Effect of Dietary Vitamin K_1 on Selected Plasma Characteristics and Bone Ash in Young Turkeys Fed Diets Adequate or Deficient in Vitamin D_3

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ABSTRACT Three experiments were conducted to determine the effect of dietary vitamin K_1 (K_1) on selected plasma characteristics and bone ash in poults. In Experiment 1, diets were supplemented with 0, 0.5, 1.0, or 2.0 mg of K_1/kg. All diets contained 1,650 IU of vitamin D_3 (D_3)/kg. Dietary K_1 had no effect on tibia ash at 7 d or incidence of a severe, rickets-like condition. Tibia ash of poults fed 2.0 mg of K_1/kg, however, was greater at 14 d of age than that of poults fed the basal diet. Dietary inclusion of 0.5 mg of K_1/kg was as effective as 1 or 2 mg of K_1/kg in reducing plasma prothrombin time. In Experiment 2, a 2 × 4 factorial arrangement was used consisting of 1,650 or 550 IU of D_3/kg and 0.1, 0.45, 1.0, and 2.0 mg of K_1/kg. Dietary D_3 and K_1 had no effect on bone ash. Dietary inclusion of 0.1 mg of K_1/kg seemed to be enough to minimize plasma prothrombin time. In Experiment 3, dietary treatments consisted of a control (1,650 IU of D_3 and 2.0 mg of K_1/kg) and K_1 concentrations of 0, 0.37, 2.28, or 5.33 mg/kg in diets containing 275 IU of D_3/kg. Poults fed the low-D_3 diet without K_1 consumed less feed, gained less weight, and had increased plasma alkaline phosphatase activity, decreased inorganic phosphorus level, and decreased tibia ash ($P < 0.05$) compared with those of poults fed the control diet. Feed intake and body weight gain were improved, plasma alkaline phosphatase activity decreased, and plasma inorganic phosphorus increased or tended to increase when poults were fed the low-D_3 diet supplemented with 0.37 or 2.88 mg of K_1/kg compared with poults fed the low-D_3 diet without K_1 supplementation. Tibia ash of poults fed the low-D_3 diet was not affected by K_1 supplementation. The results of this research show that dietary K_1 concentration had little, if any, effect on bone development in 1- to 14-d-old turkeys.

(*Key words*: vitamin D_3, vitamin K_1, alkaline phosphatase, phosphorus, bone ash)

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INTRODUCTION

Since its discovery in the late 1940s and up to the early 1970s, vitamin K was thought to be needed only for the synthesis of four plasma clotting proteins in the liver, namely factors II (prothrombin), VII, IX, and X. Research during the past two decades, however, has identified a substantial list of γ-carboxyglutamic acid (GLA)-containing proteins in various tissues, such as the kidneys (Booth, 1997) and uterine smooth muscle (Luo et al., 1995). Other than the liver, some cells in the skeleton, such as osteoblasts in bone (Hauschka and Reid, 1978) and chondrocytic lineage cells in cartilage, also actively synthesize the GLA proteins, such as osteocalcin and GLA matrix protein (Luo et al., 1995). The process of posttranslational carboxylation of glutamyl residues to γ-carboxyglutamyl residues in these GLA proteins requires the presence of vitamin K. The GLA residues in the vitamin K-dependent proteins are essential for their full biological activities (Will and Suttie, 1992).

There is evidence indicating an interaction between the biological functions of vitamins D and K. Leeuwen et al. (1996) reported that the expression of osteocalcin genes was regulated by vitamin D. Takede et al. (1994) postulated that the vitamin D receptor, which is a nuclear transcription factor, binds to the vitamin D response elements of the osteocalcin genes and regulates their expressions. After translation the nascent osteocalcin is carboxylated in a vitamin K-dependent process (Hauschka et al., 1989). Sergeev and Norman (1992) reported that the 1,25-$(OH)_2$ D_3 receptor (VDR) underwent γ-carboxylation in the presence of K_1 in vitro, and 15–25% of Glu residues in the VDR were carboxylated in vivo. It was suggested that the carboxylation of VDR might regulate its binding to DNA (Sergeev and Norman, 1992).

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Abbreviation Key: D_3 = vitamin D_3; GLA = γ-carboxyglutamic acid; IROC = immunoreactive osteocalcin; K_1 = vitamin K_1 (phylloquinone); MK-4 = menaquinone-4; VDR = 1,25-$(OH)_2$ D_3 receptor.

Vitamin K has been reported to affect some plasma parameters related to bone. Knapen et al. (1989) reported that administration of K_1 (1 mg/d) increased the concentration and the hydroxyapatite-binding capacity of circulating immunoreactive osteocalcin (IROC) in postmenopausal women, and this increase was due to the increased serum IROC with a high affinity for hydroxyapatite; whereas, the IROC with a low affinity for hydroxyapatite remained unaffected (Knapen et al., 1993). Knapen et al. (1993) reported that K_1 supplementation in elderly women also resulted in an increase of plasma bone-specific alkaline phosphatase activity and a parallel increase in total plasma alkaline phosphatase. Akedo et al. (1992) reported that inclusion of menaquinone-4 (MK-4) in culture medium suppressed proliferation of osteoblasts and increased alkaline phosphatase activity in vitro. Effect of vitamin K on bone morphology reported in the literature is not consistent. Fleming et al. (1998) reported that supplementation of high levels of menadione nicotinamide bisulfite in diets significantly reduced the loss of cancellous bone in the proximal tarsometatarsus in laying hens between 15 and 25 wk of age compared with hens fed a diet containing a low level of menadione nicotinamide bisulfite. Lavelle et al. (1994) reported that menadione sodium bisulfite supplementation had no effect on gross and histological morphology of bone, cartilaginous tissues, or bone ash in young growing chicks.

Information in the literature on the effect of vitamin K on bone or related parameters in young growing animals is scarce. Thus, the objective of the research reported here was to determine the influence of dietary K_1 on bone development and related plasma parameters in young turkeys when dietary D_3 was adequate or deficient. Concurrently, the dietary concentration of K_1 needed to minimize plasma prothrombin time was evaluated.

MATERIALS AND METHODS

Experimental procedures used in this research were approved by the Laboratory Animal Care Committee, Iowa State University.

Diets

In Experiment 1, four dietary treatments were used that consisted of a basal diet (Table 1) supplemented with 0, 0.5, 1.0, or 2.0 mg of K_1[3]/kg. The diets were fed as pellets from Days 1 to 7 and then were switched to mash form from 8 to 14 d of age. Each treatment was fed to four replicate pens with seven poults per pen from 1 to 14 d of age. Eleven poults in an extra pen were fed the basal diet in mash form from 1 to 14 d of age for observation. In Experiment 2, a 2 × 4 factorial arrangement was used, consisting of two concentrations of D_3 (1,650 or 550 IU/kg) and four concentrations of K_1 (0.1, 0.45, 1.0, or 2.0 mg/kg, by analysis[4]) based on the basal diet shown in Table 1, except the vitamin premix contained no D_3. Each treatment was fed to four replicate pens with eight poults per pen from Days 1 to 14. The diets were fed in mash form. In Experiment 3, dietary treatments consisted of a control (1,650 IU of D_3/kg and 2.0 mg of K_1/kg) and diets containing K_1 concentrations of 0, 0.37, 2.28, or 5.33 (by analysis) and 275 IU of D_3/kg. The same basal diet shown in Table 1 was used to obtain these dietary treatments, except the D_3 concentration was adjusted accordingly. Each treatment was fed to four replicate pens with eight poults per pen from 1 to 19 d, and the diets were fed in mash form.

TABLE 1. Composition of the basal diet

Ingredients	(g/kg)
Soybean meal (48% CP)	567.03
Corn starch	267.70
Sunflower meal	80.00
Stripped corn oil	39.02
Dicalcium phosphate	23.83
Limestone	12.67
Vitamin premix[1]	3.00
Mineral premix[2]	3.00
DL-methionine	2.00
NaCl	1.50
BMD[3]	0.25
Calculated nutrient composition	
ME, kcal/kg	2,850
CP, %	28.5
TSAA, %	1.05
Lysine, %	1.69
Calcium, %	1.20
Available phosphorus, %	0.60
Vitamin K_1, mg/kg	<0.02[4]

[1]Provided per kilogram of diet: vitamin A (retinyl acetate), 8,190 IU; dl-α-tocopheryl acetate, 30 IU; vitamin D_3 (cholecalciferol), 1,650 IU; vitamin B_{12}, 15.9 μg; pantothenic acid, 13.2 mg; niacin, 75 mg; choline, 509 mg; folic acid, 2.4; biotin, 0.27 mg; pyrodixine HCl, 6.0 mg; thiamine mononitrate, 2.4.

[2]Supplied per kilogram of diet: Mn, 70 mg; Zn, 40 mg; Fe, 37 mg; Cu, 6 mg; Se, 0.15 mg; NaCl (iodized), 2.60 g.

[3]Bacitracin dimethylene salicylate, A. L. Pharma, Inc., Ft. Lee, NJ 07024.

[4]Detection limit for vitamin K_1.

Animals

One-day-old male Nicholas poults were obtained from a commercial hatchery and were randomly assigned to each experimental pen. The poults were kept in heated, thermostatically controlled batteries with raised wire floors. Plastic sleeves, designed to filter out ultraviolet light, were put on all light sources in the room and in the batteries in Experiments 2 and 3. Water and feed were provided ad libitum. Water troughs were cleaned daily to reduce the possibility of production of vitamin K by fermentation. Excreta observed in feeders were removed daily. In Experiments 1 and 2, feed consumption and body weight were recorded weekly. Two blood samples were obtained from two poults from each pen

[3]Sigma Diagnostics, St. Louis, MO 63178.
[4]CN Laboratory, Courtland, MN 56021.

on Days 7 and 14. Citrate was used as an anticoagulant for the blood samples from which plasmas were used to determine prothrombin time. Heparin was used in blood samples from which plasmas were used for determination of K_1, MK-4, and 25-OH-D_3. After blood samples were obtained, the poults were euthanatized by inhalation of Halothene®.[5] In Experiment 1, the right and left tibias were then collected. The right tibia was used for histological evaluation, and the left tibia was used to determine bone ash. In Experiment 2, only the left tibia was collected for bone ash determination. Body weight and feed consumption data were recorded on Days 6, 13, and 19 in Experiment 3. Blood samples also were obtained on these same days from two poults per pen as described for Experiments 1 and 2. When poults were 19 d old, left tibias were collected from two poults per pen for the determination of bone ash. Plasmas, obtained when citrate was used as the anticoagulant, were used to determine prothrombin clotting time, whereas plasmas obtained from heparinized blood were used to determine alkaline phosphatase activity and calcium and inorganic phosphorus concentrations. Plasma calcium and inorganic phosphorus were determined on plasmas obtained on Days 13 and 19 only.

Analytical Procedures

Plasma K_1 and MK-4 levels were determined at the University of Wisconsin[6] with the HPLC method of Haroon et al. (1986). In the analysis, plasma samples were extracted through a liquid phase with a volume ratio of plasma, water, ethanol, and hexane of 1:2:3:9. After centrifugation, the organic layer at the top of the supernatant was harvested and evaporated to dryness. The dried sample was resuspended in hexane, filtered through a silica Sep-Pak cartridge, and eluted with a 3% mixture of ether in hexane. K_1 and MK-4 were analyzed by HPLC with a Zorbax C18 reverse-phase chromatography column, a zinc reduction column, and a fluorescent detector. The zinc reduction column reduced vitamin K compounds to their fluorescent hydroquinones. The mobile solvent used was 15% methylene chloride in methanol with 5 mL/L reductive solvent additive. The rate of solvent flow was set to 1.0 mL/min, and pressure was 7,757 cm mercury. Excitation was performed at 244 nm, and emission was monitored at 418 nm. The internal standard used was di-hydro K_1.

Plasma 25-OH-D_3 was determined[7] using the radioimmunoassay of Hollis et al. (1993). The primary goat antibody against 25-OH-D_3 and the anti-goat second antibody were obtained from Incstar Corp.[3] The radio-iodinated D_3 was prepared from 23, 24, 25, 26, 27-pentanor-C22-carboxylic acid of D_3. Briefly, a solution of the C22 acid of D_3 and 1,1-carbonyldiimidazole in dimethylforamide was allowed to react at 4 C for 2 h. 1,3-Diaminopropane was then added and allowed to react at 4 C for 16 h and at 25 C for 3 h. The 3-aminopropyl derivative of D_3-C22-amide produced was combined with Bolton-Hunter reagent in dioxane. The resulting side-chain-radio-iodinated D_3 analogue was isolated and purified by silica gel chromatography. The assay calibrators were prepared from serum and stripped free of 25-OH-D_3 by activated charcoal, and crystalline 25-OH-D_3 was dissolved in absolute ethanol. 25-OH-D_3 in the plasmas and calibrators was extracted with acetonitrile, and after centrifugation, the supernatants were harvested to analyze 25-OH-D_3.

One-stage prothrombin time was determined manually using a commercial thromboplastin preparation, Simplastin® Excel.[3] Plasma inorganic phosphorus, calcium, and total alkaline phosphatase activity were determined using commercial kits of procedures No. 670, No. 587, and No. 104,[3] respectively, obtained from Sigma Diagnostics.

The left tibias were cooked in boiling water and cleaned of flesh. The cleaned tibias were dried and then ashed in a muffle furnace at 600 C for 12 h to determine ash content. The right tibias were trimmed of flesh at the time of dissection, and the proximal ends were cut longitudinally at the middle. Care was taken not to displace the growth plate. The slice of the proximal end was fixed in 10% neutral buffered formalin for 2 to 3 d. The bone was then demineralized through a graded series of ethanol solutions and infiltrated with paraffin. The bone tissue was then sectioned at 5 μm, mounted, and stained with hematoxylin and eosin. The histological structure of the tibia was examined under a microscope.

Statistical Methods

Data were analyzed using the general linear model (SAS Institute, 1996). When appropriate, linear and quadratic responses of the dependent variable to independent variable were tested. Single degree of freedom contrasts were used to determine the differences among treatment means when appropriate. Significance was declared when probability was less than 5%, and a trend was noted when probability was less than 10%.

RESULTS

Experiment 1

Dietary K_1 concentration did not affect body weight gain, feed intake, or feed efficiency (feed-to-gain ratio) of poults through 14 d of age (Table 2). Weight gain from 1 to 7 d was exceptionally good, averaging 124 g/poult. At 7 d, however, more than 50% of poults in all dietary treatment groups developed leg weakness. Affected poults were reluctant to move, often sitting on their hocks. Some of them used wings to move around

[5]Halocarbon Laboratories, River Edge, NJ 07661.
[6]J. W. Suttie and his group, Department of Biochemistry, University of Wisconsin, Madison, WI 53706.
[7]Ronald Horst, USDA, ARS, National Animal Disease Center, Ames, IA 50010-0070.

TABLE 2. Effect of dietary vitamin K_1 concentrations on body weight gain, feed intake, and feed efficiency in young poults fed a diet containing 1,650 IU of D_3/kg; Experiment 1

Supplemental K_1	Body weight 14 d	Weight gain 1 to 14 d	Feed intake 1 to 14 d	Feed efficiency[1] 1 to 14 d
(mg/kg)		(g/poult[2])		
0	314[2]	257	330	1.29
0.5	316	258	331	1.28
1.0	320	263	343	1.30
2.0	323	267	340	1.28
SEM	8.2	5.5	7.9	0.02
		(*P*)		
Sources of variation				
Linear	0.38	0.34	0.80	0.28
Quadratic	0.92	0.91	0.62	0.87

[1]Feed-to-gain ratio.

[2]Means of four pens per treatment, seven poults per pen.

or keep their balance. Gross examination of longitudinal slices of tibias taken at 7 d showed that the growth plates were thickened to about 2 mm. Histological examination of tibias confirmed a rickets-like condition in all instances of poults fed the pelleted diets. In contrast, poults fed the basal diet that had not been pelleted showed no signs of leg weakness. Because only part of each experimental diet had been pelleted, the poults were changed to the nonpelleted, mash form of the respective experimental diets from 8 to 14 d. By Day 14, all poults, showed few signs of leg weakness, irrespective of dietary K_1 concentration.

Bone ash concentrations of the tibias were relatively low at 7 d, irrespective of dietary K_1 concentration (Table 3). At 14 d, bone ash was determined on only the tibias from poults fed the diet containing 2.0 mg of K_1/kg and from poults fed the basal diet. The poults fed the basal diet included those in the observation group that were fed the basal diet in mash form from Day 1 and those in the experimental group that were fed the pelleted diet from Days 1 to 7 and the mash diet from Days 8 to 14. These data showed that the percentage of bone ash in the experimental groups increased substantially as compared with the 7-d data. The percentage of bone ash of poults fed 2.0 mg of K_1/kg was greater ($P = 0.05$) than that of poults fed the basal diet. Supplementation of K_1 in diets had no effect on plasma 25-OH-D_3 levels (Table 3). At 7 d of age, plasma 25-OH-D_3 levels in poults of all dietary treatments were lower (6.8 ng/ml) than values (11.6 ng/mL) observed with normal poults at 8 d of age (Sell and Horst, 1983). Although poults in the experimental groups recovered from the leg weakness by 14 d of age, their plasma 25-OH-D_3 levels were still less than those of poults fed the mash diet from Day 1.

Plasma prothrombin time decreased with the increase of dietary K_1 at 7 and 14 d of age (Table 4). Comparisons by single degree of freedom contrasts showed that the prothrombin times of poults fed diets containing 0.5, 1.0, or 2.0 mg of K_1/kg did not differ from each other, but they were all less than that of poults fed the diet without K_1. At 7 d of age, plasma K_1 concentration increased curvilinearly as dietary K_1 level increased and

TABLE 3. Effect of dietary vitamin K_1 concentrations on bone ash and plasma 25-OH vitamin D_3 levels in young poults fed a diet containing 1,650 IU of vitamin D_3/kg; Experiment 1

Item	Bone ash 7 d	Bone ash 14 d	25-OH vitamin D_3 7 d	25-OH vitamin D_3 14 d
	(%)		(ng/ml)	
Supplemental K_1 (mg/kg)				
0	30.4[1]	34.3[b]	6.56	6.65
0.5	31.2	ND[2]	4.84	8.84
1.0	31.3	ND	7.06	7.59
2.0	31.0	36.9[a]	8.92	8.06
0 (mash)[3]	ND	37.9	ND	11.70
SEM	0.29	0.40	1.63	1.41
		(*P*)		
Source of variation				
Treatments	0.26	0.05	0.33	0.33

[a,b]Means within a column without a common superscript differ significantly ($P \leq 0.05$).

[1]Mean of four pens per treatment, two poults per pen except for 0 (mash), for which there was one pen of four poults.

[2]ND = not determined.

[3]The data were analyzed without inclusion of data from poults fed the basal diet in mash form from Day 1.

TABLE 4. Effect of dietary vitamin K_1 (K_1) concentrations on prothrombin time (PT) and plasma K_1 (K_1) and menaquinone-4 (MK-4) concentrations in young poults fed a diet containing 1,650 IU of vitamin D_3/kg; Experiment 1

	PT		K_1		MK-4	
Item	7 d	14 d	7 d	14 d	7 d	14 d
	(s)		(ng/mL)			
Supplemental K_1 (mg/kg)						
0	437[1]	356	0.10	0.05	0.32	0.61
0.5	155	156	4.25	1.68	1.16	2.94
1.0	147	116	4.86	3.36	4.12	5.41
2.0	121	114	2.95	7.29	6.19	9.73
SEM	36.4	34.7	1.54	2.23	1.69	2.29
	(*P*)					
Sources of variation						
Linear	0.08	0.006	0.30	0.002	0.001	0.0001
Quadratic	0.05	0.001	0.0005	0.14	0.003	0.21

[1]Means of four pens, two poults per pen.

reached an apparent plateau when the dietary level of K_1 reached 1.0 mg/kg (Table 4). Plasma MK-4 concentration also increased curvilinearly as dietary K_1 level increased (Table 4). At 14 d of age, plasma K_1 and MK-4 concentrations, however, increased linearly with the increase of dietary K_1 (Table 4).

Experiment 2

Poults fed diets containing 550 IU of D_3/kg gained less weight during the 14-d experiment than poults fed diets containing 1,650 IU of D_3/kg (Table 5). Dietary D_3 had no effect on feed intake. The dietary K_1 effect on body weight gain and feed intake was not significant (Table 5). A significant interaction between dietary D_3 and K_1 was detected on efficiency of feed utilization (Table 5). Dietary K_1 did not affect efficiency of feed utilization in poults fed diets containing 1,650 IU of D_3/kg. However, the efficiency of feed utilization was poorer statistically or numerically in poults fed diets containing 550 IU of D_3/kg when supplemented with high levels of K_1 (0.45 mg or more /kg) compared with that of poults fed diets containing 550 IU of D_3/kg with 0.1 mg of K_1/kg. It should be noted that the biological meaning of this interaction was questionable on the basis of the relatively small numerical difference.

TABLE 5. Effect of dietary concentrations of vitamin D_3 (D_3) and vitamin K_1 (K_1) on weight gain, feed intake, and feed efficiency in poults; Experiment 2

Supplemental		Weight gain	Feed intake	Feed efficiency[1]
D_3 (IU/kg)	K_1 (mg/kg)	1 to 14 d	1 to 14 d	1 to 14 d
		(g/poult)		
1,650	0.1	321[2]	372	1.15
	0.45	321	368	1.14
	1.0	324	371	1.14
	2.0	322	369	1.14
550	0.1	324	374	1.15
	0.45	302	377	1.25
	1.0	312	367	1.17
	2.0	316	372	1.17
SEM		5.5	6.4	0.01
Main effect means				
D_3	550	314	373	1.18
	1,650	322	370	1.14
K_1	0.1	323	373	1.15
	0.45	312	373	1.20
	1.0	318	369	1.16
	2.0	319	371	1.16
		(*P*)		
Sources of variation				
D_3		0.05	0.13	0.001
K_1		0.24	0.92	0.001
D_3 * K_1		0.24	0.48	0.001

[1]Feed-to-gain ratio.

[2]Means of four replicate pens, eight poults per pen.

TABLE 6. Effect of dietary levels of vitamin D_3 (D_3) and vitamin K_1 (K_1) on prothrombin time, plasma K_1 levels, and bone ash; Experiment 2

Supplemental D_3 (IU/kg)	Supplemental K_1 (mg/kg)	Prothrombin time (s) 7 d	Prothrombin time (s) 14 d	Plasma K_1 14 d	Bone ash 14 d
		(s)		(ng/ml)	(%)
1,650	0.1	146[1]	138	0.06	40.1
	0.45	135	142	1.44	40.6
	1.0	132	115	3.79	41.1
	2.0	130	113	7.87	41.4
550	0.1	149	105	0.18	41.4
	0.45	140	94	1.75	39.9
	1.0	124	91	2.81	40.4
	2.0	122	94	8.03	40.5
SEM		13.5	11.5	0.98	0.46
Means of main effect					
D_3	550	100	96	3.19	40.6
	1,650	136	127	3.29	40.9
K_1	0.1	147	122	0.12	40.8
	0.45	138	118	1.60	40.3
	1.0	128	103	3.30	40.9
	2.0	126	104	7.95	41.0
			(*P*)		
Source of variation					
D_3		0.99	0.07	0.92	0.96
K_1		0.59	0.20	0.002	0.61
D_3 * K_1		0.99	0.57	0.96	0.10

[1]Means of four replicate pens, two poults per pen.

Plasma prothrombin times of poults fed diets containing 1,650 IU of D_3/kg tended to be longer ($P = 0.07$) than those of poults fed diets containing 550 IU of D_3/kg at 14 d of age but not at 7 d of age (Table 6). However, dietary D_3 had no effect on plasma K_1 concentrations and bone ash (Table 6). Dietary K_1 concentration of 0.1 mg/kg was as effective as 0.45, 1.0, or 2.0 mg/kg in reducing plasma prothrombin time. Dietary supplementation of K_1 had no effect on bone ash of poults at 14 d of age. Plasma K_1 increased linearly with the increase of dietary K_1 level.

Experiment 3

Poults fed diets that contained 275 IU of D_3/kg gained less weight and consumed less feed during the 19-d trial than did poults fed the control diet, which contained 1,650 IU of D_3/kg (Table 7). These differences occurred irrespective of K_1 supplementation of the low D_3 diet. Weight gain and feed intake of poults fed the low D_3 diet were increased ($P \leq 0.05$) by K_1 supplementation only when 2.88 mg of K_1 were added to the diet. Supplementation of the low D_3 diet with 0.37 or 5.37 mg of K_1/kg did not affect weight gain or feed intake ($P > 0.05$), and no dietary treatment effects on feed efficiency were observed.

Analysis of the data showed that supplementation of the low D_3 diets with 0.37 mg or more of K_1 decreased ($P = 0.018$) plasma alkaline phosphatase activity at 13 d of age as compared with alkaline phosphatase activity of poults fed the low D_3 diet not supplemented with K_1 (Table 8). At that age, alkaline phosphatase activity in plasmas of poults fed K_1-supplemented diets was similar to that of poults fed the control diet. Vitamin K_1 supplementation had no consistent effects on alkaline phosphatase activity when poults were 6 or 19 d old, compared with poults fed the diet not supplemented with K_1. Plasma calcium concentration was not affected by dietary treatment when poults were 13 d old. However, lower plasma calcium concentrations were indicated ($P = 0.07$) for 19-d-old poults fed the low D_3 supplemented

TABLE 7. Effect of supplementation of vitamin K_1 (K_1) on body weight gain, feed intake, and feed efficiency of poults fed low-vitamin D_3 diets (Experiment 3)

Item	Weight gain 1 to 19 d	Feed intake 1 to 19 d	Feed efficiency[1] 1 to 19 d
	(g/poult)		
Dietary K_1 (mg/kg)			
Control[2]	483[a,3]	627[a]	1.30
0	408[c]	540[c]	1.32
0.37	440[bc]	576[bc]	1.31
2.28	453[b]	582[b]	1.29
5.37	420[bc]	551[bc]	1.31
SEM	10.5	12.6	0.02
		(*P*)	
Source of variation			
Treatment	0.001	0.002	0.57

[a,b]Means not followed by a common superscript differ significantly ($P \leq 0.05$).

[1]Feed-to-gain ratio.

[2]The control diet contained 1,650 IU vitamin D_3 and 2.0 mg K_1 per kilogram, whereas diets supplemented with increments of K_1 contained 275 IU of vitamin D_3/kg.

[3]Means of four pens per dietary treatment, eight poults per pen.

TABLE 8. Effect of supplemental vitamin K_1 (K_1) on plasma characteristics related to bone metabolism and bone ash in poults fed low vitamin D_3 diets (Experiment 3)

Item	Bone ash Day 19	Alkaline phosphatase Day 6[1]	Alkaline phosphatase Day 13	Alkaline phosphatase Day 19	Calcium Day 13	Calcium Day 19	Phosphorus Day 13	Phosphorus Day 19
	(%)	(μmol p-nitrophenol released/mL/15 min)			(mg/dL)			
Dietary K_1 (mg/kg)								
Control[2]	42.9[a,3]	79.3	70.0[b]	54.6	7.2	9.9	4.9	7.1
0	39.1[b]	75.3	103.9[a]	91.5	8.6	9.2	3.9	5.0
0.37	40.2[b]	84.1	74.3[b]	67.7	8.9	8.7	5.5	6.2
2.28	39.4[b]	78.6	74.9[b]	81.4	8.9	8.7	6.2	6.2
5.33	39.4[b]	84.7	80.6[b]	69.8	8.5	8.7	4.7	6.1
SEM	0.52	5.68	6.56	8.36	1.11	0.31	0.64	0.70
	(*P*)							
Source of variation								
Diet effect	0.002	0.77	0.018	0.063	0.77	0.07	0.06	0.08

[a,b]Means within a column not followed by a common superscript differ significantly ($P < 0.05$).

[1]Days of age.

[2]The control diet contained 1,650 IU vitamin D_3 and 2.0 mg K_1/kg, whereas diets supplemented with increments of K_1 contained 275 IU of vitamin D_3/kg.

[3]Means of four pens per treatment, two poults per pen.

with 0.37 mg or more of K_1/kg. Although dietary treatment effects on plasma inorganic phosphorus bordered on significance ($P = 0.06$ and 0.08 at 13 and 19 d, respectively), the inconsistencies among responses made any meaningful interpretation impossible. Percentage bone ash of poults fed the control diet was greater ($P < 0.05$) than that of poults fed the lower D_3 diets, with or without K_1 supplementation.

DISCUSSION

The main objective of the research reported here was to determine whether dietary K_1 affected bone development when dietary D_3 was adequate or deficient. Results of Experiment 1 showed that dietary K_1 had no effect on bone development or incidence of leg weakness among poults fed 1,650 IU of D_3/kg, with more than 50% of the poults showing leg weakness and histologic signs of rickets at 7 d of age, irrespective of dietary K_1 concentration. Feeding the same dietary treatments in nonpelleted form from 8 to 14 d alleviated the leg weakness condition of most poults, regardless of the dietary K_1 concentration. Change in percentage of bone ash during this time showed a favorable effect of supplementing 2.0 mg of K_1/kg compared with no K_1 supplementation, although supplementation of K_1 had no effect on plasma 25-OH-D_3 concentration. There was a general increase in bone ash percentage when the nonpelleted diets were fed, but there was no corresponding change in plasma 25-OH-D_3 concentration. Plasma 25-OH-D_3 concentration remained relatively low through 14 d of age as compared with the 25-OH-D_3 concentration reported by Sell and Horst (1983). An explanation for the adverse effect of pelleting on early leg weakness and bone development observed in this experiment is unknown. The occurrence of the problem, however, corresponds with observations of Riddell (1983), who reported that field rickets and leg weakness occurred most often when poults were 10 to 14 d old. He also reported that diets fed to these poults were pelleted, and when analyzed, they contained adequate calcium, phosphorus, and D_3. Regardless of the reason for the adverse effect of diet as pellets on early leg weakness, the current study showed that the dietary K_1 concentrations used did not prevent the problem.

Dietary K_1 concentration had no effect on 14-d weight gain or feed efficiency (feed-to-gain ratio) in Experiment 1. The same was true in Experiment 2, although one seemingly aberrant value for feed efficiency resulted in statistical significance of questionable meaning. Weight gain and feed efficiency of poults fed 1,650 IU of D_3/kg were superior to those of poults fed 550 IU of D_3/kg. Percentage of bone ash in tibias, however, was not affected by dietary concentration of D_3 or K_1, even though the dietary D_3 concentration of 550 IU of D_3/kg was 50% of the 1,100 IU of D_3/kg recommended by the National Research Council (1994). It should be noted that all lights used in Experiments 2 and 3 were covered with plastic shields that filtered the UV light.

To further evaluate the potential effect of dietary K_1 on bone development, diets containing 275 IU of D_3/kg were used in Experiment 3. Although supplementation of the low D_3 diet with 0.37 to 5.33 mg of K_1/kg seemed to enhance weight gain and feed intake in this trial. These effects were inconsistent, and only inclusion of 2.28 mg of K_1/kg caused significant improvements; there was no effect of K_1 on feed efficiency. Vitamin K_1 supplementation of the diet containing 275 IU of D_3/kg also had no effect on percentage bone ash. The only indication that K_1 supplementation of the low D_3 diet influenced a plasma characteristic, which may be related to bone development, was observed with plasma alkaline phosphatase activity of 13-d-old poults. At this age, the inclusion of 0.37 to 5.33 mg of K_1/kg in the low D_3 diet decreased plasma alkaline phosphase activity as compared with when no K_1 was included. However, no such effect of K_1 supplementation was observed when

poults were 6 or 19 d old, and plasma Ca and inorganic P concentrations were not affected by K_1 supplementation when poults were 13 and 19 d old. These data, together with the bone ash data, suggest that dietary K_1 has little, if any, effect on bone development in young turkeys.

Plasma K_1 and MK-4 concentrations generally increased as the dietary concentration of K_1 increased. These increases in plasma K_1 and MK-4 concentrations were linear when poults were 14 d of age in Experiments 1 and 2. However, plasma K_1 and MK-4 concentrations increased curvilinearly with increments of dietary K_1 at Day 7 in Experiment 1. This latter observation might have resulted as a consequence of the leg weakness, which might have impaired feed intake for a time before blood samples were collected. Plasma K_1 is cleared from the circulation system very quickly. Shearer et al. (1974) reported that at 2 and 8 h after oral or intravenous administration of radioactively labeled K_1, only about 10 and 1% of the initial radioactivity, respectively, remained in the plasma.

The results of Experiment 1 showed that the prothrombin time of poults fed a diet containing 0.5 mg of K_1/kg was comparable to that of poults fed a diet supplemented with 1 or 2 mg of K_1/kg. The results of Experiment 2 indicated that dietary supplementation of 0.1 mg of K_1 was as effective as 0.45,1.0, or 2.0 mg of K_1 in reducing the plasma prothrombin time. These data indicated that the dietary requirement of K_1 for young turkeys might be less than 0.1 mg of K_1/kg. The NRC (1994) lists a vitamin K requirement of 1.75 mg/kg. However, this value is based on studies in which menadione sodium bisulfite was the source of vitamin K.

The results of this research show that dietary K_1 concentration had little, if any, effect on bone development in 1-to-14-d-old turkeys. The data also indicate that the dietary K_1 requirement of young turkeys is less than the value listed by the NRC (1994). The dietary K_1 requirement of young turkeys is the subject of a companion paper (Jin and Sell, 2001).

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