Changes in serum levels of 1,25-dihydroxyvitamin D_3, calcium and phosphorus with age and vitamin D status in chickens

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1. The effects of vitamin D_3 (D_3) on serum levels of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), ionic calcium, total Ca and phosphorus in chicks were studied from the time of hatching until sexual maturity.

2. Chicks fed on a diet low in D_3 showed a serum level of $1,25(OH)_2D_3$ higher than that in chicks on a normal-D_3 diet, for both sexes and at any given age.

3. A dramatic increase in the serum level of $1,25(OH)_2D_3$ occurred in female birds approaching sexual maturity and in laying hens raised on the low-D_3 diet the level was five times that of their counterparts raised on a normal-D_3 diet.

4. The serum $1,25(OH)_2D_3$ level in adult males in the low-D_3 groups was seven times that of those on the normal-D_3 diet.

5. The serum level of 25-hydroxyvitamin D_3 remained relatively unchanged at weeks 2 and 15 in birds on a low D_3 intake as well as in those fed on a normal-D_3 diet. Nevertheless, the levels of 25-hydroxyvitamin D_3 were different between the two groups.

6. No significant change was observed in the level of ionized serum Ca in relation to dietary regimen, but there was an increase in total Ca concentration in females with the onset of reproduction.

7. The serum P level decreased gradually with age, reaching a minimum value 3 and 8 weeks before laying commenced in the groups on low- and normal-D_3 diets respectively. An increase was observed when the hens began laying.

8. Chicks adapted to a low-D_3 diet by elevation of their plasma level of $1,25(OH)_2D_3$. The mechanism by which this is achieved is not known, but the results suggest that parathyroid hormone, Ca and P are unlikely to play roles in the adaptive increase in the level of $1,25(OH)_2D_3$ in the blood of chicks given a minimal amount of D_3. The possibility that the rate of degradation of $1,25(OH)_2D_3$ is greatly reduced under these conditions cannot be excluded and this could account for the level of this metabolite in those birds.

Before it exerts its effect on calcium and phosphorus metabolism, vitamin D_3 (D_3) is bioactivated in the liver (Ponchon *et al.* 1969) and in other organs (Tucker *et al.* 1973) to 25-hydroxyvitamin D_3 ($25(OH)D_3$). The $25(OH)D_3$ is then further hydroxylated in the kidney to produce either 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$; Fraser & Kodicek, 1970) or 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$; Holick *et al.* 1972), depending on the physiological state of the animal and the need for Ca. The 1,25-dihydroxylated metabolite is considered to be the most active form of D_3 derivatives in stimulating Ca and P absorption (Boyle *et al.* 1972) and in Ca mobilization from the bone (Raisz *et al.* 1972).

The regulation of the secretion of $1,25(OH)_2D_3$ has been the subject of extensive research which has revealed that the enzyme $25(OH)D_3$-1-hydroxylase (25-hydroxycholecalciferol-1-monooxygenase; *EC* 1.14.13.13) is regulated by complicated interactions involving ionic and hormonal factors (Fraser, 1980). In vitamin D-deficient animals the activity of $25(OH)D_3$-1-hydroxylase predominates and $25(OH)D_3$-24-hydroxylase is hardly detectable. Administration of D_3 or $1,25(OH)_2D_3$ into such animals (Galante *et al.* 1973; Henry *et al.* 1974; Tanaka & De Luca, 1974; Horiuchi *et al.* 1976; MacIntyre *et al.* 1976; Henry, 1977) or the addition of $1,25(OH)_2D_3$ to an incubation mixture (Larkins *et al.* 1974; Henry, 1977; Spanos *et al.* 1978) results in a decrease in $25(OH)D_3$-1-hydroxylase. Injection of gonadal hormones into birds (Tanaka *et al.* 1978) stimulates the activity of $25(OH)D_3$-1-hydroxylase. Furthermore, the activity of this enzyme remains elevated in spite of the associated increase in the level of $1,25(OH)_2D_3$ (Tanaka *et al.* 1978). It seems that

$1,25(OH)_2D_3$ has no effect on its own biosynthesis under physiological circumstances, such as growth, pregnancy, lactation and egg production where $25(OH)D_3$-1-hydroxylase has been stimulated directly or indirectly by other endocrine systems (Spanos *et al.* 1976; Tanaka *et al.* 1978; Abe *et al.* 1979; Castillo *et al.* 1979; Kumar *et al.* 1979). Changes, if any, with age in the serum levels of $1,25(OH)_2D_3$ Ca and P in birds raised on a low-D_3 diet from the time of hatching until sexual maturity have not been reported, since previous studies on the metabolism or regulation of D_3, or both, have used vitamin D-depleted birds for relatively short periods.

The objectives of the present study were to compare the effects of low- and normal-D_3 diets on the serum level of $1,25(OH)_2D_3$ and on serum Ca and P levels in chicks from the time of hatching until the first egg appeared in the oviduct, and to provide additional evidence for the increased production of $1,25(OH)_2D_3$ when birds approach sexual maturity.

MATERIALS AND METHODS

Experiments were performed on White Leghorn chicks. After hatching the birds were housed in battery cages for 1 week. Then they were transferred to a temperature-controlled room, from which sunlight was excluded, until laying commenced. The photoperiod was 14 h light–10 h dark. The experimental birds were fed *ad lib.* on a diet containing (/kg): 34 g Ca, 6·3 g P, 5·5 μg D_3. The control birds were fed on the same diet except that it contained 40 μg D_3/kg diet (Grain Silos and Flour Mill Organization, Fed Mill, Riyadh). After hatching, at the end of weeks 1 and 2, twelve to fifteen chicks of each sex from each dietary group were killed. Thereafter, four to five chicks of each sex on the low-D_3 diet were killed weekly and a group of five birds of each sex on the normal-D_3 diet were killed at the end of weeks 8, 15 and 23 for the measurement of serum $1,25(OH)_2D_3$, ionic Ca, total Ca and P. Serum samples from birds of up to 5 weeks of age were pooled according to age, sex and dietary regimen, but samples from older birds were treated separately. Samples (2 ml) were labelled with [^{3}H]$1,25(OH)_2D_3$, 6000 disintegrations/min (specific activity 148 Ci/mmol; Amersham International plc, Amersham, Bucks), extracted and purified as described by O'Riodan *et al.* (1982) with slight modification. The position of $1,25(OH)_2D_3$ on the high-pressure liquid chromatogram was determined using crystalline standard $1,25(OH)_2D_3$ and [^{3}H]$1,25(OH)_2D_3$. The fraction containing $1,25(OH)_2D_3$ was collected and prepared for radioimmunoassay as described by Bouillon *et al.* (1980). Standard curves were constructed by dispensing adequate volumes of ethanolic solutions containing increasing amounts of $1,25(OH)_2D_3$ (0·49–1000 pg/l) in triplicate into tubes. In each assay the total counts (*I*, bound + free), non-specific binding (*D*) and maximum binding of tracer to antisera (*B*) were assessed in triplicate. The value for bound as opposed to free was calculated.

The intra- and inter-assay coefficients of variation were 15 (n 20) and 11 (n 7) % respectively. The final recovery (mean and SD) of $1,25(OH)_2D_3$ was 74·7 (5·2) %. Quantitative determination of $25(OH)D_3$ was performed as described by Edelstein *et al.* (1974), after adding [^{3}H]$25(OH)D_3$ to 1 ml serum for recovery monitoring, extraction by organic solvent and purification using a 500 mm Sephadex LH-20 column.

Serum ionic Ca was measured as quickly as possible using an AVL electrolyte analyser. Total Ca and P were determined by conventional colorimetric methods.

The results obtained were analysed by Statistical Analysis System (SAS) at the King Saud University Computer Centre; values for means, standard deviations, probabilities and t tests were obtained.

RESULTS

The serum $1,25(OH)_2D_3$ concentrations in the two dietary groups in relation to age for both sexes are shown in Table 1. The levels of $1,25(OH)_2D_3$ in the low-D_3 dietary group were higher than in the normal-D_3 dietary group for both males and females at any given age and remained relatively high throughout life. In both dietary groups, the concentrations of $1,25(OH)_2D_3$ were significantly higher ($P < 0{\cdot}005$) in the laying hens than in the prelaying hens and the level was higher in laying hens raised on the low-D_3 diet than in laying hens fed on the normal-D_3 diet. In the males of both dietary groups, the mean of the circulating levels of $1,25(OH)_2D_3$ decreased with age but, in the low-D_3 dietary group, was seven times that of the normal-D_3 dietary group at week 23.

To assess the vitamin D status of the birds during the study, the circulating levels of $25(OH)D_3$ were measured in both dietary groups at weeks 2 and 15 (Table 1). Birds fed on the low-D_3 diet had $25(OH)D_3$ levels lower than birds fed on the normal-D_3 diet. As shown in Table 1, there were no significant changes in the levels of $25(OH)D_3$ at weeks 2 and 15 in the low-D_3 group as well as in normal-D_3 group.

Table 2 shows the serum levels of ionic Ca, total Ca and P in females of both dietary groups. The ionic Ca was measured relative to age up to the 14th week and showed no changes during that period in either group. In contrast, serum total Ca levels increased at the commencement of the laying phase in these hens and serum P fell during the course of the experiment to about half of the concentration found in 1-week-old birds. An increase from the minimum value was observed when the hens reached sexual maturity.

DISCUSSION

The results presented here demonstrate a significant increase in the serum level of $1,25(OH)_2D_3$ associated with a diet low in D_3 as compared with a normal diet. The increased $1,25(OH)_2D_3$ remained relatively high throughout life in both sexes, as long as the low-D_3 diet was supplied to the birds. These results support the findings of Hughes *et al.* (1977) who observed that chicks raised on an optimal amount of D_3 (35 μg/kg diet) for 3–4 weeks had plasma levels of $1,25(OH)_2D_3$ higher than those raised on a fifty-fold excess of D_3 irrespective of increasing the dietary Ca. Although there are several reports concerning the regulation by $1,25(OH)_2D_3$ of its own production in vitamin D-depleted animals (Galante *et al.* 1973; Henry *et al.* 1974; Tanaka & De Luca, 1974; Horiuchi *et al.* 1976; MacIntyre *et al.* 1976; Henry, 1977), our results seem to indicate the absence of this effect under conditions of low dietary D_3 intake in which the enzyme $25(OH)D_3$-1-hydroxylase is probably stimulated in response to the supplementation with only a minimal level of D_3 (5·5 μg D_3/kg diet). However, the inhibitory effect of $1,25(OH)_2D_3$ on $25(OH)D_3$-1-hydroxylase is lost in circumstances where the activity of the enzyme is enhanced (Spanos *et al.* 1976; Boass *et al.* 1977; Turton *et al.* 1977; Tanaka *et al.* 1978; Abe *et al.* 1979; Castillo *et al.* 1979; Halloran *et al.* 1979; Kumar *et al.* 1979; Lund & Selnes, 1979; Pike *et al.* 1979). Therefore it is more probable that D_3 or its metabolites, or both, suppress the production of the active form when D_3 is given in doses intermittently to vitamin D-deficient animals.

The results shown in Table 2 may indicate the exclusion of the involvement of parathyroid hormone (PTH) and of serum ionic Ca and P in the increased serum level of $1,25(OH)_2D_3$ in the low-D_3 group, since the secretion of PTH is unlikely to be altered, as shown by the relatively constant serum ionic Ca level during the prelaying phase. Unfortunately, a sensitive assay for avian PTH is not yet available and thus it was not possible to determine changes in the rate of secretion of PTH in relation to diet. Despite the gradual decrease in the serum P level in both dietary groups, the elevated level of $1,25(OH)_2D_3$ was evident

Table 1. *The effect of dietary vitamin D_3 (D_3) level on serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) concentrations in female and male chicks*

(Mean values and standard deviations; no. of chicks in parentheses)

	Low-D_3 (5·5 μg/kg) diet								Normal-D_3 (40 μg/kg) diet							
	$25(OH)D_3$ (nmol/l)				$1,25(OH)_2D_3$ (pmol/l)				$25(OH)D_3$ (nmol/l)				$1,25(OH)_2D_3$ (pmol/l)			
	♀		♂		♀		♂		♀		♂		♀		♂	
Age (weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	—	—	—	—	638·1(15)	—	602(15)	—	—	—	—	—	213·1(12)	—	195·0(12)	—
2	10·6(14)	—	9·4(14)	—	650(14)	—	578·6(15)	—	28·3	—	22·5	—	185·2(12)	—	204·8(12)	—
3–7	—	—	—	—	604·8	236·9(20)	516·4	167·9(23)	—	—	—	—	—	—	—	—
8	—	—	—	—	659·5	161·9(5)	519	69·5(5)	—	—	—	—	167·1	44(5)	157·6	27·4(5)
9–14	—	—	—	—	664·3	169(28)	615	172(33)	—	—	—	—	—	—	—	—
15	12·3	2·25(5)	11·3	2·05(5)	678·6	85·7(5)	589	105(5)	30·9	5·3	20·3	4·2	127·6	20·2(5)	115·2	21·4(5)
16–21	—	—	—	—	494	154·8(28)	431·9	141·9(25)	—	—	—	—	—	—	—	—
23	—	—	—	—	1270·5***	113(5)	403·6 NS	57·4(4)	—	—	—	—	338·6***	27·9(5)	57·1**	15·7(4)

NS, not significantly different from age 16–21 weeks. Values were significantly different from those at 15 weeks of age: ** $P < 0·005$, *** $P < 0·001$.
All values at weeks 1 and 2 represent pooled serum samples from twelve to fifteen birds.

Table 2. *Serum levels of ionic calcium (Ca^{2+}), total Ca and phosphorus in relation to dietary vitamin D_3 (D_3) intake in female chicks*

(Mean values and standard deviations (mmol/l); no. of chicks in parentheses)

	Low-D_3 (5·5 μg/kg) diet						Normal-D_3 (40 μg/kg) diet					
	Ca^{2+}		Ca		P		Ca^{2+}		Ca		P	
Age (weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1·14(15)	—	2·25(15)	—	2·19(15)	—	1·09(12)	—	2·46(12)	—	2·09(12)	—
2	1·11(14)	—	2·6(14)	—	2·1(14)	—	1·1(12)	—	2·55(12)	—	2·03(12)	—
3–7	1·11	0·12(20)	2·58	0·5(20)	2·06	0·19(20)	—	—	—	—	—	—
8	1·18	0·03(5)	2·5	0·22(5)	1·52	0·2(5)	1·16	0·07(5)	2·5	0·11(5)	1·65	0·11(5)
9–14	1·18	0·08(28)	2·53	0·34(28)	1·32	0·28(28)	—	—	—	—	—	—
15	—	—	2·53	0·14(5)	1·0	0·2(5)	—	—	2·55	0·14(5)	1·14	0·17(5)
16–21	—	—	2·55	0·29(28)	0·81	0·25(28)	—	—	—	—	—	—
23	—	—	4·73	0·3(5)	1·14	0·18(5)	—	—	3·81	0·17(4)	1·21	0·05(5)

All values at weeks 1 and 2 represent pooled serum samples from twelve to fifteen birds.

only in the low-D_3 group. The dramatic increase in total Ca with the onset of reproductive activity is restricted mainly to the non-ultrafiltrable fraction which is associated with the appearance of a phospholipoprotein with a high capacity for the binding of Ca (Urist *et al.* 1960).

There was a dramatic increase in the serum level of $1,25(OH)_2D_3$ when the female birds approached sexual maturity, probably under the influence of oestradiol (Spanos *et al.* 1976; Castillo *et al.* 1979; Sedrani, 1979). The increased production of $1,25(OH)_2D_3$ is physiologically required for the provision of Ca for the mineralization of the egg shell and medullary bone.

In conclusion, it appears that the chick is able to adapt to a low dietary D_3 intake by increasing the serum level of $1,25(OH)_2D_3$. The mechanism by which the adaptation occurs is not known but probably does not involve PTH, Ca and P. A mechanism for regulating the $25(OH)D_3$-1-hydroxylase activity as suggested by Fraser (1980) involves the means of presenting the substrate, $25(OH)D_3$, to the enzyme. The system for transferring $25(OH)D_3$ to the inner mitochondrial membrane, after entry to the renal cell, may be sensitive to low levels of substrate. A correlation between plasma levels of $25(OH)D_3$ and $1,25(OH)_2D_3$ has been reported at low concentrations of $25(OH)D_3$ (Mawer *et al.* 1975; Sedrani, 1984). This is consistent with the existence of such a system. Consequently the renal $25(OH)D_3$-1-hydroxylase in the chick on a low-D_3 diet may be responding to the low $25(OH)D_3$ levels with an increased output of $1,25(OH)_2D_3$.

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