table 3). Significant statistically differences ($p \leq 0.05$) were only found in the content of offals in carcass; the mentioned differences concerned weight of heart muscle in males and gizzard in females. The highest contribution of heart muscle in the carcasses of males and females was found in group III; in case of males, the differences mentioned were statistically significant ($p \leq 0.05$). A weak tendency towards increasing percentage of heart muscle in carcasses was observed in the chickens receiving a lower dose of calcidiol in the feed mixture. A somewhat higher contribution of abdominal fat in the carcasses of females in a comparison to males, irrespectively of the feeding group, was also recorded.

Partial replacement of vitamin D_3 in feed for chickens with calcidiol did not have any significant effect on the chemical composition of their breast muscles. On the other hand, in the leg muscles of the chickens fed a feed mixture in which 1500 IU of vitamin D_3 were replaced with calcidiol (group II), a higher protein content and lower water content was found in comparison to leg muscles of the chickens from the other groups (Table 4). The results concerning physicochemical traits of breast and leg muscles of the chickens are provided in Table 5. The results demonstrate that, irrespective of the addition of calcidiol to a feed mixture, pH of the breast muscles of the chickens was lower by *ca.* 0.5–0.6 units than that of the leg muscles and reached 5.8–5.9. The breast and leg muscles of the chickens from the experimental groups (II and III) were characterised by a significantly higher water absorption and lower thermal drip in comparison to the birds from the control group.

TABLE 3. Results of slaughter analysis (%).

Feeding group	Dressing percentage	Breast muscle	Leg muscles	Gizzard	Heart	Liver	Fat
Males							
I	76.04	28.69	20.20	0.70	0.39^{b}	1.66	1.39
II	76.47	30.14	20.10	0.64	0.39^{b}	1.59	1.47
III	75.95	28.59	19.40	0.64	0.43^{a}	1.59	1.37
SEM	0.4	0.7	0.2	0.3	0.1	0.6	0.1
Females							
I	76.33	29.39	20.06	0.74^{ab}	0.38	1.63	1.06
II	76.32	29.91	19.34	0.77^{a}	0.38	1.61	1.89
III	76.89	28.69	18.92	0.69^{b}	0.40	1.62	2.15
SEM	0.3	0.5	0.2	0.3	0.2	0.8	0.2

^{a,b} – Means with different superscripts differ significantly at $p \leq 0.05$.

TABLE 4. Proximate chemical composition of chicken meat (g/100 g).

Feeding group	Moisture content		Protein		Fat		Ash	
	BM	LM	BM	LM	BM	LM	BM	LM
I	73.9	74.6^{ab}	22.5	18.5^{b}	1.1	5.3	0.9	1.0
II	74.2	74.3^{b}	23.4	19.1^{a}	0.9	5.1	0.9	1.0
III	74.0	74.8^{a}	22.8	18.4^{b}	0.9	4.9	0.9	1.0
SEM	0.332	0.125	0.338	0.054	0.079	0.161	<0.001	<0.001

BM – breast muscles, LM – leg muscles. ^{a,b} – Means with different superscripts differ significantly at $p \leq 0.05$.

TABLE 5. Physicochemical traits of chicken meat.

Feeding group	pH		Water absorption (%)		Thermal drip (%)	
	BM	LM	BM	LM	BM	LM
I	5.8	6.4	23.4^{b}	45.8^{Bb}	3.3^{Aa}	3.3
II	5.9	6.4	28.4^{ab}	53.2^{Aab}	3.0^{ABb}	3.2
III	5.9	6.4	29.1^{a}	51.1^{ABa}	2.9^{Bab}	3.0
SEM	0.027	0.019	1.453	1.113	0.058	0.144

BM – breast muscles, LM – leg muscles. ^{A,B} – Means with different superscripts differ significantly at $p \leq 0.01$; ^{a,b} – Means with different superscripts differ significantly at $p \leq 0.05$.

The results of determination of fatty acid profile in intramuscular and abdominal fat of the chickens are given in Table 6. They show that partial replacement of vitamin D_3 with calcidiol in feed mixtures of chickens did not have any significant effect on the profile of fatty acids in breast fat muscles. On the other hand, it was found that leg fat muscles and abdominal fat of the chickens from experimental groups (II and III) were characterised by a significantly higher content of monounsaturated fatty acids and a lower content of polyunsaturated fatty acids, especially from the *n*-6 family, in comparison to the control group.

Oxidative stability of abdominal fat of the chickens was evaluated based on the TBA indicator and the results obtained were provided in Table 7. According to this data, it may be stated that values of the TBA indicator in abdominal fat of the chickens administered the feed mixture containing calcidiol (group II and III) were significantly lower in respect of the control group, both 72 h after slaughter as well as after 56 days of storage in frozen state (-18°C).

TABLE 6. Fatty acid composition in abdominal fat of chickens and fat of chicken meat (g/100 g).

Feeding group	SFA	MUFA	PUFA	PUFA *n*-3	PUFA *n*-6
Breast muscles					
1	30.5	35.0	34.5	3.5	30.3
2	31.0	34.7	34.3	3.7	29.9
3	30.6	36.2	33.2	3.7	29.0
SEM	0.324	0.629	0.733	0.141	0.621
Leg muscles[1]					
1	27.8^{Ba}	36.7^{B}	35.5^{A}	3.3	31.8^{A}
2	28.7^{Aab}	38.9^{A}	32.3^{B}	2.9	29.1^{B}
3	27.3^{Bb}	39.7^{A}	32.9^{B}	3.1	29.5^{B}
SEM	0.114	0.319	0.321	0.108	0.218
Abdominal fat[2]					
1	26.9^{B}	40.4^{B}	32.7^{Aab}	2.8^{Aab}	29.6^{Aab}
2	26.9^{B}	42.0^{A}	31.1^{Ba}	2.7^{Ba}	28.2^{Ba}
3	27.2^{A}	42.1^{A}	30.7^{Bb}	2.6^{Bb}	27.9^{Bb}
SEM	0.022	0.099	0.114	0.009	0.103

^{A,B} – Means with different superscripts differ significantly at p≤0.01; ^{a,b} – Means with different superscripts differ; SFA – 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 22:0; MUFA – 14:1; 16:1; 17:1; 18:1; 20:1; 24:1; PUFA *n*-3 – 18:3; 20:5; 22:5; 22:6; PUFA *n*-6 – 18:2; 20:4. [1] without SFA – 12,0; PUFA *n*-3 – 20:5; [2] without MUFA – 24:1; PUFA *n*-3 – 20:5; 22:5; 22:6.

TABLE 7. Oxidative changes in chicken abdominal fat during storage.

Feeding group	TBA index		
	Temp 4-6°C		-18 °C
	2 days	7 days	60 days
1	0.34^{A}	0.76	1.35^{A}
2	0.20^{B}	0.79	0.78^{B}
3	0.20^{B}	0.77	0.76^{B}
SEM	0.009	0.041	0.038

^{A,B} – Means with different superscripts differ significantly at p≤0.01.

DISCUSSION AND CONCLUSION

Poor quality of skeleton of broiler chickens is currently a serious problem and therefore has a negative influence on economic results of rearing and welfare of the birds. Investigations have demonstrated that the level of vitamin D_3 in the diet, which is allowed in the existing nutritional practice, may be too low to ensure a good quality of bones and to limit the incidence of tibial bone (TB) dyschondroplasia in quickly growing broilers [Williams *et al*., 2000; Świątkiewicz *et al.,* 2006]. In turn, Whitehead *et al.* [2004] stated that the application of the hydrolyzed form of vitamin D_3, *i.e.* 25-OH-D_3 (calcidiol), may be an alternative to a significant increase of its level in feed mixtures for chickens.

The results of the present study suggest partial replacement of vitamin D_3 in the mixture with its metabolite, *i.e.* calcidiol, to affect the production results of chickens (group II; 2500 IU of vit. D_3 and 1500 IU of calcidiol). Świątkiewicz *et al.* [2006] replaced 50% of vitamin D_3 in feed for chickens with calcidiol (25-OH-D_3) and recorded improvement in chicken body weight gains by 11.5% an in feed conversion ratio by 8.7%. They stated that statistically significant differences which they had observed in the production results till the 21st day of rearing, might be due to the favourable effect of calcidiol in the initial period of growth when not all chickens have developed an effective enzymatic system necessary for hydroxylation of vitamin D_3 in liver.

Mireles *et al.* [1996] observed a favourable effect of calcidiol (25-OH-D_3) in feeding chickens on their body weight gains and fed conversion ratio. On the other hand, Yarger *et al*. [1995] demonstrated that calcidiol was the most effective once applied at the level of 69 μg/kg of feed, *i.e.* 2760 IU.

The results of the tests, which determine the effect of the type and level of feed additives on production results, dressing percentage of the birds and meat and fat quality, are of a significant importance to the producers of slaughter poultry in respect of the choice of the composition of feed mixtures. The latter trait (meat and fat quality) is extremely important for the producers of poultry products and consumers – it often determined the product's success on the market. According to various authors [Rennie & Whitehead, 1996; Fritts & Waldroup, 2003; Świątkiewicz *et al.,* 2006], partial replacement of vitamin D_3 addition with calcidiol in the feed mixtures has a favourable effect on the quality of skeleton and decreases the incidence of TD disease. No information has, however, been found in the available literature on the effect of such a replacement on poultry meat and fat quality.

Based on the results obtained, it may be concluded that the chemical composition of breast and leg muscles of chickens was similar to that reported by other authors [Skomiał *et al*., 2003; Pietrzak *et al.,* 2005, 2006; Szkucik *et al.,* 2007]. Breast muscles, in contrary to the leg muscles, contain less fat and their content of fat is determined genetically. Although, it lowers their sensory values, it improves their dietetic value. Replacement of a part of vitamin D_3 with calcidiol in the feed mixture (group II and III) had a favourable effect on the physicochemical properties of the breast as well as leg muscles of the chickens (higher water absorption and lower drip after thermal treatment). WBC of leg muscles was

almost twice higher than that of breast muscles, which is connected with the higher pH of the leg muscles. On the other hand, the amount of drip after heat treatment of breast and leg muscles was found at a similar level.

When analysing the results of determinations of fatty acid profile in the intramuscular and abdominal fat of chickens it was observed that the percentage of saturated and monounsaturated acids was considerably lower and that of monounsaturated acids was higher, in comparison to the values reported earlier in other publications [Pietrzak *et al.,* 2005; 2007; Pisarski *et al*., 2006; Szkucik *et al.*, 2007]. Attention should also be paid to a relatively high percentage of unsaturated fatty acids of the *n*-3 family in fat of the experimental chickens (2.9––3.7 g/100 g), which has a positive effect on health. A high level of polyunsaturated fatty acids in poultry meat and fat improves its nutritional value. Acids from the *n*-3 family are especially important. It has been demonstrated that a daily intake of those acids at a level of 0.3–1.0 g prevents coronary disease [Lopez-Ferrer *et al*., 2001]. Moreover, they have been shown to exert a therapeutic and prophylactic effect on such diseases as arthritis and cancer of breast and pancreas [Lopez-Ferrer *et al*., 2001]. The high level of polyunsaturated fatty acids in poultry fat has, however, an unfavourable effect on its stability. Rapid oxidative changes of lipids and the resulting products of oxidation may deteriorate the quality of meat and meat products and considerably shorten their shelf-life [Pikul, 1996]. The quantity of polyunsaturated fatty acids in fat of leg muscles and in abdominal fat of chickens receiving feed mixtures with the addition of calcidiol (group II and III) was significantly lower in comparison to the control group which considerably decelerated the rate of fat oxidation during storage.

Reassuming, partial replacement of vitamin D_3 with calcidiol in feed mixtures for broiler chickens had a favourable effect on their production results and meat quality.

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