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Effects of u.v. irradiation of very young chickens on growth and bone development

H. M. Edwards Jr

Department of Poultry Science, The University of Georgia, Athens, GA 30602-2772, USA

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Six experiments were conducted to study the effects of exposure of young chickens to u.v. radiation. Chickens were fed a cholecalciferol (D_3)-deficient diet and exposed to u.v. radiation from fluorescent lights giving total radiance (285–365 nm) at 0·15 m of 99·9 mJ/s per m^2. In Expt 1, chickens had increased body weight, bone ash and plasma Ca and decreased incidence of rickets and tibial dyschondroplasia (TD) when exposed to fluorescent light radiation 24 h per d, 24 h every 2 d, or 24 h every 3 d starting with exposure on day 1 after hatching. However, when not exposed on day 1, but on days 4, 7, 10, 13 and 16, the bone ash was reduced, and the incidence of TD and rickets was increased, compared with chickens exposed on day 1 after hatching. When chickens were exposed at 1 d of age to radiation from two lamps, each of which gave a radiance (285–365 nm) at 0·26 m of 856 mJ/s per m^2, both the length of time of radiation and location of the lamps (above or below the chicken) influenced the response as measured by body weight, bone ash, plasma Ca and incidence of rickets. When chickens that received a TD-inducing diet were exposed to 30 min u.v. radiation from below at 1 d of age they developed significantly less TD than did those not exposed when fed either 27·5 or 55·0 μg D_3/kg diet.

Cholecalciferol: Ultraviolet radiation: Rickets: Tibial dyschondroplasia

Hess & Weinstock (1924), Steenbock (1924) and Rosenheim & Webster (1926) demonstrated that the sterol fraction of oils and feedstuffs became antirachitic when irradiated with u.v. radiation. This effect of u.v. radiation is now known to convert ergosterol and 7-dehydrocholesterol to ergocalciferol and cholecalciferol (D_3) respectively. It has been used as a method to fortify food with D_3 and as a method to prepare ergocalciferol and D_3 in more purified forms for fortification of poultry and livestock diets. The chapter by Holick *et al.* (1982) provides a good chronological review of photochemistry and photobiology of vitamin D_3.

The importance of D_3 and some of its derivatives in the prevention of rickets and tibial dyschondroplasia (TD) in young chickens has been reviewed (Edwards, 2000). Rickets in young chickens that receive no u.v. radiation and are fed a D_3-deficient diet is prevented by D_3, 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$), 1α-hydroxycholecalciferol, 25-hydroxycholecalciferol and 1α,24*R*,25-trihydroxycholecalciferol and u.v. radiation (Scott *et al.* 1929; Haussler *et al.* 1973; Boris *et al.* 1977). TD that develops in young chickens fed a diet low in Ca but adequate in P and D_3 is prevented by 1α-hydroxycholecalciferol, 1,25-$(OH)_2D_3$, 1α,24,25-trihydroxycholecalciferol,1,25-dihydroxy-26, 27-hexadenterocholecalciferol, 1,25-dihydroxy-24*R*-fluorocholecalciferol and u.v. radiation (Edwards, 1989, 1990; Elliot & Edwards, 1997). Studies indicate that fluorescent lighting (providing 3·4 % power (W) in the u.v. range 260–400 nm) and dietary supplementation with 1,25-$(OH)_2D_3$ under certain conditions are effective in reducing the development of TD and rickets in chickens (Elliot & Edwards, 1997). When broiler chickens fed diets containing no D_3 were exposed to fluorescent lights from 1 to 16 d old, the lights were estimated to provide the equivalent of 20·0–40·0 μg D_3/kg (Edwards *et al.* 1994). While these studies show that u.v. radiation is beneficial to growing broiler chickens, whether they are receiving diets adequate in D_3 and Ca or deficient in these nutrients, little is known about the effects of specific amounts of u.v. radiation.

Therefore, studies have been conducted to determine the effectiveness of low-level u.v. radiation of chickens with fluorescent lights at various time intervals during their early growing period. The effectiveness of 30–120 min radiation of 1-d-old broiler chicks with a stronger source of u.v. radiation was also determined. Studies were also conducted to determine the effect of this u.v. radiation at 1 d of age on both D_3 deficiency and the development of TD in young broiler chickens.

Materials and methods

General

Male broiler chickens (1 d old) were used in all experiments. All experiments were conducted in electrically heated battery brooders (Petersime Incubation Co.,

Abbreviations: D_3, cholecalciferol; TD, tibial dyschondroplasia; 1,25-$(OH)_2D_3$, 1,25-dihydroxycholecalciferol.

Corresponding author: Dr Hardy M. Edwards, fax +1 706 5421827, email hedwards@uga.edu

Gettysburg, OH, USA) with wire-mesh floors; feed and water were always available. The overhead fluorescent lights in the room were all fitted with Arm-a-Lite® sleeves (FR312W-T-12; Arm-a-Lite® Thermoplastic Processes, Stirling, NJ, USA) to prevent emission of u.v. radiation to the whole room. The fluorescent lights used in the battery were General Electric (F15T8-CW; General Electric Co., Cleveland, OH, USA), providing 3·4 % energy (W) in the u.v. range (260–400 nm) and total radiance (285–365 nm) at 0·15 m of 9·99 mJ/s per m^2. The battery brooders contained six decks with four pens per deck. For a description of the light location and the effectiveness of the sleeves, see Edwards *et al.* (1994). The temperature of the room was maintained at 22°C. In Expt 1, Arm-a-Lite® sleeves (FR312W-T-12; Arm-a-Lite® Thermoplastic Processes) were used to expose the chickens to various amounts of u.v. radiation from the battery fluorescent tubes. In Expts 2 to 6, all the fluorescent tubes in the batteries were covered with the sleeves to exclude all u.v. radiation to the birds. In these experiments, the birds were exposed in groups of nine or ten birds in a 0·18 × 0·25 m area to the radiation from two lamps each containing two Hg-vapour tubes. The chickens stood on a 0·012 m wire-mesh floor. The lamps were 0·31 m long and each lamp contained two tubes 0·23 m long and 0·01 m apart. They were placed in a position either above or below the chickens 0·17 m apart at an angle to focus on the centre of the chickens, which were 0·26 m from the tubes. Each lamp (two tubes) gave a radiance (285–365 nm) at 0·26 m of 856 mJ/s per m^2. The diets used in these experiments are shown in Table 1. The D_3-deficient diet was used in Expts 1, 2, 3 and 4 while the diet adequate in D_3 but low in Ca was used in Expts 5 and 6. The D_3 added to the diets in all six experiments was obtained from Sigma Chemical Co. (St Louis, MO, USA), designated Sigma Reference Standard (a pure crystalline D_3), meeting or exceeding US Pharmacopoeia specifications. The 1,25-$(OH)_2D_3$ used in the experiment was supplied by M. Uskokovic (Hoffman-LaRoche, Inc., Nutley, NJ, USA).

All experiments were conducted for 16 d except Expt 4, which was conducted for 21 d. Before being weighed, one bird was randomly selected from each pen and a blood sample obtained by heart puncture for plasma total Ca (section N-31, Technicon Autoanalyser Methodology; Technicon Corp., Tarrytown, NY, USA) and dialysable P (section 7N-46, Technicon Autoanalyser Methodology; Technicon Corp.) contents. At the termination of the experiments, birds were weighed by pens and their feed consumption recorded. They were then killed by CO_2 asphyxiation and examined for TD and rickets without knowledge of treatment. The birds were diagnosed as having rickets when the subepiphyseal growth plate band was lengthened and increased in opacity (Long *et al.* 1984). In this size of chicken with rickets, the growth plate band is usually 2–5 mm wide, compared with 1 mm wide in birds not showing rickets. In rickets the widened growth plate is very striated, in contrast to the general opaque look with no apparent organization or striation of cartilage, which occurs in chickens with TD. TD was determined as described by Edwards & Veltmann (1983). The left tibia was removed for bone ash determination on a dry fat-free basis (Association of Official Analytical Chemists, 1995).

Analysis of variance was computed using the General Linear Models procedure of SAS® (version 6, 1990; SAS Institute Inc., Cary, NC, USA). Where appropriate, mean values were separated by Duncan's multiple range test. All statistics were conducted on pen averages or a single value for a pen (*n* 4, 4, 4, 6, 6 and 4 for Expts 1, 2, 3, 4, 5 and 6 respectively.

Expt 1

The aim of this part of the study was to determine whether the duration and age of exposure of 1-d-old chickens to low-level u.v. radiation from fluorescent lights influenced their growth and bone development when they were fed D_3-deficient diets. There were six treatments: (1) birds that received no u.v. radiation (pens were fitted with Arm-a-Lite® sleeves (FR312W-T-12; Arm-a-Lite® Thermoplastic Processes)); (2) birds receiving no u.v. radiation but 12·5 μg D_3 was added/kg diet; (3) birds exposed to u.v. radiation (fluorescent lights in pen) continuously during the 16 d of the experiment; (4) birds exposed to u.v. radiation for 24 h/d on days 1, 3, 5, 7, 9, 11, 13 and 15; (5) birds exposed to u.v. radiation for 24 h/d on days 1, 4, 7, 10, 13 and 16; (6) birds exposed to u.v. radiation for 24 h/d on days 4, 7, 10, 13 and 16. The pen assignments could not be completely randomized because the exposure to u.v. radiation, or not, had to be done for two pens under the same fluorescent light (see Fig. 1 in Edwards *et al.* 1994). There were four pens of ten birds per treatment.

Table 1. Composition of the basal diets used in the experiments

	Amounts (g/kg)	
	Expts 1, 2, 3 and 4	Expts 5 and 6
Ingredients		
Ground yellow maize	558·5	571·7
Soyabean meal (dehulled)	350·0	350·0
Poultry fat (stabilized)	50·0	50·0
Iodized NaCl	4·5	4·5
DL-Methionine (980 g/kg)	2·0	2·0
Vitamin premix*†	2·8	2·5
Trace mineral premix‡	0·8	0·8
Ca_2PO_4 (feed grade)	18·6	18·6
Limestone	12·8	–
Calculated analysis		
Protein	219·3	220·0
Ca	10·0	5·1
Total P	7·3	7·3
Non-phytate P	4·7	4·7
Metabolizable energy (MJ/kg)	13·3	13·5

* Vitamin premix provided the following (mg/kg diet): vitamin A (as all-*trans*-retinyl acetate) 1·89, cholecalciferol 27·5 μg, vitamin E (all-*rac*-α-tocopheryl acetate) 1·1, riboflavin 4·4, calcium pantothenate 12, nicotinic acid 44, choline chloride 220, cyanocobalamin 9 μg, pyridoxine 3·0, menadione (as menadione sodium bisulfite) 1·1, thiamin (as thiamin mononitrate) 2·2, folic acid 3·0, biotin 0·3, ethoxyquin 125.

† The vitamin mixture used in Expts 1, 2, 3 and 4 did not contain any cholecalciferol.

‡ Trace mineral premix provided (mg/kg diet): MnO_2 222, ZnO 150, $FeSO_4.7H_2O$ 200, $FeCO_3$ 83, $CuSO_4.5H_2O$ 29, $Ca(IO_3)_2$ 15.

Expt 2

The aim of this part of the study was to determine how much exposure of 1-d-old chicks to u.v. radiation from Hg-vapour lamps (826 mJ/s per m^2) is needed to give maximum protection from developing D_3 deficiency. The basal diet was fed to four pens of ten chicks each that received at 1 d of age 0, 30, 60, 120 or 240 min of radiation from above with the Hg-vapour lamps. Four pens of ten chicks each that were not irradiated received the basal diet supplemented with 5·0 μg D_3/kg.

Expt 3

The aim of this part of the study was to determine whether the effectiveness of exposure of 1-d-old chickens to u.v. radiation on bone growth and development was influenced by whether the radiation source was above or below the birds. The treatments were: (1) birds receiving no u.v. radiation, (2) birds irradiated from above for 30 or 60 min; (3) birds irradiated from below for 30 or 60 min; (4) birds receiving no u.v. radiation but 5·0 μg D_3 was added/kg diet. There were four pens of ten chickens each per treatment.

Expt 4

The aim of this part of the study was to determine whether the effects of the 30 or 60 min radiation of 1 d old chicks persist in promoting growth and bone development when the chicks were fed a vitamin D_3-deficient diet. The treatments included: (1) birds receiving no u.v. radiation; (2) birds irradiated at 1 d of age from below for 30 or 60 min; (3) birds receiving the basal D_3-deficient diet supplemented with 5·0 μg D_3/kg. There were six pens of eighteen birds per treatment. The weight and gain per kg feed at days 7, 14 and 21 represented eighteen, twelve and six chickens per pen respectively. At days 7, 14 and 21, six chickens per pen were killed after taking a blood sample from two of them; all were examined for rickets and TD and the left tibia removed for bone ash determination.

Expts 5 and 6

The aim of this part of the study was to determine whether 30 min exposure of 1-d-old chicks to radiation offers protection against the development of TD when chicks were fed a low-Ca, high-P, TD-inducing diet (Table 1). In Expt 5, the chicks were irradiated from above and in Expt 6 they were irradiated from below. In addition, in Expt 6, the 2×2 factorial of D_3 levels and receiving or not receiving u.v. radiation was expanded to a 2×3 factorial by including the variable of adding 10·0 μg 1,25-$(OH)_2D_3$/kg to the diets containing 27·5 or 55·0 μg D_3/kg.

Results

Expt 1

The chicks receiving the D_3-supplemented diet or any exposed to u.v. radiation had greater body weight on day16 than the chicks receiving the basal D_3-deficient diet (Table 2). The birds receiving the deficient diet also had the lowest gain:feed ratio. The chicks receiving continual exposure to u.v. radiation had the highest bone ash followed by those receiving the u.v. radiation every other or every third day, or those receiving the D_3 in the diet. The chicks receiving the deficient diet had the lowest bone ash and those exposed to u.v. radiation every third day from the fourth day had bone ash lower than all other chicks exposed to u.v. radiation, but higher than the basal fed chicks. The plasma Ca levels of the birds fed the basal diet were lower than those of chicks in all the other treatments and there was no difference in their plasma Ca levels. The birds receiving the D_3-supplemented diet had higher plasma dialysable P values than the chickens of all the other treatments including the basal. Of the chickens fed the deficient diet, 87 % showed symptoms of rickets. The birds receiving the D_3-supplemented diet had an incidence of rickets of 23 % while those receiving continuous u.v. radiation had 0 % rickets. There was no difference between the incidence of rickets in birds receiving continuous, every other day, or every third day exposure to u.v. radiation. However, the birds that received the u.v. radiation every third day starting with the fourth day had a significantly ($P \leq 0{\cdot}05$) higher incidence of rickets than birds that were exposed to u.v. radiation every third day including the first day. The incidence of TD followed the same general pattern between treatments as the incidence of rickets, and once again the birds that did not receive first day exposure to u.v. radiation had a high incidence of TD. The D_3 supplementation did not significantly reduce the incidence of TD as compared with basal chicks; however, all the u.v. radiation treatments that had first day exposure significantly ($P \leq 0{\cdot}05$) reduced the incidence as compared with the basal group.

Expt 2

The mortality in the order of treatments in Table 3 was two, one, three, five, seven and eleven per forty chickens started. This high mortality in the chickens receiving the greatest exposure to the u.v. radiation at 1 d of age was due to chickens starving as a result of eye damage from the u.v. radiation. Mortality of two, one and three chickens per forty birds started would be in the normal range. The birds that received the D_3-supplemented diet had significantly greater body weight at day16 than all the other treatments, except the chickens exposed to u.v. radiation for 30 min.

There were significant differences in gain:feed ratio between treatments; however, the mortality mentioned previously has probably influenced the gain:feed ratio of some treatments. The bone ash values were increased by the D_3 supplementation and almost equally by the lowest time exposure to u.v. radiation (30 min). Increasing time exposure to u.v. radiation gave increasing percentage bone ash (linear regression, $P \leq 0{\cdot}001$). Plasma Ca values also increased with increased exposure of 1-d-old chicks to the u.v. radiation, but dialysable P values were not affected. The incidence of TD was very low in this experiment, but the incidence of rickets was high as expected and increasing time exposure of the 1-d-old chick to u.v. radiation reduced the incidence from 95 to 16 %.

Table 2. Effect of exposure to radiation from fluorescent lights on growth, feed efficiency, bone ash and plasma minerals, and on the incidence of rickets and tibial dyschondroplasia (TD) in broiler males (Expt 1)*

Treatments						Plasma mineral (mg/l)			
Addition to basal diet	Exposure to fluorescent light	*n*†	Body weight at day 16 (g)	Gain:feed ratio	Bone ash (mg/g)	Ca	dP	Rickets incidence (%)	TD incidence (%)
None	None	4	324^{b}	0·672^{b}	248^{d}	67·8^{b}	67·1^{b}	87^{a}	27^{a}
12·5 μg cholecalciferol/kg	None	4	402^{a}	0·723^{a}	397^{ab}	119·4^{a}	90·5^{a}	23^{bc}	16^{ab}
None	24 h/d every d	4	397^{a}	0·706^{ab}	409^{a}	115·0^{a}	74·6^{b}	0^{d}	0^{b}
None	24 h/d every other d	4	405^{a}	0·697^{ab}	399^{ab}	112·4^{a}	77·3^{b}	5^{cd}	3^{b}
None	24 h/d; days 1, 4, 7, 10, 13 and 16	4	402^{a}	0·698^{ab}	388^{b}	113·3^{a}	72·8^{b}	13^{cd}	0^{b}
None	24 h/d; days 4, 7, 10, 13 and 16	4	412^{a}	0·735^{a}	371^{c}	111·4^{a}	76·1^{b}	40^{b}	26^{a}
Mean			390	0·705	369	106·5	76·4	28	12
SEM			12	0·014	5	3·9	3·1	7	6
Statistical significance of effect of treatment (ANOVA, 5 df): *P*			0·001	0·060	<0·001	<0·001	0·002	<0·001	0·004

dP, dialysable phosphorus.
[a,b,c,d]Mean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 152.
† Number of replicates per mean value.

Table 3. Effect of exposure of 1-d-old broilers to u. v. radiation from mercury-vapour lamps on growth, feed efficiency, bone ash, plasma minerals and on the incidence of rickets and tibial dyschondroplasia (TD) (Expt 2)*

Treatments						Plasma mineral (mg/l)			
Addition to basal diet	Exposure to Hg-vapour lamps (min)	*n*†	Body weight at day 16 (g)	Gain:feed ratio	Bone ash (mg/g)	Ca	dP	Rickets incidence (%)	TD incidence (%)
None	0	4	328^{b}	0·660^{b}	256^{e}	69	54	95^{a}	5^{ab}
5 μg cholecalciferol/kg	0	4	370^{a}	0·677^{ab}	299^{d}	87	70	81^{ab}	10^{a}
None	30	4	334^{ab}	0·696^{ab}	290^{d}	79	61	71^{ab}	0^{b}
None	60	4	324^{b}	0·712^{a}	317^{c}	77	66	58^{b}	0^{b}
None	120	4	319^{b}	0·698^{ab}	329^{b}	88	57	38^{c}	0^{b}
None	240	4	316^{b}	0·715^{a}	342^{a}	93	56	16^{c}	0^{b}
Mean			332	0·693	306	82	61	58	3
SEM			13	0·014	4	7	7	8	2
Statistical significance of effect of treatment (ANOVA, 5 df): *P*			0·105	0·092	<0·001	0·262	0·566	<0·001	0·008
Regression of light exposure									
Linear (1 df)			0·338	0·062	<0·001	0·009	0·732	<0·001	0·127
Quadratic (1 df)			0·843	0·223	<0·001	0·422	0·446	0·010	0·063

dP, dialysable phosphorus.
[a,b,c,d,e]Mean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 152.
† Number of replicates per mean value.

Table 4. Effect of exposure of 1-d-old broilers to u. v. radiation from mercury-vapour lamps on growth, feed efficiency, bone ash and plasma minerals, and on the incidence of rickets and tibial dyschondroplasia (TD) (Expt 3)*

Treatments						Plasma mineral (mg/l)			
Addition to basal diet	Exposure to Hg-vapour lamps (min)	*n*†	Body weight at day 16 (g)	Gain:feed ratio	Bone ash (mg/g)	Ca	dP	Rickets incidence (%)	TD incidence (%)
None	0	4	308^{b}	0·655^{b}	243^{d}	67·2^{b}	51·5	84^{ab}	38^{a}
5 μg cholecalciferol/kg	0	4	391^{a}	0·706^{ab}	348^{b}	100·9^{a}	54·6	69^{b}	38^{a}
None	Exposure from above 30	4	317^{b}	0·672^{b}	261^{c}	65·2^{b}	53·8	96^{a}	19^{ab}
None	Exposure from above 60	4	319^{b}	0·695^{ab}	275^{c}	72·4^{b}	48·5	98^{a}	24^{a}
None	Exposure from below 30	4	390^{a}	0·742^{a}	354^{ab}	91·5^{a}	56·1	46^{c}	3^{b}
None	Exposure from below 60	4	377^{a}	0·715^{ab}	366^{a}	94·2^{a}	63·0	31^{c}	5^{b}
Mean			350	0·697	308	81·9	54·6	70	21
SEM			14	0·020	5	5·2	4·0	6	6
Statistical significance of effect of treatment (ANOVA, 5 df): *P*			<0·001	0·069	<0·001	<0·001	0·230	<0·001	0·002

dP, dialysable phosphorus.
a,b,c,dMean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 152.
† Number of replicates per mean value.

Expt 3

The birds that received the D_3 supplementation or the u.v. radiation for 30 or 60 min from below had significantly greater body weight, bone ash and plasma Ca at16 d than chicks fed the basal diet or irradiated for 30 or 60 min from above (Table 4). The birds irradiated from above did have significantly ($P \leq 0{\cdot}05$) higher bone ash than the basal group. The ANOVA indicates no significant treatment effects on plasma dialysable P levels. The treatment effects on incidence of rickets and TD were quite different. Birds receiving 30 or 60 min of radiation from below had very low incidence of TD and the incidence of rickets was significantly ($P \leq 0{\cdot}05$) lower than the birds receiving 5·0 μg D_3/kg feed. The radiation from above did not prevent the development of rickets or TD.

Expt 4

This experiment was a time study of four of the treatments that were present in Expt 3. The birds receiving radiation for 30 or 60 min had significantly lower body weight and incidence of rickets at day 7, but the bone ash and plasma dialysable P were higher than either the chicks receiving the basal diet or the diet supplemented with 5·0 μg D_3/kg (Table 5). At day 7, the birds receiving the D_3-supplemented diet differed from the birds receiving the basal diet only in bone ash where they had a significantly higher value. By day 14, the birds receiving the D_3 supplement or the u.v. radiation either for 30 or 60 min had higher body weight, plasma Ca and bone ash than birds receiving the basal diet at day 14. The birds receiving 30 or 60 min radiation had significantly ($P \leq 0{\cdot}05$) lower incidence of both rickets and TD than those receiving the basal diet or the basal diet supplemented with D_3. The irradiated birds also had bone ash values greater than the birds receiving D_3. At day 21, the birds receiving the D_3 had greater body weight, bone ash and plasma Ca than the birds receiving the basal diet, or the irradiated birds. However, the irradiated birds had greater body weight and bone ash than the birds receiving the basal diet at day 21. All of the birds had a high incidence of rickets, but only the birds receiving the D_3 had a high incidence of TD. As would be expected from the results cited earlier, there were significant treatment × time interactions for body weight, TD, plasma Ca and bone ash at day 14.

Expt 5

The radiation of chicks for 30 min from above with u.v. light had no significant effect when compared with chicks receiving the basal diet on any of the criteria measured in this experiment (Table 6). When the level of D_3 supplementation was raised from 27·5 to 55·0 μg/kg there was a significant ($P \leq 0{\cdot}05$) increase in bone ash and plasma dialysable P compared with birds receiving the basal diet. The birds that received both the high level of D_3 plus u.v. radiation were not significantly different from the basal in any criteria measured.

Table 5. Effect of exposure of 1-d-old broilers to u. v. radiation from mercury-vapour lamps on the growth on days 7, 14 and 21, feed efficiency, bone ash and plasma minerals, and incidence of rickets and tibial dyschondroplasia (TD) (Expt 4)*

Treatments						Plasma mineral (mg/l)			
Time (d)	Additions and radiation	*n*†	Body weight (g)	Gain:feed ratio	Bone ash (mg/g)	Ca	dP	Rickets incidence (%)	TD incidence (%)
7	None	6	126^{a}	0·727	306^{c}	94·3	45·7^{b}	69	0
7	5 μg cholecalciferol/kg	6	121^{ab}	0·721	337^{b}	99·9	52·8^{b}	64	0
7	Exposure from below 30 min	6	109^{b}	0·817	357^{a}	98·4	56·4^{ab}	37	0
7	Exposure from below 60 min	6	108^{b}	0·720	350^{ab}	101·5	66·1^{a}	39	0
7	Mean		116	0·746	337	98·5	56·5	52	0
7	SEM		5	0·04	6	3·0	5·1	12	0
7	Statistical significance of effect of treatment (7 d) (ANOVA, 3 df): *P*		0·074	0·214	<0·001	0·270	0·017	0·132	–
14	None	6	254^{b}	0·496^{b}	246^{c}	69·4^{b}	47·9	100^{a}	17^{a}
14	5 μg cholecalciferol/kg	6	290^{a}	0·519^{ab}	300^{b}	87·6^{a}	55·1	96^{a}	24^{a}
14	Exposure from below 30 min	6	296^{a}	0·591^{a}	348^{a}	89·7^{a}	52·3	66^{b}	0^{b}
14	Exposure from below 60 min	6	299^{a}	0·549^{ab}	366^{a}	96·7^{a}	53·1	43^{c}	0^{b}
14	Mean		282	0·539	315	85·8	52·1	76	10
14	SEM		8	0·026	6·6	3·8	3·8	7	5
14	Statistical significance of effect of treatment (14 d) (ANOVA, 3 df): *P*		0·005	0·089	<0·001	<0·001	0·596	<0·001	0·004
21	None	6	359^{c}	0·491^{b}	245^{c}	64·9^{b}	57·5	100^{a}	13^{b}
21	5 μg cholecalciferol/kg	6	537^{a}	0·681^{a}	323^{a}	91·9^{a}	54·9	85^{b}	71^{a}
21	Exposure from below 30 min	6	488^{b}	0·675^{a}	290^{b}	67·9^{b}	62·7	98^{ab}	10^{b}
21	Exposure from below 60 min	6	494^{b}	0·575^{ab}	306^{ab}	74·3^{b}	67·7	96^{ab}	0^{b}
21	Mean		469	0·606	291	74·8	60·7	94	24
21	SEM		14	0·042	8	4·2	5·9	4	7
21	Statistical significance of effect of treatment (21 d) (ANOVA, 3 df): *P*		<0·001	0·012	<0·001	0·001	0·446	0·083	<0·001
	Statistical significance of effect (combined 7, 14 and 21 d): *P*								
	Treatment (3 df)		<0·001	0·012	<0·001	<0·001	0·018	<0·001	<0·001
	Days (2 df)		<0·001	<0·001	<0·001	<0·001	0·254	<0·001	0·004
	Treatment × days (3 df)		<0·001	0·194	0·029	<0·016	0·525	0·065	0·001

dP, dialysable phosphorus.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 152.
† Number of replicates per mean value.

Table 6. Effect of dietary cholecalciferol and exposure of 1-d-old broilers to u. v. radiation from mercury-vapour lamps on growth, feed efficiency, bone ash and plasma minerals, and the development of tibial dyschondroplasia (TD) (Expt 5)*

Treatments						Plasma mineral (mg/l)		
Cholecalciferol in diet (μg/kg)	Exposure to mercury-vapour lamps (min)	*n*†	Body weight (g)	Gain:feed ratio	Bone ash (mg/g)	Ca	dP	TD incidence (%)
27·5	0	6	343	0·725	315[b]	92·0	94·6[ab]	95
55	0	6	382	0·712	328[a]	103·0	101·6[a]	89
27·5	Exposure from above 30	6	353	0·716	315[b]	99·8	84·1[b]	97
55	Exposure from above 30	6	351	0·722	323[ab]	103·9	94·1[ab]	97
Mean			357	0·719	320	99·7	93·6	94
SEM			15	0·019	4	3·8	7·6	4
Statistical significance of effect (ANOVA): *P*								
Treatment (3 df)			0·293	0·962	0·085	0·142	0·118	0·565
Cholecalciferol (1 df)			0·220	0·853	0·016	0·060	0·095	0·502
Radiation (1 df)			0·480	0·979	0·535	0·263	0·078	0·296
Cholecalciferol × radiation (1 df)			0·187	0·622	0·598	0·380	0·760	0·502

dP, dialysable phosphorus.
[a,b]Mean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 152.
† Number of replicates per mean value.

Expt 6

In this experiment, the chicks receiving the diet containing 55·0 μg D_3/kg had increased body weight, bone ash and reduced incidence of rickets at day 16 compared with the chicks fed the basal diet containing 27·5 μg D_3/kg diet (Table 7). The chicks that were irradiated for 30 min from below had increased body weight, bone ash and a reduced incidence of TD compared with chicks receiving the basal diet at day 16. The birds receiving the 1,25-$(OH)_2D_3$ had increased body weight, bone ash and reduced incidence of both rickets and TD at day 16. Chicks fed the high level of D_3 (55·0 μg/kg) plus u.v. radiation did not grow faster than the basal, and their tibia bone ash, while higher than the basal, was not higher than the bone ash of chickens receiving the high D_3 or only u.v. radiation. The incidence of rickets was reduced by the high level of D_3 in the diet and was almost completely prevented by the addition of 1,25-$(OH)_2D_3$. While the radiation of the chickens did not significantly reduce the incidence of rickets at both D_3 levels, it did reduce the incidence of TD and the addition of 1,25-$(OH)_2D_3$ reduced the incidence of TD to very low levels.

There was a significant ($P \leq 0{\cdot}05$) interaction between radiation and D_3 level on body weight at day 16. Chicks exposed to u.v. radiation and fed the low D_3 diet grew significantly faster than chickens irradiated but fed the high-D_3 diet.

Discussion

The effect of continuous exposure of broiler chickens fed a D_3-deficient diet to radiation from fluorescent lights confirms the results of Edwards *et al.* (1994), showing that the radiation is equivalent to 10·0–20·0 μg D_3/kg diet. There was little effect from the decrease in exposure of chickens to the radiation from fluorescent lights from 24 h every day to 24 h every third day, only bone ash values showed a significant decrease. However, when the chicken was not exposed during the first 24 h of its life to radiation from fluorescent light in the every third day exposure rotation, bone ash was significantly ($P \leq 0{\cdot}05$) lower and the incidence of rickets and TD was higher at 16 d of age. This indicates that the 1-d-old chick, which probably has adequate stores of D_3 at hatch, is in need of additional D_3 during the first 4 d of life. This susceptibility to D_3 deficiency very early in its life is clear from the results of Expt 4 where the broilers fed the D_3-deficient diet had a very low bone ash (306 mg/g) at 7 d of age.

The results obtained in Expt 2 clearly show that there is a long-term (16 d) effect to the relatively short-term (30–240 min) exposure of the 1 d old chicken to u.v. radiation. Exposure of the birds to u.v. radiation from below appeared to be more than twice as effective as from above. Koch & Koch (1941) found that the skin from the feet and legs contained eight times as much pro-vitamin D potency than body skin. Studies by Tian *et al.* (1994) working with female chickens found the concentration of 7-dehydrocholecalciferol in the back, legs and feet to be 1·20, 35·24 and 28·56 ng/mm^2 and the concentration of pre-D_3 after u.v. light exposure none was detected, 0·44

Table 7. Effect of dietary cholecalciferol and exposure of 1-d-old broilers to mercury-vapour lamps on growth, feed efficiency, bone ash and plasma minerals, and the development of tibial dyschondroplasia (TD) (Expt 6)*

Treatments										
Amount in diet (μg/kg)		Exposure from below to Hg-vapour lamps (min)	*n*†	Body weight at day 16 (g)	Gain:feed ratio	Bone ash (mg/g)	Plasma mineral (mg/l)		Rickets incidence (%)	TD incidence (%)
Cholecalciferol	1,25-$(OH)_2D_3$						Ca	dP		
27·5	0	0	4	330^{c}	0·703	305^{c}	99·2	90·3	87^{a}	67^{a}
27·5	0	30	4	381^{a}	0·697	320^{b}	97·9	78·8	84^{a}	30^{b}
27·5	10	0	4	372^{ab}	0·718	359^{a}	99·6	82·4	18^{b}	5^{c}
55	0	0	4	371^{ab}	0·702	322^{b}	101·0	88·1	72^{a}	62^{a}
55	0	30	4	344^{bc}	0·698	319^{b}	98·1	95·8	70^{a}	40^{b}
55	10	0	4	387^{a}	0·703	361^{a}	97·0	80·5	15^{b}	5^{c}
Mean				364	0·703	331	98·8	86·0	58	35
SEM				11	0·011	3	3·2	6·1	8	6
Main effect mean values										
Cholecalciferol levels (μg/kg)										
27·5			12	367	0·706	32·8^{b}	9·89	8·38	63	34
55·0			12	361	0·701	33·4^{a}	9·87	8·82	52	36
1,25-$(OH)_2D_3$ or u. v. light										
None			8	350^{b}	0·702	31·4^{b}	10·01	8·92	80^{a}	65^{a}
u. v. light			8	362^{ab}	0·698	31·9^{b}	9·80	8·73	77^{a}	35^{b}
1,25-$(OH)_2D_3$			8	379^{a}	0·711	36·0^{a}	9·83	8·14	16^{b}	5^{c}
Statistical significance of effect (ANOVA, 5 df): *P*										
Treatment				0·012	0·770	<0·001	0·960	0·378	<0·001	<0·001
Cholecalciferol level				0·496	0·592	0·022	0·943	0·399	0·116	0·738
None, u. v. 1,25-$(OH)_2D_3$				0·053	0·474	<0·001	0·782	0·437	<0·001	<0·001
Cholecalciferol levels × none, u.v. and 1,25-$(OH)_2D_3$				0·008	0·721	0·026	0·789	0·230	0·664	0·433

1,25-$(OH)_2D_3$, 1,25-dihydroxycholecalciferol; dP, dialysable phosphorus.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different (Duncan's new multiple range test, $P \leq 0{\cdot}05$).
*For details of diets and procedures, see Table 1 and p. 152.
†Number of replicates per mean value.

and 0·54 ng/mm^2 respectively. Thus, it is not surprising that the radiation from below was so much more effective in the studies reported in the present paper. The skin on the legs and feet of a 1-d-old chick is approximately 400 mm^2; therefore, if irradiated with the same amount as Tian *et al.* (1994), it would have 200 ng pre-D_3. When the diet contains 5·0 μg D_3/kg, the chick would have to consume 40 g feed to take in 200 ng D_3. The bird would probably take in this much feed in the first 3 d. However, the 7-d-old chicks irradiated for 30 min from below in Expt 4 had higher bone ash than the chicks receiving 5·0 μg D_3/kg diet, which would have taken in approximately 500 ng D_3. Therefore, the dietary D_3 must be poorly absorbed and utilized or the u.v. radiation is producing a much greater amount of D_3, or D_3 is being used much more effectively than that from the diet. There is a high probability that the 1-d-old chicks in the present studies were very efficient in synthesizing D_3 compared with the older females of Tian *et al.* (1994), especially since Holick *et al.* (1989) has presented evidence indicating that young human volunteers (age range 20–30 years) were much more efficient in synthesis of D_3 from u.v. radiation than older human volunteers (age range 62–80 years).

The chicks of Tian *et al.* (1994) received 0·0050 J/mm^2. The chicks irradiated at 1 d of age in the present studies received 0·0015, 0·0030, 0·0060 and 0·0120 J/mm^2 from 30, 60, 120 and 240 min exposure respectively. The kinetic model of Tian *et al.* (1994) (see Fig. 5 in Tian *et al.* (1994)) may be applicable to our present chicks that received short-term radiation: 30 and 60 min at 1 d of age. This would mean that the concentration of D_3 in the blood probably peaked at 30 h after radiation of the 1-d-old chicks followed by a slower disappearance of D_3 from the blood, as pre-D_3 in the epidermis is continuing its thermoisomerization to D_3 and entering the circulation. However, the results in most of the experiments in the present paper indicate that u.v. radiation had very long-term effects on body weight, plasma Ca, bone ash and incidences of rickets and TD. Thus, D_3 synthesized in the skin and translocated may be stored in adipose tissue (Norman & DeLuca, 1963; Mawer *et al.* 1972) and the 25-hydroxycholecalciferol made from it may also be conserved. However, other studies (Holick *et al.* 1981, 1982) indicated that pre-D_3 synthesis occurs throughout the layers of the entire epidermis. The highest concentrations were in the basal layer and the malpighian layer. Thus, there may be a continual amount of D_3 entering the circulation of the animal some time after radiation even if the amount is very small it is known that it is transported on the vitamin D-binding protein and is very active compared with oral D_3 (Fraser, 1983; Haddad *et al.* 1993). It is apparent from Expt 4, where data were collected at 7 d intervals, that by the third week the supplies of D_3 in chickens irradiated at 1 d of age were very low, since body weight fell below the body weight of the birds receiving D_3 in the feed. The effect on plasma Ca, bone ash and rickets also indicate that the effects of u.v. radiation were becoming less. However, these results indicate a definite ability of the young chicken to conserve and utilize efficiently the relatively small amount of D_3 synthesized by u.v. light radiation at 1 d of age.

The results obtained on the incidence of TD are of special interest in Expt 4 where birds were scored at days 7, 14 and 21. A high incidence of TD was detected at day 21 by gross observation when the birds received 5·0 μg D_3/kg. These birds grew the fastest and had the highest bone ash and plasma Ca compared with the basal and the irradiated treatments. However, this high incidence of TD may have been present at days 7 and 14 but it was not differentiated by gross examination from the rickets at this early age. This is a clear case indicating that histopathology studies should be conducted when examining for both rickets and TD when the birds are very young. The results still suggest that u.v. radiation at day 1 influences the fate of growth plate chondrocytes that will not appear for 10–20 d.

A point should be made that in all of the Expts, except Expt 5, there was lower TD when the birds were exposed to u.v. radiation regardless of what level of D_3 was fed. In Expts 1, 2, 3 and 4 the low levels of D_3 fed, 12·5, 5·0, 5·0 and 5·0 μg/kg diet respectively, may just not have provided sufficient D_3 to prevent TD. However, the results of Expt 6 indicate that the early exposure to u.v. radiation prevented the development of TD even when the vitamin D_3 level was 55·0 μg/kg diet. This special response to u.v. radiation in preventing the development of TD confirms the results reported earlier from this laboratory (Elliot & Edwards, 1997). The results from several of the experiments indicate that the development of TD may be especially sensitive to exposure to u.v. radiation when the bird is very young; 24 h exposure to fluorescent lights on day 1 in Expt 1 or 30 to 60 min exposure from below to the Hg-vapour lamps in Expts 3, 4 and 6 prevented the development of TD.

The effect of u.v. radiation on the incidence of TD in the chicken in Expts 5 and 6 once again emphasizes the importance of u.v. light striking the feet and legs during this short 30 min exposure period used in these experiments. When the 30 min exposure to u.v. radiation was from above there was no effect on any of the criteria measured at dietary vitamin D_3 levels of 27·5 and 55·0 μg/kg. Exposure from below decreased the incidence of TD similar to the effect reported by Elliot & Edwards (1997). The exposure to u.v. radiation for just 30 min did not offer the protection given by feeding 10 μg 1,25-$(OH)_2D_3$/kg (Edwards, 1990) in the feed for the 16 d experimental period. The results of Expt 6 also confirm the results of Elliot & Edwards (1997), indicating that higher levels of D_3 in the diet will not reduce the incidence of TD while u.v. radiation and 1,25-$(OH)_2D_3$ will. These present results should stimulate further studies on the effects of exposure of chickens to u.v. radiation and in particular exactly what happens to the D_3 that is synthesized in this manner compared with dietary D_3.

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References

Association of Official Analytical Chemists (1995)? In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th ed., vol. 2, chapter 45, pp. 57–58. Washington, DC: AOAC.

Boris A, Hurley JF & Trmal T (1977) Relative activities of some metabolites and analogs of cholecalciferol in stimulation of tibia ash weight in chicks otherwise deprived of vitamin D. *J Nutr* **107**, 194–198.

Edwards HM Jr (1989) The effect of dietary cholecalciferol, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol on the development of tibial dyschondroplasia in broiler chickens in the absence and presence of disulfiram. *J Nutr* **119**, 647–652.

Edwards HM Jr (1990) Efficacy of several vitamin D compounds in the prevention of tibial dyschondroplasia in broiler chickens. *J Nutr* **120**, 1054–1061.

Edwards HM Jr (2000) Nutrition and skeletal problems in poultry. *Poult Sci* **79**, 1018–1023.

Edwards HM Jr, Elliot MA, Sooncharernying S & Britton WM (1994) Quantitative requirement for cholecalciferol in the absence of ultraviolet light. *Poult Sci* **73**, 288–294.

Edwards HM Jr & Veltmann JR Jr (1983) The role of calcium and phosphorus in the etiology of tibial dyschondroplasia. *J Nutr* **113**, 1568–1575.

Elliot MA & Edwards HM Jr (1997) Effect of 1,25-dihydroxycholecalciferol, cholecalciferol, and fluorescent lights on the development of tibial dyschondroplasia and rickets in broiler chickens. *Poult Sci* **76**, 570–580.

Fraser D (1983) The physiological economy of vitamin D. *Lancet* **i**, 969–972.

Haddad JG, Matsuoka LY, Hollis BC, Hu YZ & Wortsman J (1993) Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* **91**, 2552–2555.

Haussler MR, Zerwekh JE, Hesse RH, Rizzardo E & Pechet MM (1973) Biological activity of 1-α-hydroxycholecalciferol, a synthetic analog of the hormonal form of vitamin D_3. *Proc Nat Acad Sci USA* **70**, 2248–2252.

Hess A & Weinstock M (1924) Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation. *J Biol Chem* **62**, 301–313.

Holick MF, MacLaughlin JA & Doppelt SH (1981) Regulation of cutaneous previtamin D_3 photosynthesis in man: skin pigment is not an essential regulator. *Science* **211**, 590–593.

Holick MF, MacLaughlin JA, Parrish JA & Anderson RR (1982) The photochemistry and photobiology of vitamin D_3. In *The Science of Photomedicine*, pp. 195–218 [JD Regan and JA Parrish, editors]. New York: Plenum.

Holick MF, Matsuoka LY & Wortsman J (1989) Age, vitamin D, and solar ultraviolet radiation. *Lancet* **4**, 1104–1105.

Koch EM & Koch FC (1941) The provitamin D of the covering tissues of chickens. *Poult Sci* **20**, 33–35.

Long PH, Lee SR, Rowland GN & Britton WM (1984) Experimental rickets in broilers: Gross microscopic and radiographic lesions. II Calcium deficiency. *Avian Dis* **28**, 921–932.

Mawer EB, Backhouse J, Holman CA, Lumb GA & Stanbury SW (1972) The distribution and storage of vitamin D and its metabolites in human tissues. *Clin Sci* **43**, 413–431.

Norman AW & DeLuca HF (1963) The preparation of ^{3}H-vitamins D_2 and D_3 and their localization in the rat. *Biochem J* **2**, 1160–1168.

Rosenheim O & Webster TA (1926) The anti-rachitic properties of irradiated sterols. *Biochem J* **20**, 537–544.

Scott HT, Hart EB & Halpin JG (1929) Winter sunlight, ultraviolet light and glass substitutes in the prevention of rickets in growing chicks. *Poult Sci* **9**, 65–76.

Steenbock H (1924) The induction of growth promoting and calcifying properties in a ration exposed to light. *Science* **60**, 224–225.

Tian XQ, Chen TC, Lu Z, Shao Q & Holick MF (1994) Characterization of the translocation process of vitamin D_3 from the skin into the circulation. *Endocrinology* **135**, 655–661.