Effect of dietary vitamin K1 on selected plasma characteristics and bone ash and the requirement of vitamin K1 in young turkeys

Shihou Jin
Iowa State University

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Effect of dietary vitamin K1 on selected plasma characteristics and bone ash and the requirement of vitamin K1 in young turkeys

by

Shihou Jin

A dissertation submitted to the graduate faculty in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

Major: Animal Nutrition

Major Professor: Jerry L. Sell

Iowa State University

Ames, Iowa

2000
This is to certify the Doctoral dissertation of

Shihou Jin

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

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For Major Program

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For the Graduate College
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EFFECT OF DIETARY VITAMIN K1 (K1) ON SELECTED PLASMA CHARACTERISTICS AND BONE ASH IN YOUNG TURKEY POULTS FED ADEQUATE OR DEFICIENT VITAMIN D3 69

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willingness to shoulder some responsibility of taking care of my son during the course of my
graduate study.
GENERAL INTRODUCTION

Vitamin K is a general term that refers to a series of compounds with the general structure of polyisoprenoid-substituted naphthoquinone. Vitamin K is traditionally classified into K1 (phyloquinone) and K2 (menaquiones), according to its origin from plants or bacteria, respectively. A group of synthetic menadione derivatives, such as menadione sodium bisulfite, also have biological activity of vitamin K. The K vitamins are essential cofactors for microsomal vitamin K-dependent carboxylases, which catalyze a post-translational conversion of glutamyl residues in the nascent precursors to γ-carboxyglutamyl residues (Gla) in their respective mature proteins. Vitamin K is required for the maintenance of normal levels of blood clotting factors such as Factors II, VII, IX and X. Recent research progress indicates that vitamin K is also involved in a large variety of physiological functions. The influence of vitamin K status on bones has received substantial attention in recent years. Vitamin K status of postmenopausal women is related to the incidence of osteoporosis and to some related plasma parameters, such as osteocalcin. Dietary supplemental vitamin K reduces the loss of cancellous bone in laying hens during reproduction. However, information in the literature on the effect of vitamin K on bone or related parameters in young growing animals is scarce and inconclusive. There continues to be a high incidence of leg problems in fast growing young turkeys. Young turkeys, therefore, should serve as a good model for studies to determine the influence of dietary vitamin K on bone development and related parameters.

Recent evidence indicates an interaction between the biological functions of vitamin D and vitamin K. Leeuwen et al. (1996) reported that 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. Sergeev and Norman (1992) reported that 1,25-(OH)₂D₃ receptor (VDR) underwent γ-carboxylation in the presence of K₁ in vitro, and 15 to 25% of Glu residues in the VDR were carboxylated in vivo.

The objectives of the research reported here are 1) to determine the K₁ requirement of turkey poult, and 2) to determine the influence of dietary K₁ on bone mineralization and related plasma parameters in young turkeys fed diets that were adequate or deficient in D₃.

**Dissertation Organization**

The dissertation is divided into a Literature Review followed by two manuscripts and a General Conclusion.

The first manuscript will be submitted to publish in *Poultry Science* with the authorship of Shihou Jin and Jerry L. Sell. The second manuscript will also be submitted for publishing in *Poultry Science* under the authorship of Shihou Jin, Jerry L. Sell and John W. Suttie. Shihou Jin is the senior author for both of these two manuscript.

**References**


Chemistry and Sources of K Vitamins

Chemistry and Nomenclature

K vitamins (from the Danish, Koagulation) are polyisoprenoid-substituted naphthoquinones. The parent structure of the vitamin K series is 2-methyl-1, 4-naphthoquinone (commonly known as menadione). This compound, as far as is known, does not occur in nature but does possess biological activities in vertebrates after it has been alkylated to one of the menaquinones by animal tissues in a process whereby a geranylgeranyl side chain is added at the three position of the parent ring. All naturally occurring K vitamins possess the same naphthoquinone ring structure as menadione but differ in the structures of the side chains at the three-position. They are traditionally classified into two groups according to their origin from either plants or bacteria. The primary form of vitamin K in plants is phylloquinone (vitamin K1). Bacteria, on other hand, synthesize a family of compounds called menaquinones (vitamin K2) with side chains based on different number of repeating isoprenoid units. These compounds are generally referred to as MK-n. where n is the number of isoprenoid units of which the side chain is composed. Natural menaquinones ranging from MK-4 to MK-13 are produced by bacteria. The double bonds in the side chain of menaquinones may be in either trans or cis configuration. Some bacteria also synthesize menaquinones with partially saturated side chains. The additional hydrogen atoms in these menaquinones are indicated by the prefix such as dihydro-, tetrahydro- and so forth.
Sources of K Vitamins

Until recently, there has been little accurate and comprehensive data on the levels of K vitamins in feed and foods. In part, as Shearer et al (1996) indicated, this has been due to the lack of any perceived threat to health or well-being from a dietary deficiency of vitamin K as manifested by its classical function in blood coagulation, and partly due to the lack of suitable analytical techniques for measuring vitamin K in feed or food. As a consequence, the feed composition tables in the most recent NRC (1994) publication for poultry and NRC (1998) for swine do not include data for vitamin K.

Evidence obtained in recent years indicates that a subclinical deficiency of vitamin K, while having no impact on homeostasis, may have an effect on bone in aged humans. These observations have altered the perception of the functions and health effects of vitamin K, and provide a new impetus to obtain reliable data for the vitamin K contents in human foods. However, there is still little new information about vitamin K in animal feed. Therefore, most information cited in the following review is from data in foods that were analyzed by high performance liquid chromatography in recent years.

Phylloquinone (Vitamin K1)

Vitamin K1 is assumed to be widely distributed in nature. Booth et al. (1993, 1995) reported that the dark green, leafy vegetables such as collards, and spinach as well as other green vegetables such as broccoli contain the greatest amounts of phylloquinone (110–960 μg/100g). Ferland and Sadowski (1992) reported that vitamin K1 concentrations in green leafy vegetables are greatly affected by level of maturity and geographic locations where they are grown. In general, vitamin K1 content increases during maturation, and on a wet
weight basis vitamin K1 is more concentrated in mature green vegetables than in young ones. Vitamin K1 concentrations in green vegetables are influenced by both the soil and climatological conditions (Ferland and Sadowski, 1992). Ferland and Sadowski (1992) also reported that the distribution of vitamin K1 is not uniform even in the same plant; the outer leaves of cabbage contain 3 to 6 times more vitamin K1 than that of the inner leaves.

Other good sources of vitamin K1 include some species of algae, such as purple laver and hijiki, which contain about 1385 and 327 μg vitamin K1/100g, respectively. Some oil seeds are also good vitamin K1 sources. Booth et al. (1993) reported that dry soybean seeds contain substantial amount of vitamin K1, however, the contents of vitamin K1 in other oil seeds are very low. Raw peanut and sesame seeds contain only 0.2 and 8 μg of vitamin K1/100g, respectively. Vegetable oils that are derived from vegetables or seeds contain large amount of vitamin K1 are also good sources of vitamin K1. The vitamin K1 concentrations of some commonly used vegetable oils are listed in Table 1.

Booth et al. (1996) reported that hydrogenation of vitamin K1-rich vegetable oils, such as soybean, canola, and cottonseed oil, converts vitamin K1 to dihydro-vitamin K1, with an overall loss of the vitamin that is proportional to the degree of hydrogenation. Therefore, any foods or diets that are otherwise poor sources of vitamin K1 might contain appreciable amounts of dihydro-vitamin K1 if they contain certain kinds of hydrogenated oils or are processed with these kinds of oils.

Cereal grains and their milled products appear to be low in vitamin K1. Shearer et al (1996) reported that vitamin K1 contents in maize and wheat are 0.3 and 8 μg/100g, respectively; and 7 and 10 μg/100g for barley and oats. Booth et al. (1993) reported that
TABLE 1. Phylloquinone concentrations in some commonly used vegetable oils

<table>
<thead>
<tr>
<th>Oil</th>
<th>Booth et al. (1993)</th>
<th>Shearer et al. (1996)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/100 g</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Palm oil</td>
<td>--</td>
<td>8</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>0.7</td>
<td>--</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>10</td>
<td>--</td>
</tr>
<tr>
<td>Olive oil</td>
<td>49</td>
<td>80</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>193</td>
<td>173</td>
</tr>
</tbody>
</table>

vitamin K1 content in corn flakes is 0.04 µg/100g whereas the analogous values for wheat flour and barley flour are a little bit higher with values of 7 and 1 µg/100g, respectively.

Menaquinones

Compared with phylloquinone, information about foods or feeds as a source of bacterially synthesized menaquinones is scarce. The livers of various animal species were long though to be a good source of a wide variety of long-chain menaquinones with side chains ranging from MK-6 to MK-13 (Duello and Matschiner, 1971; Mitschiner and Amelotti, 1968). However, recent quantitative analysis by high performance liquid chromatography revealed that only livers of ruminant species contain high enough concentrations of some menaquinones likely to be of nutritional significance (Hirauchi et
al., 1989). Cheese (Shearer et al., 1996) and yogurt (Vermeer et al., 1995) also have been reported to contain great concentrations of menaquinones.

The intestinal microbial flora produce menaquinones that might be absorbed by host animals. Ramotar et al. (1984) concluded that *Escherichia coli* and *Bacteroids* species and some gram-positive, anaerobic, non-spore-forming bacilli commonly colonizing in the intestines all produce menaquinones. Quantitative culture of human intestinal content showed that the most prevalent organisms from the terminal ileum down to distal colon are *Escherichia coli* and *Bacteroids* species (Conly and Stein, 1992). Therefore, *Escherichia coli* and *Bacteroids* species are among the most dominant microbial flora in the intestines that produce menaquinones. The menaquinone concentrations in the different segments of human intestines are listed in Table 2.

**Synthetic menadione derivatives**

There are three major menadione derivatives that are commonly used in diet fortification. These are menadione sodium bisulfite, menadione dimethylpyrimidinol bisulfate, and a recently available compound, menadione nicotinamide bisulfite. All these compounds have to be alkylated first in animal tissues to be functional as K vitamins *in vivo*. Menadione nicotinamide bisulfite in addition to being a source of vitamin K, is also a bioactive source of niacin.
TABLE 2. Menaquinone concentrations in the contents of different segments of human intestines (Conly and Stein, 1992)

<table>
<thead>
<tr>
<th>Site</th>
<th>MK4 to Mk-9</th>
<th>Mk-9, MK-10</th>
<th>Total MK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/g dry weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal Colon</td>
<td>5.55</td>
<td>14.54</td>
<td>19.85</td>
</tr>
<tr>
<td>Colon</td>
<td>1.64</td>
<td>4.50</td>
<td>6.10</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.69</td>
<td>1.16</td>
<td>1.85</td>
</tr>
<tr>
<td>Terminal ileum</td>
<td>0.92</td>
<td>7.93</td>
<td>8.85</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Absorption of K Vitamins

The absorption site of K vitamins is believed to be in the proximal part of the intestines (Shearer et al. 1974; Hollander and Rim, 1976). However, absorption of menaquinones has been reported to occur in the distal intestines including the jejunum (Ichinashi et al. 1992; Hollander et al., 1977), ileum (Hollander and Rim, 1974; Hollander et al., 1977) and the colon (Ichinashi et al. 1992; Hollander et al., 1977). Compared with absorption in the colon, the absorption of MK-4 in the jejunum was much more efficient (Ichinashi et al. 1992). Groenen-van Dooren et al. (1995), after comparison of bioavailability of phylloquinone and menaquinones administered orally or colorectally, respectively, concluded that the colonic absorption of K vitamins is extremely poor.
The absorption mechanism of K vitamins is similar to other lipid substances. It requires the presence of bile salts and products of pancreatic lipolysis to form micelles. Shearer et al. (1974) reported that biliary obstruction or the inclusion of nonabsorbable anion exchange resin cholestyramine that binds to bile salts impaired the absorption of phylloquinone. This impairment was also observed under conditions of pancreatic insufficiency or inadequate ingestion of lipid that leads to a reduced generation of solutes of mixed micelles (Shearer et al., 1974; Vermeer et al., 1995). Hollander et al. (1997) reported that the absorption rate of menaquinone (MK-9) in situ in the ileum increased as bile salt concentrations increased in the perfusate. However, these authors observed that unsaturated long chain fatty acids, such as oleic or linoleic acid adversely affected the absorption of menaquinones. When these types of fatty acids were added in the perfusate the absorption of menaquinone was significantly decreased.

The absorption rate of phylloquinone administered in free-form is very rapid. Vermeer et al. (1995) reported that 1 hour after oral administration of phylloquinone a very high level of vitamin K1 was detected in plasma, and about 4-5 hours after administration plasma concentrations reached a plateau. The absorbability of free-form phylloquinone is believed to be very high, about 80%, under normal conditions (Shearer et al., 1974). However, phylloquinone occurs naturally as a component of the photosynthetic electron transport system in plants: it exists exclusively in the thylakiod membranes of the chloroplast in combination with other substances (Lichtenthaler, 1993). The binding is very tight and leads to very poor and slow absorption of phylloquinone from vegetable sources. Vermeer et al. (1995) reported that even with simultaneous ingestion of fat, no significant concentration of phylloquinone could be detected in plasma until 5 hours after ingestion of spinach. The
absorbability of phylloquinone from vegetables is very low averaging less than 10% 
(Vermeer et al., 1995)

The newly recognized dihydro-vitamin K1 that is produced primarily during the 
hydrogenation of vitamin K1-rich vegetable oils, has also been shown to be absorbable. 
Booth et al. (1996) reported that, after dietary intake of a hydrogenated vitamin K1-rich 
vegetable oil, dihydro-vitamin K1 is detectable in human plasma.

Most of the naturally occurring menaquinones have been confirmed to be absorbable 
by in vitro (Hollander and Rim, 1976), in situ (Ichihashi et al. 1992) and in vivo (Hollander et 
al., 1977) procedures. The absorption rates of menaquinones are affected by their side chains. 
Compared to phylloquinone, menaquinones with short side chains such as MK-4 might be 
absorbed more quickly, as indicated by the more rapid increase in plasma concentration 
following oral administration, compared to that of phylloquinone (Sakamoto et al. 1994). 
This finding is consistent with the observation that plasma vitamin K1 level decreased more 
slowly compared to that of MK-4 following the peak after oral administration (Sakamoto et 
al. 1996). However, menaquinones with long side chains such as MK-9 might be absorbed 
more slowly compared to phylloquinone. Will and Suttie (1992) reported that MK-9 
concentration in rat plasma was lower during the first few hours after ingestion of a diet 
containing MK-9 compared to phylloquinone concentrations in rats that ingested 
phylloquinone. Plasma phylloquinone increased dramatically following ingestion and then 
decreased sharply a few hours later. Akiyama et al. (1995) compared the intestinal absorption 
of menaquinones containing varying number of isoprene units (from 1 to 14) and observed 
that 6 hours after oral administration, the concentrations of corresponding MKs in plasma 
were increased in rats that received MK-0, and MK-4 to 11, but there were no or very little
change of the concentrations of corresponding MKs in plasma in rats that received MK-1, 2, 3 and MKs with more than 12 isoprene units. They concluded that the number of isoprene units of MKs is an important factor in its absorption.

Menaquinones can be synthesized by the microbial flora in the lower intestinal tract, and there is little doubt that some of these menaquinones are absorbed. However, the question of whether intestinally synthesized menaquinones can be absorbed in nutritionally significant amounts by the host is long-debated for some species, including the human being and rat. As mentioned earlier, K vitamins are absorbed mainly in the proximate small intestines and very little is absorbed in the colon, as reported by Groenen-van Dooren et al. (1995) and Ichihashi et al. (1992), whereas the segment of the human intestines that contains the high concentrations of menaquinones is the colon. These observations lead to doubts of the importance of intestinally microbially synthesized menaquinones for the host. However, there have been observations that antibiotic therapy sometime caused hypoprothrobinaemia in humans (Frech et al., 1967; Pineo et al. 1973). Furthermore, germ-free rats have a higher requirement for vitamin K than normal rats (Gustafsson et al., 1962). These observations indicate some role for menaquinones produced by intestinal microbial flora for the host. Recent analysis showed that the content of terminal ileum also contains a substantial amount of menaquinones (total menaquinones 8.85 μg/ g DM)(Conly and Stein, 1992), and the ileum has been shown to absorb menaquinones very well (Hollander et al., 1977). On the basis of this information, Shearer concluded (1996) “the most promising site of absorption would seem to be the terminal ileum, which contains reasonable concentration of menaquinones and of bile salts.” Conly (1992), after reviewing available literature regarding production and liberation of menaquinones from bacteria, stated that the preponderance of the evidence
suggests that bacterially synthesized menaquinones, particularly in the ileum, can and do contribute toward vitamin K requirements in humans in maintaining coagulation homeostasis, especially during periods of episodic dietary lack of the vitamin.

**Transport, Storage of K Vitamins**

*Transport of K Vitamins in Plasma*

After enteral absorption, phylloquinone is incorporated into chylomicrons and transported into the circulatory system by the lymphatic system in mammals or by the hepatic portal circulation in birds. The transportation of menaquinones from the intestines to the circulatory system most likely is similar to that of phylloquinone, although there might be some minor differences. Ichihashi *et al.* (1992) reported that even in mammals such as rats menaquinones could be absorbed directly into hepatic portal circulation. This was especially true for the menaquinones absorbed from the colon.

Once in the circulatory system, K vitamins mainly, phylloquinone, MK-7, and possible MK-8 and MK-9, are predominately associated with the triglyceride-rich lipoproteins (*Kohlmeier et al.*, 1996). These lipoproteins are from two families: very low density lipoproteins and their derivatives, which are formed in the liver, and chylomicrons and their derivatives which are formed in the intestines. There are much smaller fractions of K vitamins carried by low density or high density lipoproteins. The proportion of phylloquinone and MK-4 carried by the triglyceride-rich lipoproteins was more than 50% of the total amount in the plasma (*Kohlmeier et al.*, 1996). The observation of a strong positive correlation between phylloquinone and plasma glyceride (*Sadowki et al.*, 1989; *Saupe et al.*, 1993) is consistent with the conclusion that phylloquinone is primarily transported by
triglyceride-rich lipoproteins. Further delineation of this association comes from the demonstration that fasting plasma phylloquinone concentration is strongly influenced by the common genetic polymorphism of apolipoprotein E (apoE), being greatest in individuals with the apoE2 variant, intermediate in apoE3, and lowest in apoE4 (Shearer, 1995). This fits well with the conclusion that the rate at which chylomicron remnants are cleared from circulation in individuals, differs according to the variant of apoE: fastest for individual with apoE4, intermediate for apoE3 and slowest for people with variant of apoE2 (Kohlmeier et al., 1996; Shearer, 1995).

Chylomicrons and their remnants, and the associated vitamin K, are thought to be primarily taken up by the liver. However, Hussain et al. (1989) reported that bone marrow also plays an important role in the removal of chylomicrons and their remnants. Kohlmeier et al. (1996) concluded on the basis of animal experiments that nearly all intestinal lipoproteins are removed from the circulation by the liver, spleen and bone marrow within less than 1 hour. Accumulation of the intestinal lipoproteins by these three tissues is because the receptor-bearing cells in these tissues have direct access to circulation and acquire lipoproteins preferentially. Among the bone marrow cells that have direct access to the intestinal lipoproteins are stromal and mesenchymal cells (Kohlmeier et al., 1996). These cells are the obligatory precursors of all bone forming cells (Baron, 1993). Kohlmeier et al. (1996) postulated that the migration of these stromal and mesenchymal stem cells to the sites of bone resorption responding to appropriate stimuli and carrying the amounts of vitamin K they extract while residing in bone marrow, is the final step of vitamin K transportation from the circulatory system to bone tissue.
**Clearance of K Vitamins from Plasma**

Being firmly associated with the lipoproteins of intestinal origin and other triglyceride-rich lipoproteins. K vitamins share the same metabolic fate as that of these lipoproteins. Many acquired and constitutional factors are known to affect clearance of chylomicrons and their remnants and other triglyceride-rich lipoproteins from the circulatory system. One such example is the different variants of apolipoprotein E.

Under normal conditions, radioactive labeled phylloquinone was cleared from the circulation very rapidly following either oral or intravenous administration (Shearer et al., 1972). Two and eight hours after administration only about 10 and 1% of the initial radioactivity remained in the plasma, respectively. Shearer et al. (1974) made an estimate of the clearance kinetics of phylloquinone, and concluded that the half-time for the first and second exponential phases of phylloquinone clearance in plasma was 20-24 and 120-150 minutes, respectively. This result was consistent with the report of Bjornson et al. (1980) who showed that the half-time values of first and second exponential phases of phylloquinone in plasma were 26 and 199 minutes, respectively.

Compared with phylloquinone, Sakamoto et al. (1996) reported that after intravenous administration, MK-4 was cleared quicker. One hour after administration, plasma MK-4 level was about 3% of the original concentration, whereas the value for K1 was 74%. They postulated that vitamin K1 may be more hydrophobic and might have a greater affinity than MK-4 for lipoproteins in plasma.
Phyloquinone in plasma might be taken up by different tissues. Of these tissues, the liver plays a major role. Bell and Matschiner (1969) reported that, after parenteral administration of vitamin K1, as much as 50% of the administered dose appears in the liver within 1 hour. After oral administration, the liver contains about 20% of the administered dose within 2 hours. This is consistent with the observation by Wiss and Floor (1966) and Kindberg and Suttie (1989) who reported that after an oral ingestion the concentration of vitamin K1 in the liver increased dramatically and reached a peak within a few hours. The vitamin K1 taken up by the liver after the administration is released from the liver to other tissues. Wiss and Gloor (1996) and Kindberg and Suttie (1989; 1992) reported that the vitamin K1 level in the liver decreased to a very low level within 20–24 hours following the peak after administration. Wiss and Gloor (1996) observed that the level of vitamin K1 in extrahepatic tissues concomitantly increased with the decline of vitamin K1 in the liver. These observations indicate that the liver serves as a temporary storage for part of absorbed vitamin K1. Of the total vitamin K1 store in the body, 10% is stored in the liver in humans (Shearer et al., 1988). and this store is very labile. Under conditions of severe dietary depletion. the liver store is reduced to about 25% of the original concentration (Usui et al. 1990). Within hepatocytes. vitamin K1 is mainly located in microsomes. Bell and Matschiner (1969) reported that the relative distribution of radioactivity after administration of $^3$H-phyloquinone in hepatocytes was: nuclei, 13%; mitochondria, 9%; microsome, 63%; and cytosol, 14%.
Although the liver is a major site for vitamin K1 storage, phylloquinone is widely distributed in the body and can be detected in most tissues, such as muscle, spleen, kidney (Hirauchi et al., 1989) and bone (Hodges et al., 1993). Concentrations of phylloquinone in different tissues of some farm animals are listed in Table 3.

**TABLE 3. Phylloquinone concentrations of different tissues in some farm animals (Hiraushi et al., 1989)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Liver (ng/g)</th>
<th>Spleen (ng/g)</th>
<th>Kidney (ng/g)</th>
<th>Heart (ng/g)</th>
<th>Muscle (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>25.2</td>
<td>7.7</td>
<td>3.3</td>
<td>5.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Pig</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Horse</td>
<td>76.7</td>
<td>23.7</td>
<td>5.8</td>
<td>14.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Bovine</td>
<td>18.4</td>
<td>2.8</td>
<td>1.9</td>
<td>2.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

There is evidence to suggest that mobilization of menaquinones in animal tissues is different from that of phylloquinone. Sakamoto et al. (1996) reported that, compared with phylloquinone, intravenous administered MK-4 was taken up by the liver more rapidly, which leads to a more rapid accumulation of MK-4 in liver. MK-4 stores in liver also might be more easily mobilized compared with these of phylloquinone. Sakamoto et al. (1996) observed that concentrations of MK-4 in liver decreased to 8.5% of peak values within 5 hours after an intravenous administration, whereas values for K1 were 53% 6 hours after its
Will and Suttie (1992) compared the metabolism of phylloquinone and menaquinone with a long side chain (MK-9). Their results showed that the initial rate of hepatic turnover of phylloquinone was two to three times as rapid as that of MK-9.

The storage and distribution of menaquinones are also different from that of phylloquinone. Shearer et al. (1996) stated that the liver store of menaquinones, some with long side chains, constitutes 90% of the total body pool in the human. This amount is much larger than that of phylloquinone, which is only about 10% (Shearer et al., 1988). However, this might not be true for all MKs, especially for some of MKs with short side chains such as MK-4. Miura et al. (1990) and Thijssen and Dritlij-Reijinder (1994) reported that some extrahepatic tissues, such as brain, pancreas, and bone etc. contain very high level of MK-4. Hirauch et al. (1989) reported that MK-4 concentrations in spleen, heart and muscle of chicken were 17, 26, 39 times, respectively, of the concentrations of vitamin K1 in these tissues. Menaquinone concentrations of different tissues in some farm animals are listed in Table 4.

**Metabolism of K Vitamins**

**Conversion of Phylloquinone to Menaquinone-4**

The major form of K vitamins from plant sources is phylloquinone. Studies of vitamin K metabolism demonstrated that orally ingested phylloquinone could be converted to menaquinone-4 (Billeter and Martius, 1960: Billeter et al., 1964). More recently, Will et al. (1992) reported that chickens fed diets containing phylloquinone as the only source of vitamin K have a very high level of MK-4 in plasma and liver. Suzuki et al. (1997) reported that as phylloquinone level increased, the concentrations of MK-4 in yolks increased in parallel. This conversion of phylloquinone to MK-4 also occurs in rats. Will et al. (1992)
TABLE 4. Concentrations of menaquinones in different tissues of farm animals (Hrauchi et al. 1989)

<table>
<thead>
<tr>
<th>Species</th>
<th>liver</th>
<th>Spleen</th>
<th>muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MK-4</td>
<td>MK-7</td>
<td>MK-9</td>
</tr>
<tr>
<td>Bovine</td>
<td>8.2</td>
<td>181.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Horse</td>
<td>2.1</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Pig</td>
<td>5.9</td>
<td>6.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>39.6</td>
<td>ND¹</td>
<td>0.4</td>
</tr>
</tbody>
</table>

¹ ND stands for not detectable

reported that rats fed diets containing phylloquinone as the sole source of vitamin K contained detectable MK-4 in the liver and plasma but in much lower concentrations than those observed in chickens. Thijssen and Drittij-Reijnders (1994) reported that supplementation of K1 in diets resulted in increased MK-4 levels in the pancreas, salivary glands and sternum of rats.

The mechanism of conversion of phylloquinone to MK-4 has been proposed to be dealkylation of orally ingested phylloquinone by bacteria in the gut, and then alkylation in animal tissues (Will et al. 1992). However, the observation that intravenous administration of phylloquinone also increased the levels of MK-4 in the liver and plasma of chickens (Will et al. 1992), and the pancreas and sternum of rats (Thijssen and Drittij-Reijners, 1994), suggests the possibility of an alternate pathway that has not yet been determined, for conversion of phylloquinone to MK-4 in animal tissues.
Conversion of Menadione to Menaquinone-4

The commonly supplemented vitamin K sources in diets are menadione derivatives such as menadione sodium bisulfite. These derivatives have to be alkylated in animal tissues before they are functional. Dialameh et al. (1970) reported that menadione could be enzymatically alkylated to menaquinones by chicken liver in vitro and by animal tissues in vivo. When radioactively labeled menadione was administered to chickens and rats, labeled MK-4 was isolated from the livers of the two species. However, the conversion from menadione to MK-4 is not an efficient process. Dialamen et al. (1971) reported that the extent of conversion, at best, by liver is 0.01-1% of the administered dose of menadione, with the highest conversion occurring in the chick.

Vitamin K-vitamin K-2,3 Epoxide Cycle

Fiesh and his colleagues (1941) first synthesized the 2,3-epoxide of phylloquinone and showed that it was rapidly converted to vitamin K in animal tissues. Approximately 30 years later, Matschner et al. (1970) reported that phylloquinone-2,3-epoxide is an intermediary metabolite in phylloquinone metabolism in rats. These observations provide the brief outline of the vitamin K-vitamin K-2,3-epoxide cycle. Many details of the cycle are provided through the study of enzymes involved in the cycle, notably vitamin K epoxidase, vitamin K-2,3-epoxide reductase and vitamin K reductase.

Willingham and Matschiner (1974) first reported that isolated liver microsomes had phylloquinone "epoxidase" activity. A subsequent study by Sadowshi et al. (1977) demonstrated that it is the hydroquinone form of phylloquinone that is converted to the 2,3-epoxide form. and O₂ is the source of the epoxide oxygen. They concluded that the overall
reaction of epoxidation of phyloquinone seems to be catalyzed by a mixed-function oxidase, with the required reducing equivalent coming from the quinone form of vitamin K.

Zimmerman and Matschiner (1974) reported that liver microsomes contain epoxide reductase activity that can convert the vitamin K-2,3-epoxide back to the vitamin, and this enzyme is inhibited by warfarin. Whitlon et al. (1978) stated that this enzyme has very little activity in the presence of NAD(P)H but is very active when dithiothreitol is used as reducing agent.

The active form of K vitamins is the hydroquinone form. Therefore, the regenerated quinone through the vitamin K-vitamin K-2,3 epoxide cycle and newly introduced dietary K vitamins need to be converted to KH2 form. There are several enzymes in animal tissues that can reduce K vitamins to their hydroquinone forms (Olson, 1984). It was concluded by Wallin et al. (1978) that DT-diophorase (E.C.1.6.99.2) is one of the physiologically relevant vitamin K reductases. The enzyme is a flavoprotein that can use either NADH or NAD(P)H as cofactor and is inhibited by dicumarol (Ernster et al., 1960; 1962). More recently, Wallin and Huston (1982) reported that only about 45% of the NAD(P)H activity of solublized microsomes could be neutralized by pure antibody against DT-diaphorase, and concluded that about half of the NAD(P)H dependent reductive activity is from a Warfarin-insensitive reductase that is different from DT-diophorase. Fasco and Principe (1980) showed that the hydroquinone of vitamin K resulted from the reduction of vitamin K by dithiothreitol in intact rat liver microsomes, and concluded that dithiothreitol might be another mechanism to reduce vitamin K to its KH2 form in the liver. The proposed vitamin K cycle is showed in Figure 1.
Difference Between the Avian and Mammal

It is believed that the vitamin K requirement of chicks (0.5 mg/kg, NRC, 1994) is 5 to 10 times that of rats (0.05 mg/kg, National Academy of Science, 1978) (Suttie, 1991). The metabolic basis for the high vitamin K requirement for chicks was studied by Will et al. (1992). These authors observed that one major difference between chicks and rats is that chick livers contain much higher MK-4 levels compared with those of rats when they were both fed the same diet. In rat liver, MK-4 was detected at a concentration of about ~10% of that of phyloquinone, whereas in chick liver the concentration of MK-4 was more than twice that of phyloquinone. Another major difference between the two species is that there is a very high level of phyloquino-2,3-epoxide in liver and plasma of chicks but not in rats, and the activity of vitamin K epoxide reductase is very low in chick liver, about 10% of that of rats. Based on these observations, Will et al. (1992) concluded that the inability of chicks to effectively recycle the epoxide of vitamin K seems to be the major factor related to its relatively high requirement of vitamin.

Catabolism of K Vitamins

The liver plays an exclusive role in the metabolic transformation of K vitamins that leads to their excretion from the body (Shearer et al. 1996). Wiss and Gloor (1966) reported that all K vitamins, in spite of differences in the quinone part and the side chains, are metabolized in the same way. The side chains are shortened to seven carbon atoms, yielding a carboxylic group that forms γ-lactone. The major routes of excretion for K vitamin metabolites are bile and urine, of which the biliary route is quantitatively of greater significance than the urinary route (Shearer et al., 1974). Tracer experiments with labeled
phylloquinone have shown that a sizable fraction of a single dose of phylloquinone is rapidly metabolized and excreted. The fraction of the vitamin excreted was not dependent on the dose of administration (Shearer et al., 1996). Shearer et al. (1974) reported that regardless of whether the ingested dose was 1 mg or 45 μg, ~20% was excreted in the urine within 3 days, whereas ~40-50% was excreted via the bile in the feces. This extensive catabolism of phylloquinone by the liver is consistent with the observation of the rapid turnover and depletion of hepatic reserves in rats within a few hours after the removal of diets (Will and Suttie, 1992; Kinberg and Suttie, 1989). Shearer et al. (1996) stated that the body stores of phylloquinone are being constantly replenished.

**Functions of Vitamin K**

*The Biochemical Basis for Functions of K Vitamins*

The major, if not only, function of K vitamins is to serve as an essential cofactor for vitamin K-dependent carboxylase that mediates the post-translational carboxylation of some specific glutamyl residues in nascent vitamin K-dependent proteins to γ-carboxyglutamyl residues (Gla) in the corresponding mature proteins (Will et al., 1992). The only known function of the Gla residues in all these proteins is binding to calcium ions (Knapen et al., 1993). The vitamin K-dependent carboxylation is shown in Figure 2.

The exact mechanism of the vitamin K-dependent carboxylation remains unsettled. Olson (1984) stated that the general hypothesis for the action is: first, the carboxylase enzyme combines with the hydroquinone form of vitamin K (KH₂) and O₂ to yield a ternary enzyme-substrate complex and a possible vitamin KH₂-(O₂) compound. The peptide that needs to be carboxylated, then binds to the complex. The metabolism of KH₂-(O₂) to
Protein precursor

\[ \text{CH}_2 \]
\[ \text{CH}_2 \]
\[ \text{COOH} \]

Vitamin K

\[ \text{CO}_2 \]
\[ \text{O}_2 \]

Completed proteins

\[ \text{CH}_2 \]
\[ \text{CH}-\text{COOH} \]
\[ \text{COOH} \]

Glutamyl residue

\[ \gamma\text{-carboxyglutamyl residue} \]

**FIGURE 2.** The vitamin K-dependent carboxylation reaction

Vitamin K-2. 3-epoxide through an unknown intermediate(s) couples with the removal of the \(\gamma\)-methyl proton from the specific glutamyl residues in the peptide and then carboxylation.

The Gla residues in the vitamin K-dependent proteins are essential for expression of their full biological activity (Will *et al.*, 1992). Therefore, the normal physiological functions of these Gla proteins are closely related to the vitamin K status of the animal, and the knowledge of these Gla protein physiological functions are really key to the understanding of the functions of vitamin K.

**Some of the Vitamin K-dependent Proteins and Their Functions**

From its discovery 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. However, research during the past two decades has identified a whole list of Gla proteins in various of tissues that require vitamin K for their synthesis. Cells and tissues that actively synthesize Gla proteins include macrophages (Chapman, 1985), vascular endothelium (Stern *et al.*, 1986), kidney (Booth, 1997), uterine smooth muscle (Luo *et al.*,}
1995). and bone (Hauschka and Reid, 1978). Cells of the chondrocytic lineage, such as the ones in cartilage and trachea or bronchi, also actively synthesize Gla proteins (Luo et al., 1995). In most of these tissues, the Gla proteins have not been fully characterized. For example, very little is known about the Gla protein in kidney, nephrocalcin (Booth, 1997). Among all the Gla proteins identified only a few of them have been studied in some degree. These include the Gla proteins related to blood coagulation eg., factors II, VII, IX, X and proteins S and C, and those related to mineralization and remodelling of bone and/or calcification of some soft tissues, eg., osteocalcin.

The four classical factors (II, VII, IX and X) in blood coagulation all exist in the forms of proenzymes in the circulation and they need to be activated to be functional. Prothrombin, when activated, catalyzes a proteolytic reaction to cleave fibrinopeptides from fibrinogen, which converts fibrinogen to fibrin and results in the formation of a soft blood clot. Prothrombin is activated by activated factor X which in turn is activated through the intrinsic or extrinsic pathway of blood coagulation. One of the factors in the extrinsic pathway to activate factor X is factor VII, and the one in the intrinsic pathway to activate factor X is factor IX. Both factors VII and IX themselves need to be activated to be functional. The part of the blood clotting cascade related to Gla proteins is shown in Figure 3.

It is clearly shown in the above figure that the conversion of prothrombin to thrombin is the key for the formation of blood clotting. Prothrombin sits at a cross point of the intrinsic and extrinsic pathways. The concentration of prothrombin in plasma is many times greater than the other three classic blood coagulation factors (X, IX, VII) (Olson, 1984). These facts make prothrombin an ideal protein to studying the function of vitamin K. There are several methods to determine the prothrombin concentration in plasma or the accumulation of its
precursor protein in microsomes in livers. Shah et al. (1984) reported an amidolytic assay to determine the prothrombin concentration in plasma. Suttie (1973) reported that an accumulation of a precursor protein of prothrombin exists in the microsomes of warfarin-treated or vitamin K-deficient rat livers. This accumulated microsomal precursor protein could be carboxylated by injection of vitamin K into anticoagulated rats, resulting its conversion to normal prothrombin and secretion into plasma.

Two other Gla proteins related to blood clotting and their functions are well-documented are proteins S and C. Branson et al. (1983) reported that protein C inhibits coagulation and promotes fibrinolysis. Activated protein C can rapidly and selectively inactivate both normal and thrombin-modified factors V and VIII in vitro (Marlar et al., 1982). Bahlback et al. (1986) reported that protein S plays an inhibitory role in blood coagulation by acting as a cofactor for activated protein C.

One of the Gla proteins that is not related to blood clotting but has been intensively studied is osteocalcin. Osteocalcin is an osteoblast-specific vitamin K-dependent protein that
is the most abundant of all the noncollagenous proteins found in bone matrix (Hauschka et al., 1989). The expression of osteocalcin genes is regulated by vitamin D. Leeuwen et al. (1996) reported that 1.25-(OH)2 D3 stimulated osteocalcin production in vitro in some osteoblastic cell lines such as ROS 17/2.8 or MG-63. 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 its expression. The biosynthesis and secretion of osteocalcin by the osteoblast is very similar to that of prothrombin by the hepatocytes. The transcriptive peptides need to be cleaved by the signal peptidase first, and then subjected to carboxylation at specific positions of glutamyl residues to form Gla (the total number of Gla residues in osteocalcin depends on species of animal.). Under the condition of vitamin K deficiency or in presence of warfarin, partial or no carboxylated osteocalcin may be formed (Hauschka et al., 1989). The newly secreted osteocalcin might be released into blood circulation or deposited in bone matrix. Hauschka et al. (1989) stated that an estimated 90% or more of newly synthesized osteocalcin is deposited in bone matrix of 1-month-old rats, but as animals mature, a greater proportion of this protein is released directly into the blood circulation.

The precise function of osteocalcin remains unsettled, but is assumed to be related to bone mineralization and remodeling (Hauschka et al., 1989). In vitro, osteocalcin binds strongly to hydroxyapatite crystals, and is a potent inhibitor of hydroxyapatite formation (Price et al., 1976). Stein et al. (1989) reported that cultured chick and rat osteoblasts produced low levels of alkaline phosphatase and osteocalcin until they reached confluence and a dense collagenous extracellular matrix accumulated. At this time, alkaline phosphatase activity increased, and osteocalcin synthesis was enhanced by 50- to 100-fold, coincident
with the onset of mineralization of the matrix. Lian et al. (1984) reported that the carboxy-terminal part of osteocalcin possesses chemo-attractant activity for osteoclast progenitor cells that are involved in bone resorption. Ducy et al. (1996) reported that mice with the osteocalcin gene deleted were normal at birth. By 6-month of age, however, they developed long bones of increased thickness and density compared with wild-type mice, and had an increase in osteoclast surface and number. It was concluded that osteocalcin functions as a negative regulator of bone formation (Ducy et al. 1996).

Matrix Gla protein (MGP), which is not related to blood coagulation, has been studied in some extent. Luo et al. (1995) reported that the MGP gene is predominantly expressed in cells of the chondroncytic lineage that either undergo endochondral ossification, such as the chondrocytes in the growth plate cartilage, or remain cartilaginouse, such as the chondrocytes in trachea or bronchi. The mRNA coding for MGP exists at very high levels in some soft tissues such as kidney, liver, and blood vessel walls (Fraser and Price, 1988). However, only trace amounts of immunoreactive MGP can be identified in these soft tissues, whereas it is abundant in bone and cartilage (Fraser and Price, 1988). The function of MGP remains a matter of speculation. Luo et al. (1997) reported that mice with a deleted MGP gene, were normal at birth compared with their wild-type littermates. However, the MGP-deficient mice developed changes within two weeks after birth. These changes included increased heart rate, decreased stature, osteopenia, bone fracture, and ultimately death from a rupture of the thoracic or abdominal aorta because of extensive calcification. The abnormal growth plate calcification was determined to be the cause of osteopenia and stunting. It was concluded that MGP inhibited the calcification of arteries and cartilage through its mineral-binding ability (Luo et al., 1997)
There is evidence that the various Gla proteins might differ in sensitivity to vitamin K deficiency or Warfarin treatment. Price (1989) reported that when young rats were given Warfarin and supplemental vitamin K concurrently, the concentrations of the Gla proteins related to blood coagulation remain unchanged, whereas osteocalcin concentration decreased by 98%, and MGP concentration decreased by 50%. The only observed abnormalities in these rats were excessive calcification of growth plate and nasal septum.

**Indications of Vitamin K Status**

**Plasma Vitamin K Level and Liver Storage**

**Level of Vitamin K in Plasma**

Booth *et al.* (1995) concluded that plasma phylloquinone is a good biochemical measure of vitamin K status. Will and Suttie (1992) reported that plasma levels of phylloquinone or menaquinone-9 were closely related the concentrations of phylloquinone or MK-9 in diets that range from deficient to adequate. Suttie *et al.* (1988) instructed some college-aged male subjects to choose diets of low vitamin K content to restrict the intake of vitamin K, and then phylloquinone supplementation was given to these subjects at the dosage of either 50 or 500 μg/d. The phylloquinone intake of these subjects was decreased from 82 μg/d during the prestudy period to 32-40 μg/d during the restriction period. Serum phylloquinone concentration decreased from 0.87 to 0.46 ng/ml. and the functionally active prothrombin ratio decreased from 10.024 to 0.911 as the phylloquinone intake decreased. Supplementation of 50 μg phylloquinone per day increased serum phylloquinone from 0.46 to 0.56 ng/ml. and supplementation of 500 μg phylloquinone per day increased serum phylloquinone level further to 10.6 ng/ml. Supplementation of 50 or 500 μg phylloquinone
per day increased the functionally active prothrombin ratio from 0.911 to 0.980 and 0.995, respectively.

When plasma vitamin K level is used as indicator of vitamin K status, there are several factors that need to be taken into account. First, the time period that animals are off feed when blood is collected. As mentioned in an early section ("Clearance of K vitamins from plasma") plasma K vitamins are cleared very rapidly. The length of time that animals are off feed has great effect on the level of plasma vitamin K. Kindberg and Suttie (1989) reported that serum phylloquinone concentrations in rats that were fasted about 24 hours were independent of dietary concentrations of phylloquinone. Secondly, vitamin K in plasma is in close association with plasma triglyceride-rich lipoprotein (Sadowski et al., 1989). Shearer et al. (1992) reported that patients with various lipid disorders, especially those associated with hypertriglyceridemia, have very high plasma phylloquinone levels. Sadowski et al. (1989) reported that elderly adults had higher levels of phylloquinone in plasma than young adults. But, when the concentrations of phylloquinone were expressed as nmol phylloquinone per mmol of triglyceride, the elderly subjects showed decreased levels of phylloquinone compared with the young subjects. It is suggested that the ratio of plasma phylloquinone to plasma triglyceride would be a better index of vitamin K status than plasma phylloquinone alone under some of these conditions (Sadowski et al., 1989; Shearer et al., 1992). The last factor that needs to be considered is the genotype of apolipoprotein E. It has been shown that different variants of apolipoprotein E have great effect on the clearance rate of chylomicron remnants. and therefore, of plasma vitamin K. For details, see the section "Clearance of K vitamins from plasma."
Concentration of vitamin K in the liver

The liver is one of the major tissues that synthesize Gla proteins. The concentration of vitamin K in the liver, therefore, has direct effect on the carboxylation of the specific glutamyl residues of the Gla proteins related to blood coagulation. Haroon and Hauschka (1983) reported that the normal range of phylloquinone in rat liver is between 4 and 44 ng/g fresh weight of liver. Taggart and Matschiner (1969) estimated that the minimum hepatic phylloquinone concentration needed to sustain normal prothrombin level in rats was 10 pmol/g of fresh weight of liver. Knauer (1976) reported that levels of vitamin K in livers correlated with the amounts of vitamin K fed to rats regardless of strain and sex difference. More recently, Will and Suttie (1992) confirmed the above observation. They observed that the concentrations of phylloquinone or menaquinone-9 in livers increased as the levels of phylloquinone or MK-9 in diets increased from 0.2 to 5.6 μmol/kg diets, respectively. Kindberg and Suttie (1989) reported that the liver concentrations of phylloquinone in rats fed a vitamin K deficient diet with the addition of 0, 10, 100, 500, or 1500 μg of phylloquinone /kg, was closely correlated to the amount of phylloquinone intake, and plasma prothrombin levels and one-stage prothrombin time all changed in a similar pattern as that of liver phylloquinone concentrations.

Parameters Related to Blood Clotting

The concentration of prothrombin in plasma and its degree of carboxylation

Prothrombin is one of the Gla proteins that are synthesized by the liver, and is presenting the highest concentrations in plasma among all the Gla proteins related to blood coagulation. These characteristics make it an ideal subject for the study of vitamin K function
and status. Under normal conditions, it is rare to identify undercarboxylated prothrombin in circulation. However, under the condition of vitamin K deficiency or Warfarin treatment, undercarboxylated prothrombin has been identified in many animal species including the chick, rat, mouse, hamster guinea pig, rabbit, and dog (Carlisle et al., 1975). The prothrombin concentration in plasma can be determined by an amidolysis assay (Shah et al., 1984). The principle of the method is, briefly; plasma prothrombin is activated to thrombin with a physiological activation system to determine concentrations of normal prothrombin or with a specific snake venom to determine total prothrombin concentrations including the abnormal and undercarboxylated prothrombin. The formed thrombin catalyzes amidolysis of a specific chromogenic peptide substrate to release p-nitroaniline. The concentrations of p-nitroaniline can be determined spectrophotometrically. Several tests to determine concentrations of decarboxylated-prothrombin are available including an electrophoresis immunofixation method, an immunoassay and the method to determine the absorbability to barium. The most sensitive method is based on monoclonal antibodies that specifically recognize decarboxy-prothrombin (Widdershoven, 1987).

Shah et al. (1987) reported that the concentrations of both plasma total and normal prothrombin in different strains of rats fed vitamin K deficient diets or treated with Warfarin were significantly lower but undercarboxylated prothrombin levels were higher compared with that of vitamin K sufficient rats. Kindberg and Suttie (1989) reported that plasma prothrombin level increased in rats as intake of phylloquinone increased. This is also true for menaquinones such as MK-9 (Will and Suttie, 1992). The difference between phylloquinone and MK-9 was that phylloquinone at low levels was much more potent for maintaining plasma prothrombin concentrations than MK-9 on an equimolar basis.
The concentration of the precursor of prothrombin in the liver

Suttie (1973) reported an accumulation of a precursor protein in the liver microsomes of Warfarintreated or vitamin K-deficient rats. This accumulated precursor protein could be carboxylated, resulting in conversion to prothrombin and secretion into plasma. Olson (1984) concluded that vitamin K-deficient chicks accumulate only about 42% as much precursor protein per gram of liver as rats. Vitamin K deficient rats given vitamin K show a marked "burst" of prothrombin secretion in the first hour, followed by a slower rate of plasma enrichment (Suttie. 1970: Bell and Matschner. 1969). Vitamin K-deficient chicks, however, show a linear response in prothrombin secretion upon receiving a vitamin K source (Olson et al., 1968).

Esmon et al. (1975) reported that the concentration of accumulated precursor protein of prothrombin in liver is determined by a vitamin K-dependent in vitro carboxylase system. The undercarboxylated precursor protein is carboxylated in the system. The carboxylation leads to incorporate of $^{14}$C from NaH$^{14}$CO3 into the precursor protein. Radioactivity is determined and the concentration of the precursor protein calculated.

Will and Suttie (1992) reported that the concentrations of the liver microsomal vitamin K-dependent precursor protein in rats decreased as the intake of phylloquinone increased. This is consistent with an earlier report by Kindberg and Suttie (1989).

Activity of vitamin K-dependent carboxylase in the liver

The microsomal vitamin K-dependent carboxylase in liver is one of the enzymes that require vitamin K as an essential cofactor. Its activity, therefore, is closely related to the status of vitamin K. Suttie and Hageman (1976) reported that the vitamin K-dependent
carboxylase activity in liver can be determined through the assay of $^{14}$C incorporation into a synthetic peptide. The basic principle of the method is: the vitamin K-dependent carboxylase is solubilized by treatment with Triton 100. The soluble fraction is incubated with an energy-generating system, NaH$^{14}$CO$_3$, a synthetic peptide, and vitamin K to initiate the reaction. The synthetic peptide is carboxylated with radioactive $^{14}$C, and is isolated. Radioactivity is determined and used to calculate the activity of the carboxylase activity. Kindberg and Suttie (1989) reported that the peptide carboxylase activity in rat liver decreased from 7966, 5158, 4135 to 2613 dpm/mg protein as intake of phylloquinone increased from 10, 100, 500 to 1500 µg/kg diet. This observation indicated that there were more molecules of vitamin K-dependent carboxylase in vitamin K deficient rat liver than in vitamin K adequate one.

Prothrombin time

Prothrombin time determined by the one-stage method developed by Quick (1935) or the two-stage method developed by Warner et al. (1936) is widely used to evaluate vitamin K status of animals and humans. With the availability of some commercial kits it is handy to determine the status of vitamin K or factors in the extrinsic blood coagulation pathway by these methods. However, it must be noted that the assumption made in the prothrombin time method is that when an excess amount of thromboplastin in the form of a potent tissue extract is added to recalcified plasma, the formation of thrombin is regarded as a direct function of the concentration of prothrombin. As Quick (1959) stated "when the test was developed, thromboplastin and prothrombin were accepted as the two basic agents needed for the generation of thrombin ...." It is clear now that there are many more factors than thromboplastin and prothrombin involved in the conversion of prothrombin to thrombin.
Therefore, a prolonged prothrombin time would not exclusively denote a decrease of prothrombin. Kindberg and Suttie (1989) stated that one-stage prothrombin time is less sensitive to the status of vitamin K, compared with other methods such as plasma prothrombin level. Suttie et al. (1988) reported that when phylloquinone intake of college-aged male subjects decreased from 82 to 32-40 µg/day, the prothrombin times were all in the normal range. However, serum phylloquinone concentration decreased from 0.87 to 0.46 ng/ml. and functionally active prothrombin ratio decreased from 1.024 to 0.911. Nevertheless, prothrombin time provides some basic information of vitamin K status, and it is cheap, easy and simple.

Parameters Related to Bone

Osteocalcin level in plasma and its degree of carboxylation

Knapen et al. (1989) reported that in a selected group of postmenopausal women the hydroxyapatite binding capacity of the circulating immunoreactive osteocalcin (irOC) was abnormally low compared with premenopause women. Administration of vitamin K (1 mg/day) increased both the concentration and the hydroxyapatite binding capacity of circulating irOC. In a subsequent study, Knapen et al. (1993) showed that the increase of circulating irOC caused by the supplementation of vitamin K in elderly women was due to the increased level of serum irOC with a high affinity for hydroxyapatite, whereas the level of irOC with a low affinity remained unaffected even after supplementation of vitamin K for 3 months. This raises the question of whether irOC with low affinity for hydroxyapatite has a physiological role and whether its secretion is regulated by the osteoblast. It is postulated that irOC with a low affinity for hydroxyapatite might be involved in bone resorption (Knapen et
al. 1993) by serving as a chemoattractant for osteoclast-like cells (Vermeer et al., 1996).

More recently. Joe et al. (1995) reported that an impaired vitamin K status, as reflected by a low nutritional vitamin K intake, was accompanied by an increased irOC level and a reduced hydroxyapatite binding capacity of circulating osteocalcin in women with atherosclerosis.

Gla concentration in bone

Hauschka and Reid (1978) reported that the Gla concentration in pooled tibiotarsus and femur diaphysis was reduced to about 47% of the level of control chick embryos of 19 day when embryos were injected Warfarintwice at the dosage of 2.0 mg/kg egg at 13 and 16 days if incubation. Gla concentrations in different types of bones (including long bone, mandible, and calvaria) of 3-week-old chicks fed vitamin K deficient or dicoumarol containing diets. were about 56-59. and 27-36% of the level of the control chicks, respectively. However. the extent of change of the Gla concentration in bone was not strictly parallel to that observed for prothrombin time (Hauschka and Reid. 1978). More recently, Lavalle et al. (1994) similarly observed that a deficiency of vitamin K significantly decreased Gla concentration in bones of chicken embryo and growing chicks. The decrease in Gla concentration in bone. however. was not associated with any gross or histological change in bones (Hauschka and Reid. 1978; Lavalle et al. 1994). Shearer (1995) stated that the pattern of healing and the final structure and strength of repaired bone in animals seem to be normal even with a reduction of osteocalcin in bone to 2% of normal concentration.
Gla Excretion in Urine

Once Gla has been formed it is not metabolized or re-used. So eventually all Gla is excreted via the urine, most in a free amino acid form and less than 5% in a peptide-bound form (Shah et al., 1978). Hence, urinary Gla level reflects the turnover of total body pool of Gla proteins. Under a steady state, the urinary excretion of Gla is a measure for the total production of Gla that is essentially dependent on the vitamin K status (Cracium et al., 1997). Suttie et al. (1988) reported that dietary vitamin K restriction in college-aged male subjects resulted in a decrease in urinary γ-carboxyglutamic acid. When phylloquinone intake was reduced from 82 μg/d to 32 to 40 μg/d, urinary Gla excretion in these college-aged subjects decreased to 78% of the normal values. Supplementation with 50 or 500 μg of phylloquinone per day restored Gla excretion to normal values. However, Booth et al. (1995) reported that dietary intake of phylloquinone was not correlated with urinary Gla excretion in postmenopausal women. Ferland et al. (1993) also reported that elderly subjects did not demonstrate any changes in their urinary Gla levels when consuming a low phylloquinone diet, whereas young subjects did. Booth et al. (1995) indicated that there might be changes in vitamin K metabolism associated with aging which have an impact on urinary Gla levels. Using rats as a model, Cracium et al. (1997) studied the influence of vitamin K on the urinary excretion. Supplementation of extra vitamin K orally in the form of phylloquinone or MK-4 to rats (12-week-old) fed a reference diet that had been proven to be vitamin K sufficient by prothrombin time, increased urinary Gla excretion substantially. This indicated that the vitamin K requirement for liver and extrahepatic tissues might be different. However, when MK-8 was supplemented to these rats, no increase in Gla excretion was observed,
which raises the question of the bioavailability of menaquinones with long side chains, such as MK-8, for extrahepatic tissues. Cracium et al. (1997) also observed that the Gla excretion in urine was decreased when rats were fed a vitamin K-deficient diet or when the rats were treated with coumarin.

**Biological Activity of Different K Vitamins**

**Biological Activity of Vitamin K**

Vitamin K is a general term for a series of related compounds which all have a naphthoquinone ring. It includes phylloquinone, menaquinones and synthetic menaquinone derivatives.

Natural phylloquinone from plant sources occurs entirely as a trans-isomer that is fully potent (Knauer et al. 1975). However, synthetic phylloquinone contains a mixture of the both cis- and trans- isomers. Matschiner and Bell (1972) reported that although the distribution and turnover of cis-phylloquinone in liver subcellular fractions were similar to that observed with trans-phylloquinone, cis-phylloquinone had little or no biological activity. These authors indicated that most of the administered cis-phylloquinone might not be bound to an active site. In a subsequent study with rats, Knauer et al. (1975) showed that the cis-isomer of phylloquinone was a poor substrate for 2,3-epoxidation both in vivo and in vitro.

Menaquinones are a series of related compounds with various numbers of isoprenoid residues at the three position of naphthoquinone. The number of isoprenoid units of the side chains has a great effect on the biological activity of menaquinone series. Some of the biological activities reported in literature are summarized in Table 5.
### TABLE 5. Relative biological activity\(^1\) of menaquinones (vitamin K2)

<table>
<thead>
<tr>
<th>Vitamin K2</th>
<th>Matchiner and Doisy (1966)</th>
<th>Wiss et. al (1959) and Weber &amp; Wiss (1959)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-1</td>
<td>1</td>
<td>~15</td>
</tr>
<tr>
<td>MK-2</td>
<td>35</td>
<td>~40</td>
</tr>
<tr>
<td>MK-3</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>MK-4</td>
<td>100</td>
<td>~120</td>
</tr>
<tr>
<td>MK-5</td>
<td>156</td>
<td>100</td>
</tr>
<tr>
<td>MK-6</td>
<td>---</td>
<td>~70</td>
</tr>
<tr>
<td>MK-7</td>
<td>122</td>
<td>---</td>
</tr>
<tr>
<td>MK-9</td>
<td>67-78</td>
<td>---</td>
</tr>
<tr>
<td>MK-10</td>
<td>49</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^1\) The biological activity of vitamin K1 was assigned to be 100.

More recently, Akiyama et al. (1995) reported that among all the vitamin K2 analogues (MK-1 to 14), MK-4, -5, and -6 had the highest vitamin K activity as measured by the sustenance of coagulation activity and plasma prothrombin levels. MK-7 and MK-8 also had substantial vitamin K activity. However, blood coagulation activity and prothrombin level were not improved in rats fed a vitamin K-deficient diet and treated with Warfarin when supplemented with MKs with 9, 10 or 11 isoprene units. This observation is contrary to some other reports showing MKs with long side chains, such as MK-9, had vitamin K activity and were equally as or more potent than phylloquinone. Will and Suttie (1992) reported that MK-
9 was equally effective at a higher level (>1.1 μmol/kg diet) as phylloquinone to maintain the plasma prothrombin levels and the liver microsomal vitamin-K dependent protein precursor levels in rats when supplemented in a vitamin K-deficient diet. Groenen-van Dooren et al. (1995) reported that MK-9 was 50 to 80% more potent than phylloquinone for maintaining a satisfactory plasma prothrombin level when administered orally or subcutaneously 10 nmol/day to rats fed a vitamin K deficient diet. This observation was confirmed by Matschiner and Taggart (1964) who reported that MK-9 was more potent than phylloquinone for maintaining prothrombin level when administered by intracardial injection. The discrepancy in the biological activity of MK-9 and perhaps other MKs with long side chains, might be explained by the difference of dosage administered, the animal models used, and the time of sampling after the administration. Will and Suttie (1992) reported that the plasma prothrombin concentration of rats fed a diet containing low level of MK-9 (0.2 μmol/kg diet) was similar to those of rats fed a vitamin K-deficient diet whereas the prothrombin concentrations of rats fed a high level of MK-9 (1.1 μmol or more/kg diet) was similar to those of rats fed on equal molar of phylloquinone. This observation demonstrates that MK-9 is an equally potent vitamin K source as phylloquinone at high levels but not at a low level. Groenen-van Dooren et al. (1995) stated that when vitamin K-deficient animals are used to determine the biological activities of K vitamins, the vitamin cycle is operational, therefore, substantial vitamin K is recycled via vitamin-K epoxide reductase. However, when Warfarintreated animals are used for the same purpose, the reduction of vitamin K is only via the NADH-dependent pathway because of the blockage of the epoxide reductase pathway. This difference in recycling vitamin K under the two different animal model systems might influence the biological activity of vitamin K. Akiyama et al (1995) reported that MK-9
alleviated hypoprothrombinaemia within 24 hours but not within 4 hours after administration and concluded that the length of isoprene units of menaquinones affects the time needed for them to exert vitamin K activity.

The biological activity of menaquinone series is also affected by geometric isomerization. Wiss et al (1959) and Weber and Wiss (1959) reported that the biological activity of MK-3 all trans form was 86% of that of phylloquinone whereas the values for 6,7-mono-cis and 10, 11-mono-cis of MK-3 were 118 and 85% of that of phylloquinone. The biological activity of different geometric isomers of MK-5 is different, too. Wiss et al (1959) reported that the biological activity of all trans, 6,7-mono-cis, and 18, 19-mono-cis isomers of MK-5 were 58, 133 and 52%, respectively, of that of phylloquinone.

**Biological Activities of Menadione Analogues**

The comparison of biological activities among menadione derivatives or between menadione derivatives to other K vitamins should be on molar basis, and any comparison that is not on this basis should be converted to a molar basis. The molecular weight of vitamin K1 is 451 daltons, and the analogue values for menadione, menadione sodium bisulfite, menadione dimethylpyrimidinol bisulfite, menadione nicotinamide bisulfite are 172, 276, 378, and 376 daltons, respectively.

Menadione is a fat-soluble crystalline compound. Quick and Stefanini (1957) reported that menadione was slightly less effective than vitamin K1 as a vitamin K source on a molar basis. Nelson and Norris (1960) reported that the required amount of menadione to maintain normal prothrombin time was 1.03 to 1.68 times the amount of vitamin K1 needed. This indicated that the potency of menadione as a vitamin K source was about 60-97% of that
of vitamin K1. However, other results show that the potency of menadione as a vitamin K source is less than that suggested by the above values. Griminger and Donis (1960) reported that vitamin K1 was 2.5 times as active as menadione on a molar basis for decreasing prothrombin time of vitamin K-deficient chicks to normal. They also observed that, in comparison with vitamin K1, eight times as much as menadione was needed to minimize prothrombin time of chicks fed diets containing dicumarol or sulfonamides. Matschiner and Diosy (1966) reported that the potency of menadione was only 49% that of phylloquinone.

The wide variation in biological activity of menadione might be related to a difference of absorption. Nelson and Norris (1960) stated that menadione, a fat-soluble crystalline compound, is less readily absorbed than oil-like vitamin K1 or water-soluble menadione derivatives. And because menadione is a crystalline compound, it can’t be assumed that the same proportion goes into solution in dietary fats and is absorbed to the same extent in each experiment. For instance, Shelton et al. (1956) obtained different dietary requirements of menadione at different dietary fat levels. The observation of Fisher et al. (1956) that when injected intravenously, vitamin K1 and menadione had approximately the same molar biological activity, provided indirect support for the argument that the difference between biological activities of vitamin K1 and menadione might be related a difference of absorption.

Menadione sodium bisulfite is one of the water-soluble forms of menadione analogues. Its biological activity has been shown to be approximately the same as vitamin K1 on a molar basis (Nelson and Norris, 1960). However, Griminger and Donis (1960) reported that the biological activity of menadione sodium bisulfite was only about 60% of that of
vitamin K1. Several researchers have shown that the biological activity of menadione sodium bisulfite is definitely greater than that of menadione (Frost and Spruth, 1955; Frost et al 1954: Shelton et al 1956; Nelson and Norris, 1960; Griminger and Donis, 1960). However, the reported quantitative ratio of biological activity of menadione sodium bisulfite to menadione on molar basis varied considerably. Griminger and Donis (1960) reported that biological activity ratio of menadione sodium bisulfite to menadione was 1.7, and the ratios reported by Nelson and Norris (1960) varied from 1.3 to 1.8. Other research results indicate a much higher ratio. Frost and Spruth (1955) reported that biological activity ratio of menadione sodium bisulfite to menadione was 4, and Shelton et al. (1956) reported that the value was 18. This huge variation of ratio of biological activity of menadione sodium bisulfite to menadione perhaps is related to the variation of determined biological activity of menadione whereas the biological activity of menadione sodium bisulfite is relatively consitent.

Menadione dimethylpyrimidinol bisulfite is another water-soluble form of mendione derivatives. Griminger (1965) concluded that the vitamin K potency of menadione dimethylpyrimidinol bisulfite was greater than that of menadione sodium bisulfite. The conclusion was based on the observation that the amount of menadione sodium bisulfite required to obtain a short prothrombin time was 1.8 times the amount of menadione dimethylpyrimidinol bisulfite, whereas the ratio should be 1.36 if the potency of both these two compounds were same on a molar basis. These results were confirmed by Dua and Day (1966).

Recently, a new source of menaquinone analogue, menadione nicotinamide bisulfite has become available. Oduho et al (1994) compared the vitamin K activity of this new source
with that of menadione dimethylpyrimidinol bisulfite, and concluded that these two
compounds were equally potent as vitamin K source on molar basis. Menadione
nicotinamide bisulfite, however, is an effective source of niacin, too. Menadione
nicotinamide bisulfite contains about 32% of nicotinamide on weight basis.

**Vitamin K Requirement of Poultry**

*Creation of Vitamin K Deficiency in Animal Model System*

Experimentally-induced vitamin K deficiency must be created in animals to facilitate
research designed to determine dietary vitamin K requirements, obtain information on the
biopotency of dietary vitamin K sources, and determine the influence of various factors on
the vitamin K requirement. There are two strategies to create a vitamin K-deficiency in
animals. One is to reduce oral intake of vitamin K and to suppress the synthesis of vitamin K
in the intestinal tract. Another is supplementation of vitamin K antagonists such as
Warfarin or dicumarol. Both of these strategies have been used in experiments.

The vitamin K requirement of animals is met by a combination supply from dietary
source and from the microbial biosynthesis in the intestinal tract. The vitamin K synthesized
in the intestinal tract may be absorbed directly or through ingestion of feces (coprophagy).

**Vitamin K-deficient diets**

To fully induce a vitamin K deficiency in animals, they must be fed a diet devoid of
or very low in vitamin K. For studies with rats, vitamin K-deficient semi-purified or purified
diets are most commonly used. These diets consist mostly of casein or soybean protein as
protein sources, and corn starch, sucrose or glucose as energy sources. Mathers et al. (1990)
successfully developed another semi-purified, vitamin K-deficient diet that was composed of
primarily rice and casein. About 23 days were required to develop signs of vitamin K deficiency using this type of diet compared with about 5 to 7 days when purified diets were used (Vermeer et al. 1995). The form and storage conditions of diets also affect the time needed to develop vitamin K-deficiency. Vermeer et al. (1995) stated that powdered feed may be hygroscopic, and if such a feed is kept at room temperature for more than one week, menaquinone-producing bacteria may develop and thus reduce the effectiveness of the diet. Compared with rats, vitamin K deficiency is more easily developed in poultry, simply by dietary restriction of the vitamin. The common corn-soybean meal type diet has been used to successfully develop vitamin K deficiency in chicks (Frost et al. 1956) and turkeys (Griminger 1957). Semi-purified diets with soybean meal as a protein source, and purified diets have also been used to develop vitamin K deficiency in poultry. When formulating diets, special attention should be given to the sources of fat in diets. The fat sources used are usually stripped or refined to reduce the concentration of vitamin K. Examples of special fat sources used include stripped lard (Nelson and Norris, 1960) and stripped corn oil (Griminger and Donis, 1960). Occasionally corn oil is used (Oduho et al., 1993; Mathers, 1990).

Suppression of microbial synthesis of vitamin K and prevention of ingestion of feces

The microbial flora in the intestinal tract can synthesize vitamin K, and this vitamin K may be absorbed by the host animals from the ileum (Conly, 1992). Therefore, the microbial population in the intestinal tract should be suppressed to reduce the production of vitamin K if a vitamin K deficiency is wanted. Broad-spectrum antibiotics such as neomycin and sulfa drugs can be used for this purpose. These drugs are capable of essentially sterilizing the
intestinal tract and inducing hemorrhagic syndrome (Vermeer et al., 1995). Sulfa drugs such as sulfaquinoxaline have been shown to interfere with the metabolism of vitamin K and increase the requirement of vitamin K dramatically (Frost et al., 1956; Frost and Spruth, 1955). This is due to the fact that sulfamides inhibit the capacity of liver to produce prothrombin (Black et al., 1942; Mushett and Seeler, 1947).

Under normal conditions, rats eat their feces. Barnes et al. (1957) observed that rats maintained on raised wire screen still ingest 50 to 65% of their feces. This problem can be overcome in two ways. First, one may use germ-free rats. However, maintaining animals under sterile condition presents practical problems, especially when the experiments extend over long period of time and repeated handing of the animals is required. Alternatively, feces can be collected in anal cups. In chicks and turkeys, simply keeping them on wire floors and cleaning feces often enough to keep it out of reach is enough to eliminate the ingestion of vitamin K from fecal materials.

The vitamin K antagonist regimes

In early studies vitamin K antagonists such as Warfarin and dicumarol were often employed to create vitamin K deficiency because of the difficulty of creating of a consistent vitamin K deficiency with special diets. This was especially true in rats. Nowadays, vitamin K-deficient diets are easily created with the availability of ingredients containing low levels of vitamin K. and vitamin K antagonist regimes are not used very often except in some special research.
Vitamin K Requirement of Chicks

Using the minimum prothrombin time as the criterion, Griminger and Donis (1960) reported that the vitamin K1 requirement of chicks fed a purified diet containing 1% of refined corn oil was 1 mg/kg diet. Nelson and Norris (1958) estimated the requirement of vitamin K1 for chicks varied from 0.4 to 0.6 mg/kg diet. This latter observation was confirmed by Nelson and Norris (1960), who reported that the vitamin K1 requirement of chicks at 2 and 4 weeks of age were 0.524 and 0.528 mg/kg diets, respectively.

Shelton et al. (1956) reported that the menadione requirement of chicks was about 1.19 mg/kg diet when the diet was supplemented with 5.7% fat, whereas the requirement increased to 1.39 mg/kg diet when supplemental fat levels decreased to 2.7%. Griminger and Donis (1960) reported that 1.15 mg menadione /kg diet was needed to achieve a minimum prothrombin time. Nelson and Norris (1960) reported that the menadione requirement of chicks could vary from 0.205 to 0.970 mg/kg diet. However, Frost et al. (1956) reported a much higher requirement of menadione for chicks. These authors observed that menadione, even at a high level such as 2.88 mg/kg diet, could not bring prothrombin time back to normal level.

Frost and Spruth (1955) reported that a dietary concentration of menadione sodium bisulfite from 34 to 45 μg/kg only provided partial protection against hemorrhagic condition. For full protection, 90 to 180 μg menadione sodium bisulfite /kg diet was needed. Nelson and Norris (1960) reported that the menadione sodium bisulfite requirement of chicks at 2 and 4 weeks of age varied from 0.254 to 0.356 and 0.320 to 0.373 mg/kg diet, respectively. Shelton et al. (1956) reported that the menadione sodium bisulfite requirement of chicks was 0.198
mg/kg diet, regardless of the level of fat in the diet. However, other researchers showed that
the menadione sodium bisulfite requirement was much higher. Griminger and Donis (1960)
estimated that, to achieve a minimum prothrombin time, 0.96 mg menadione sodium
bisulfite/kg diet was required by chicks. Frost et al. (1956) observed that whole blood
clotting time of chicks plotted against the log of the concentrations of menadione sodium
bisulfite in diets gave a straight line in the range of 0.045 to 0.72 mg/kg diet, indicating that
0.72 mg menadione sodium bisulfite/kg diet might not meet the requirement.

The requirement of the two other menadione analogues, menadoine
dimethylprimidinol bisulfite and menadione nicotinamide bisulfite, have not been well
studied. Oduho et al. (1994) reported that prothrombin time of chicks fed diets containing 0
to 0.4 mg menadione/kg diet from menadione dimethylprimidinol bisulfite or menadione
nicotinamide bisulfite decreased linearly, but no estimate of requirement was made.

Although it has been well documented that vitamin K is required for the post-
transcription carboxylation of some glutamic acids in Gla proteins in bone to form osteocalcin
or matrix Gla protein, the effect of vitamin K on bone is not conclusive. Lavelle et al. (1994)
reported that vitamin K-deficient chicks and chick embryos developed normal skeletal
structure on the basis of gross and histological morphology and bone ash, even though they
exhibited a severe reduction in prothrombin time and bone Gla concentration when compared
to control chicks. Fleming et al. (1998) reported that the cancellous bone volume loss in the
proximal tarsometatarsus was significantly reduced in laying hen from 15 to 25 weeks of age
when these hens were fed on a diet supplemented with 10 mg menadione/kg diet compared
to control hens that were fed on a diet containing 2 mg menadione/kg diet.
The Vitamin K Requirement of Turkeys

Compared with chicks, literature on vitamin K requirement of turkeys is scarce. Griminger (1957) reported that the menadione sodium bisulfite requirement was 1.1 mg/kg diet based on prothrombin time, whereas the menadione requirement was 1.76 mg/kg diet. There is no information available in the literature on the vitamin K1 requirement of turkeys.

Some Nutrients, and Additives that Influence the Dietary Requirement of Vitamin K

The requirement of vitamin K has been shown to be influenced by several nutrients, most notably fat and fat-soluble vitamins such as vitamins A and E. Shelton et al., (1956) reported that the requirement of menadione of young chicks was affected by dietary fat levels. When the fat level was 5.7%, the requirement of menadione was 1.19 mg/kg, whereas when dietary fat level was reduced to 2.7% the requirement increased to 1.39 mg/kg. These authors, however, observed that the requirement of water-soluble form of menadione derivatives such as menadione sodium bisulfite was not affected by dietary fat levels.

It has long been known that fat-soluble vitamin A antagonizes vitamin K in rats. Light et al. (1944) reported that hypervitaminosis A in rats leads to hemorrhagic syndrome and hypoprothrombinemia that can be prevented by the administration of vitamin K. Matschiner and Diosy (1962) observed a relationship between greater than normal levels of dietary vitamin A and resulting deficiency of vitamin K as indicated by the depression of plasma prothrombin levels in rats. These authors also observed that retinoic acid was more effective than vitamin A acetate as an antagonist of vitamin K. In a subsequent study, Matschiner et al. (1967) observed that giving 50 IU retinoic acid per day to rats increased the requirement of vitamin K to 10 times that seen in germ-free rats. It was concluded that excess
vitamin A antagonizes vitamin K during the process of absorption (Matschiner et al. 1967). In chicks, however, Abawi and Sullivan (1989) failed to observe any interaction between vitamin A and vitamin K on body weight, feed efficiency and plasma vitamin A level in an experiment with factorial arrangement of the two vitamins at the levels representing deficient, optimum and excess. But they did not determine the plasma prothrombin time or other parameters related to vitamin K status.

Large amounts of dietary vitamin E also antagonize the action of vitamin K. Olson and Jones (1979, 1984) observed that a high dietary intake of vitamin E by rats antagonizes vitamin K activity and increases the requirement of vitamin K. March et al. (1973) reported that, when diets containing 2200 IU vitamin E per kg diet were fed to chicks, prothrombin times were lengthened. At a relatively low level of vitamin E (100 IU), however, Abawi and Sullivan (1989) failed to observe any negative effect on the performance of broiler chicks even when they were fed on a low vitamin K (0.22 mg menadione sodium bisulfite/kg) diet. Prolongation of prothrombin times as a result of megavitamin E therapy also has been reported in humans (Korsan-Bengsten et al., 1974).

The nature and mechanism of the antagonism between \( \alpha \)-tocopherol and vitamin K remains unsettled, but there is some evidence indicating that the antagonism might be at the level of metabolism rather than at the level of absorption. Wooley (1944) first observed that \( \alpha \)-tocopherolquinone in large doses caused hemorrhages in rats that were prevented by menadione supplementation. These observations were confirmed with pregnant rats by Rao and Mason (1975). Bettger and Olson (1982) reported that d-\( \alpha \)-tocopherolquinone was a more potent antagonist of vitamin K activity in rats than d-\( \alpha \)-tocopherol. Neither compound seemed to affect the absorption and distribution of vitamin K in the body. These authors also
observed that feeding high levels of vitamin E to rats (2 g/kg diet) resulted in an increase in d-α-tocopherolquinone in liver microsomes from 2.5 to 9.4 nmol/g.

There are several feed additives that have been shown to influence the requirement of vitamin K. One group of such additives includes broad-spectrum antibiotics such as neomycin. These antibiotics suppress or completely inhibit vitamin K synthesis by the microbial flora in the intestinal tract. Hence, supplementation of such antibiotics in diets might increase the requirement of vitamin K. Another compound that influences the requirement of vitamin K is the antioxidant, butylated hydroxytoluene (BHT). Takahashi and Hiraga (1978a) reported that BHT causes a high incidence of death due to massive hemorrhages when included in the diets of rats at concentrations of 0.69% or more. Further investigation showed that a significant dose-dependent reduction in plasma prothrombin time could be detected when the low concentration of BHT in the diet was only 0.017% (Takahashi and Hiraga, 1978b). Supplementation of vitamin K deficiency prevented the BHT-induced hypothrombinaemia, implying that vitamin K was directly or indirectly involved in the hemorrhagic death caused by BHT. More recently, Cottrell et al. (1994) reported that BHT induced clotting factor defects in vitamin K-sufficient rats, and the defects could be corrected by further supplementation of vitamin K. These authors, however, reported that no clotting factor defects were induced at a dose level of 125 mg BHT /kg body weight, and concluded that BHT was unlikely to be a human safety problem because it produced no clotting factor defect in rats receiving an intake of 1000 times the acceptable daily intake by humans.

The mechanism by which BHT antagonizes the function of vitamin K is believed to be similar to that of coumarin (Suzuki et al., 1983). Suzuki et al. (1983) reported that the
vitamin K contents of the livers of rats fed diets containing 0.25% of BHT were reduced, and
fecal excretion of vitamin K metabolites was increased compared with control rats. It has
been shown that BHT needed to be activated to exert its hemorrhagic effect. Takahashi and
Hiraga (1981) reported that quinone methide, a metabolite of BHT, inhibits vitamin K
epoxide reductase activity by 20 to 30% in rats. This mechanism was further confirmed by
Takahashi (1987) who reported that BHT quinone methide seems to be more potent than its
parent compound to cause reductions in clotting factors II, VII, IX and X.

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EFFECT OF DIETARY VITAMIN K1 (K1) ON SELECTED PLASMA CHARACTERISTICS AND BONE ASH IN YOUNG TURKEY POULTS FED ADEQUATE OR DEFICIENT VITAMIN D3 DIETS

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ABSTRACT

Three experiments (Exp.) were conducted to determine the effect of dietary K1 on selected plasma characteristics and bone ash in poults. In Exp. 1, diets were supplemented with 0, 0.5, 1.0 or 2.0 mg of K1/kg. All diets contained 1,650 IU of vitamin D3 (D3)/kg. Dietary K1 had no effect on tibia ash at 7 d or incidence or severity of the rickets-like condition. Tibia ash of poults fed 2.0 mg of K1/kg, however, was greater at 14 d of age than tibia ash of poults fed the basal diet. Dietary inclusion of 0.5 mg of K1/kg was as effective as 1 or 2 mg of K1/kg in reducing plasma prothrombin time. In Exp. 2, a 2 x 4 factorial arrangement was used consisting of 1650 or 550 IU of D3/kg and 0.1, 0.45, 1.0 and 2.0 mg of K1/kg. Dietary D3 and K1 had no effect on bone ash. Dietary inclusion of 0.1 mg of K1/kg seemed to be enough to minimize plasma prothrombin time. In Exp. 3, dietary treatments consisted of a Control (1,650 IU of D3 and 2.0 mg of K1/kg) and K1 concentrations of 0, 0.37, 2.28, or 5.33 mg/kg in diets containing 275 IU of D3/kg. Poults fed the low D3 diet without K1 consumed less feed and 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 tended to decrease, and plasma inorganic phosphorus increased when pouls were fed the low D3 diet supplemented with 0.37 or 2.88 mg of K1/kg compared with pouls fed the low D3 diet without K1 supplementation. Tibia ash of pouls fed the low D3 diet was not affected by K1 supplementation.

Keywords: vitamin D3, vitamin K1, alkaline phosphatase activity, phosphorus, bone ash.

**INTRODUCTION**

Since its discovery in the late 1940s 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 of tissues other than the liver. Soft tissues that actively synthesize Gla proteins include the kidneys (Booth, 1997), and uterine smooth muscle (Luo et al., 1995). Some cells in the skeleton tissues, such as osteoblasts in bone (Hauschka and Reid, 1978) and the chondrocytic lineage cells in cartilage, also actively synthesize Gla proteins, such as osteocalcin and Gla matrix protein (Luo et al., 1995). The process of post-translational carboxylation of glutamyl residues to γ-carboxyglutamyl residues in these Gla proteins requires the presence of enough vitamin K, because that this process is mediated by vitamin K-dependent carboxylases. The Gla residues in the vitamin K-dependent proteins are essential for their full biological activities (Will et al., 1992). Hence, vitamin K, through its effect on carboxylation, has an influence on the normal physiological functions of these Gla proteins.
There is evidence indicating an interaction between the biological functions of vitamin D and vitamin K. Leeuwen et al. (1996) reported that the expression of osteocalcin genes was regulated by vitamin D. One-25-(OH)2 D3 stimulated osteocalcin production in vitro in osteoblastic cell lines. 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. The post-translational carboxylation process of osteocalcin has been shown to affect osteocalcin synthesis and intercellular processing (Nishimoto and Price, 1985). These observations indicate that there might be an interrelationship between vitamin D and vitamin K in the synthesis and processing of nascent osteocalcin. Sergeev and Norman (1992) reported that 1,25-(OH)2 D3 receptor (VDR) underwent γ-carboxylation in the presence of K1 in vitro, and 15 to 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). Sergeev and Spirichev (1989) reported that feeding rats a diet without adequate menadione sodium bisulfite supplementation resulted in an increase in bound VDR in the crude chromatin fraction of intestinal mucosa. The 1,25-(OH)2 vitamin D3 binding capacity in the cytosol of intestinal mucosa was also increased in these vitamin K deficient rats (Sergeev and Spirichev, 1989).

Vitamin K has been reported to affect some plasma parameters related to bone. Knapen et al. (1989) reported that the hydroxyapatite binding capacity of the circulating immunoreactive osteocalcin (irOC) was abnormally low in a selected group of postmenopausal women compared with premenopausal women. Administration of K1 (1 mg/day) increased both the concentration and the hydroxyapatite binding capacity of circulating irOC. In a subsequent study, Knapen et al. (1993) showed that the increase of the
circulating irOC by the supplementation of K1 in elderly women 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) reported that K1 supplementation in elderly women also resulted in 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 MK-4 in culture medium suppressed proliferation of osteoblasts and increased alkaline phosphatase activity in vitro. In poultry, Fleming et al. (1998) reported that supplementation of high level of menadione nicotinamide bisulfite in diets significantly reduced the loss of cancellous bone in the proximal tarsometatarsus in laying hens between 15 to 25 weeks of age compared with hens fed a diet containing low level of menadione nicotinamide bisulfite. However, Lavelle et al. (1994) reported that menadione sodium bisulfite supplementation had no effect on gross and histological morphology of bone, cartilaginous tissues, and bone ash in young growing chicks, although chicks fed a diet without menadione sodium bisulfite supplementation exhibited increased plasma prothrombin times and reduced Gla contents in bone.

Information in the literature on the effect of vitamin K on bone or related parameters in young growing animals is scarce and inconclusive. All reported research was conducted with an adequate supply of vitamin D. With the indication of a interaction between vitamins D and K, it will be informative to study the effect of vitamin K on bone or related parameters under conditions of adequate and deficient dietary vitamin D. Therefore, this research was conducted to determine the influence of dietary K1 on bone mineralization and related plasma parameters in young turkeys fed diets that were adequate or deficient in D3.
Concurrently, the dietary concentration of K1 needed to minimize plasma prothrombin time was evaluated.

**MATERIALS AND METHODS**

**Diets**

In Experiment 1, four dietary treatments were used. These consisted of a basal diet (Table 1) supplemented with 0.0, 0.5, 1.0 or 2.0 mg of K1/kg. The diets were fed in pelleted form from Days 1 to 7, and then, switched to mash form from 8 to 14 d of age. Each treatment was fed to four replicate pens with seven poult's per pen from 1 to 14 d of age. Eleven poults in an extra pen were fed the basal diet in mash form for observation from 1 to 14 d of age. In Experiment 2, a 2X4 factorial arrangement was used, consisting of two concentrations of D3 (1650 or 550 IU/kg) and four concentrations of K1 (0.1, 0.45, 1.0 or 2.0 mg/kg by analysis) supplemented to the basal diet shown in Table 1, except the vitamin premix contained no D3. 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 (1650 IU of D3/kg and 2.0 mg of K1/kg) and diets containing K1 concentrations of 0.0, 0.37, 2.28 or 5.33 (by analysis) and 275 IU of D3/kg. The same basal diet shown in Table 1 was used to obtain the latter four dietary treatments, except the D3 concentration was decreased to 275 IU/kg. 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.

**Animals**

One-day-old 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 for ad libitum consumption. Water troughs were cleaned daily to reduce the possibility of production of vitamin K by fermentation. Fecal materials in feeders were removed daily, and excreta were cleaned frequently to minimize coprophagy. In Experiments 1 and 2, feed consumption and body weight were recorded weekly. Two blood samples were obtained from each of two poults from each pen on 7 and 14 d. Citrate was used as 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 K1, MK-4 and 25-OH-D3. After obtaining blood samples the poults were euthanitized by inhalation of Halothene®. In Experiment 1, the right and left tibias were 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 Experiment 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 only on plasmas obtained on Days 13 and 19.

1 Halocarbon Laboratories, River Edge, NJ 07661.
Analytical Procedures

Plasma K1 and MK-4 levels were determined by the colleagues of University of Wisconsin\textsuperscript{2} using the high performance liquid chromatography (HPLC) method of Haroon et al. (1986). The procedure to analyze plasma K1 or MK-4 was briefly; plasma samples were extracted through a liquid-phase with a ratio of plasma, water, ethanol, hexane of 1:2:3:9. After centrifugation, the organic layer at the top of the supernatent 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. K1 and MK-4 were analyzed on a HPLC with a Zorbax C18 reverse phase chromatography column and 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 5mL/L reductive solvent additive. The rate of solvent flow was set to 1.0 mL/min. and pressure was 7757 cm mercury. Excitation was performed at 244 nm and emission was monitored at 418 nm. The internal standard used was di-hydroK1.

Plasma 25-OH-D3 was determined using the radioimmunoassay of Hollis et al. (1993). The primary goat antibody against 25-OH-D3 and the antigoat second-antibody were obtained from Incstar Corp\textsuperscript{3}. The radioiodinated D3 was prepared from 23, 24, 25, 26, 27-pentanor-C22-carboxylic acid of D3. The procedure was briefly: a solution of the C22-acid of D3 and 1.1-carbonyldimidazole in dimethylforamide was allowed to react at 4 C for 2 hours. 1.3-diaminopropane was then added, and allowed to react at 4 C for 16 hours and at 25 C for 3 hours. The 3-aminopropyl derivative of D3-C22-amide produced was combined with

\textsuperscript{2} Dr. Suttie and his group, Department of Biochemistry, University of Wisconsin.
\textsuperscript{3} Incstar Corp. Stillwater, MN 55082.
Bolton–Hunter reagent in dioxane. The resulting side-chain-radioiodinated D₃ analogue was isolated and purified by silica gel chromatography. The assay calibrators were prepared from serum, stripped free of 25-OH-D₃ by activated charcoal, and crystalline 25-OH-D₃ dissolved in absolute ethanol. 25-OH-D₃ in plasma samples and in the calibrators was extracted with actonitrile, and then after centrifugation the supernatants were harvested to analyze 25-OH-D₃.

One-stage prothrombin time was determined manually using a commercial thromboplastin preparation. Simplastin® Excel® Plasma inorganic phosphorus, calcium and total alkaline phosphatase activity were determined using commercial kits of procedures No. 670, No. 587 and No. 104®, 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 hours 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 days. The bone was then demineralized through a graded series of ethanol solutions, and infiltrated with paraffin. The bone tissue was 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/or quadratic responses of the dependent variable to independent

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4 Sigma Diagnostics, St Louis, MO 63178
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 announced when probability less than 10%.

RESULTS

Experiment 1

Dietary K1 concentration did not affect body weight gain, feed intake or feed
efficiency of poults through 7 or 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 or keep their balance.
Gross examination of longitudinal slices of tibias taken at 7 d showed the prevalence of
overdeveloped growth plates with a thickness 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 no-
pelleted, mash form of the respective experimental diets from 8 to 14 d. By Day 14, poults
showed few signs of leg weakness, irrespective of dietary K1 concentration.

Bone ash concentrations of the tibias were relatively low at 7 d, irrespective of dietary
K1 concentration (Table 3). At 14 d, bone ash was determined on only the tibias from poults
fed the diet containing 2.0 mg of K1 /kg and from poults fed the basal diet. The poults fed the
basal diet included these in the observation group which were fed the basal diet in mash form
from Day 1 and these in the experimental group which were fed in pelleted form during Days
1 to 7, and mash form during Day 8 to 14. These data showed that percentage bone ash in the experimental group increased substantially as compared with the 7 d data, and was comparable to that of poults fed the basal diet in mash form from Day 1. Percentage bone ash of poults fed 2.0 mg of KI/kg was greater (P<0.05) than that of poults fed the basal diet. Supplementation of KI in diets had no effect on plasma 25-OH-D3 levels (Table 3). At 7 d of age, plasma 25-OH-D3 levels in poults of all dietary treatment were lower (6.8 vs. 12.9 ng/ml) than values observed with normal poults at same age (Sell and Horst, 1994, unpublished data). Although poults in the experimental groups recovered from the leg weakness by 14 d of age, their plasma 25-OH-D3 levels were still lower (P < 0.01) than those of poults fed mash diet from Day 1 (7.96 vs. 11.70 ng/mL).

Plasma prothrombin time decreased curvilinearly with the increase of dietary KI (Table 4). Comparisons by single degree freedom contrast showed that the prothrombin times of poults fed diets containing 0.5, 1.0 or 2.0 mg of KI/kg did not differ from each other, but they were all less than that of poults fed the diet without KI. At 7 d of age, plasma KI concentration increased curvilinearly as dietary KI level increase, and reached a apparent plateau when the dietary level of KI reached 1.0 mg/kg (Table 4 and Figure 1). Plasma MK-4 concentration also increased curvilinearly as dietary KI level increased (Table 4 and Figure 1). At 14 d of age, plasma KI and MK-4 concentrations, however, increased linearly with the increase of dietary KI (Table 4 and Figure 2).
Experiment 2

Poults fed diets containing 550 IU of D3/kg gained less weight compared with poults fed diets containing 1650 IU of D3/kg (Table 5). This effect of D3 was most evident during the 8 to 14 d age period. However, dietary D3 had no effect on feed intake. The dietary K1 effect on body weight gain was not consistent (Table 5), with some effect detected during the first 7 days but no effect detected during the second week. Dietary K1 had no effect on feed intake. A significant interaction between dietary D3 and K1 was detected with efficiency of feed utilization (Table 5). Dietary K1 did not affect efficiency of feed utilization in poults fed diets containing 1650 IU of D3/kg. However, the efficiency of feed utilization was poorer statistically or numerically in poults fed diets containing 550 IU of D3/kg when supplemented with high levels of K1 (0.45 or more mg/kg) compared with that of poults fed diet containing 550 IU of D3/kg with 0.1 mg of K1/kg.

Plasma prothrombin times of poults fed diets containing 1650 IU of D3/kg tended to be longer than that of poults fed diets containing 550 IU of D3/kg at 14 d of age but not at 7 d of age (Table 6). However, dietary D3 had no effect on bone ash and plasma K1 concentrations (Table 6). Dietary K1 concentration of 0.1 mg/kg was as effective as 0.45, 1.0 or 2.0 mg/kg in reducing plasma prothrombin time. Plasma K1 increased linearly with the increase of dietary K1 level. There was an indication of an interaction between dietary D3 and K1 on bone ash (P<0.10). Bone ash was not affected by the increase of dietary K1 when poults were fed diets containing 1650 IU of D3/kg, whereas an increase of dietary K1 tended to have an adverse effect on bone ash in poults were fed diets containing 550 IU of D3/kg.
Experiment 3

Dietary treatment effects on poult performance were evident from Day 7 and onward, whereas this effect was not detected during the first 6 days (Table 7). Poults fed the diet supplemented with 275 IU of D3/kg and 0 mg of K1/kg gained less weight (P<0.05), consumed less feed (P<0.05) compared with poults fed the Control diet. Body weight gain of poults fed the low D3 diet, when supplemented with K1 at the concentration of 0.37 or 2.88 mg/kg, was improved (P<0.05 or P<0.01) compared with that of poults fed the low D3 diet without supplementation of K1. Supplementation of K1 at 0.37 or 2.88 mg/kg to the low D3 diet also increased or tended to increase feed intake (P<0.06 or P<0.03) compared with that of poults fed the low D3 diet without supplementation of K1. However, body weight gain and feed intake were not improved in poults fed the low D3 diet when supplemented with 5.33 mg of K1/kg compared with poults fed low D3 diet without K1 supplementation. The dietary treatment effect on efficiency of feed utilization was not consistent. For example, the overall efficiency of feed utilization from Days 1 to 19 was not influenced by dietary treatments, but during 7 to 13 d age period, poults fed the low D3 diet without K1 supplementation utilized feed less efficiently than poults fed the Control diet.

Poults fed a diet containing 275 IU of D3/kg and 0 mg of K1/kg had increased plasma alkaline phosphatase activity on Days 13 (P<0.02) and 19 (P<0.05) but not on Day 6 compared with poults fed the Control diet (Table 8). The increased plasma alkaline phosphatase activity of poults fed the low D3 diet was decreased or tended to decrease (with the exception that plasma alkaline phosphatase activity on Day 19 of poults fed a diet supplemented with 2.0 K1/kg was decreased numerically) when the low D3 diet was supplemented with K1 compared with that of poults fed the low D3 diet without K1.
supplementation. The effect of dietary treatments on plasma calcium concentration was not consistent, with no effect detected on Day 13 but a trend toward decreased plasma calcium as dietary KI increased during observed at Day 19. Plasma inorganic phosphorus levels of poult fed a low D3 diet without supplementation of KI was lower compared with poult fed the Control diet (P < 0.05). Supplementation of the low D3 diets with 0.37 or 2.28 mg of KI/kg increased the plasma inorganic phosphorus significantly on Day 13 (P<0.08 or P < 0.03, respectively) and numerically on Day 19 compared with that of poult fed the low D3 diet without KI supplementation. However, supplementation with the high level of KI (5.33 mg/kg) to the low D3 diet had no effect on plasma inorganic phosphorus level compared with that of poult fed the low D3 diet without KI supplementation. Bone ash of poult fed the low D3 diet without KI supplementation was lower than that of poult fed the Control diet. The effect of supplemental KI in the low D3 diet on bone ash was not consistent. No effect of supplemental KI to the low D3 diets on bone ash was detected when the supplemental level was 0.37 or 5.33 mg/kg. whereas an adverse effect was detected when KI was supplemented at the level of 2.28 mg/kg.

**DISCUSSION**

Although substantial research has been conducted to study leg weakness of turkeys, the causes of leg weakness are not well understood. In the literature, there were several case reports in which leg weakness of turkeys was termed as "field rickets." This kind of leg weakness often occurred at very young ages, and the known nutritional factors or infectious diseases were not the causes. Walser *et al.* (1980) reported two cases of field rickets in poult at 16 to 20 d of age. The incidences of field rickets affected about 20% of the flocks, and
affected poults were reluctant to move, leaned forward and supported their weight with their wings. Bones of affected poults were soft, fragile and hypomineralized. The growth plates of tibias were thickened. In a subsequent study, Olson et al. (1981) observed that affected poults had decreased plasma phosphorus and increased alkaline phosphatase activity. Plasma 24, 25-dihydroxy D3 levels in affected poults were lower than normal values. Analysis of the rations of affected poults, however, showed that adequate concentrations of dietary calcium, phosphorus and D3 were present. Further supplementation of calcium and/or phosphorus did not prevent occurrence of the problem. Riddell (1983) reported that most outbreaks of field rickets started when poults were between 10 to 14 d of age. Skeletal lesions of affected poults were characteristics of a vitamin D3 or calcium deficiency. On the basis of chemical analysis, adequate calcium, phosphorus and D3 were present in diets. Biological tests implicated, however, that the feed was the causative factor of the field rickets. All these diets that caused leg weakness were fed in pelleted form.

The leg weakness, observed in the research reported here, had some similarities to field rickets. The problem occurred when poults were only about 7 d of age, and a high percentage of poults were affected. The poults were fed pelleted diets. The affected poults were reluctant to move, often sitting on their hocks. Gross and histological examination of tibias showed the prevalence of overdeveloped growth plates and vitamin D deficiency symptoms. Plasma 25-OH D3 levels were only about half of the values observed in normal poults of the same age (Sell and Horst, 1994, unpublished data). However, poults fed the basal diet in mash form did not show any signs of leg weakness. When the experimental diets were switched to mash form from Day 8, poults began to recover from the leg weakness. The cause of the leg weakness in this research was difficult to pin down, but it was unlikely that
the leg weakness in Experiment 1 was caused by D3 deficiency. The D3 stores from the
residual yolks and body tissues should not have been depleted at such an early age as Day 7.
A low D3 diet such as 275 IU of D3/kg in Experiment 3 did not result in similar leg
weakness. It was speculated that the pelleting process caused some chemical changes in the
nutrients or other substances in the diets which, in turn, that resulted in the high incidence of
leg weakness.

Feeding diets containing 1.650 IU of D3/kg tended to increase plasma prothrombin
time on Day 14 but not on Day 7. The absence of the effect of D3 on prothrombin time on
Day 7 perhaps reflected the fact that poultys obtained some D3 and vitamin K from the
residual yolks during the first few days posthatch. The increased prothrombin time at Day 14
observed with poultys fed 1.650 IU of D3/kg is consistent with the anticoagulant effect of D3
reported by Koyama et al. (1998). These authors reported that inclusion of 1,25-(OH)2 D3 in
culture media had an anticoagulant effect in vitro through downregulating tissue factor
expression and upregulating thrombomodulin expression. Tissue factor is one of the
accessory factors to enhance the activity of the Stuart factor (factor X) by activating
proconvertin (factor VII) in the extrinsic pathway of blood coagulation (Voet and Voet,
1995). Thrombomodulin specifically binds to thrombin so as to convert it to a form with
decreased ability to catalyze clot formation but with increased capability to activate protein
C, which is a plasma protein with the ability to limit blood clotting (Voet and Voet, 1995).

Plasma K1 and MK-4 concentrations generally increased as the dietary concentration
of K1 increase. However, Plasma K1 and MK-4 concentrations increased curvilinearly at
Day 7 (Figure 1) in Experiment 1, whereas increased linearly at Day 14 in Experiment
1(Figure 2) and in Experiment 2 (Figure 3). This curvilinear increase of plasma K1 and MK-
4 on Day 7 in Experiment 1 was probably because some of the poults might be off feed for a while as a consequence of the leg problem. It has been indicated that plasma K1 is cleared from the circulation very quickly. Shearer et al. (1972) reported that 2 and 8 hours after administration only about 10 and 1% of the initial radioactivity remained in the plasma, respectively after either oral or intravenous administration of radio-active labelled K1.

The adverse effects of vitamin K antagonists, such as warfarin, on rapidly growing bones were well documented. Warfaringiven to young rats causes premature growth plate closure (Shearer, 1995). A parallel in humans to these effects is the fatal Warfarinsyndrome, characterized by the excess calcification of the epiphyses and irregular growth of the facial and long bones (Shearer, 1995). However, The effect of vitamin K antagonists on mature bone is inconclusive. Some researchers showed that long-term intake of a vitamin K antagonist reduced bone mineral content (Fiore et al., 1990; Fesch et al., 1993) whereas others did not (Rosen et al., 1993). Information on the effect of vitamin K on bone is limited. Lavelle et al. (1994) reported that bone histological structure and bone ash were not affected in chickens although these chickens were progeny of hens fed a vitamin K deficient diet and were raised on vitamin K deficient diets themselves. However, Fleming et al. (1998) reported that dietary supplementation of 10 mg of menadione/kg from menadione nicotinamide bisulfite significantly reduced cancellous bone loss in the proximal tarsometatarsus of laying hens between 15 to 25 weeks of age. It was observed in the current research that supplementation of K1 had no effect on the histological structure of bone, or the incidence or severity of the leg weakness. However, supplementation of 2.0 mg of K1/kg significantly increased bone ash in poults during a recovery from leg weakness compared with poults fed a
diet without K1 supplementation. An interpretation of this observation is difficult because the causes of the leg weakness remained undetermined.

Compared with poults fed a diet containing adequate D3 and K1, poults fed low D3 diet without K1 had increased plasma alkaline phosphatase activity and decreased inorganic phosphorus levels. These symptoms are some of the signs of D3 deficiency. When poults were fed the low D3 diet supplemented with a low level of K1 (0.37 or 2.28 mg/kg), plasma alkaline phosphatase activity decreased or tended to decrease, and plasma inorganic phosphorus levels were increased statistically or numerically compared with that of poults fed the low D3 diet without supplementation of K1. An increase in feed intake in response to supplementation of K1 to the low D3 diet probably accounted for some, but might not all, of the changes in plasma alkaline phosphatase and inorganic phosphorus. For example, supplementation of the low D3 diet with 0.37 mg of K1/kg resulted in an increase of feed intake by 7.9% during the period of Days 7 to 13, whereas plasma alkaline phosphatase activity was decreased by 28% and plasma inorganic phosphorus level increased by 39.5% compared with poults fed the low D3 diet without K1. The decrease of plasma alkaline phosphatase activity and the increase of inorganic phosphorus, resulted by supplementation of K1 at a level of 0.37 or 2.88 mg/kg in the low D3 diet, probably also related to the suggestion that status of vitamin K-dependent carboxylation of vitamin D3 receptor might regulate its binding to DNA (Sergee and Norman, 1992), and therefore, the biological functions of D3. Sergee and Norman (1992) reported that VDR underwent γ-carboxylation in the presence of K1 in vitro, and 15 to 25% of Glu residues in the VDR were carboxylated in vivo.
REFERENCES


TABLE 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal (48%CP)</td>
<td>567.03</td>
</tr>
<tr>
<td>Corn starch</td>
<td>267.70</td>
</tr>
<tr>
<td>Sunflower meal</td>
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</tr>
<tr>
<td>Stripped corn oil</td>
<td>39.02</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>23.83</td>
</tr>
<tr>
<td>Lime stone</td>
<td>12.67</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>3.00</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>3.00</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>2.00</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.50</td>
</tr>
<tr>
<td>BMD</td>
<td>0.25</td>
</tr>
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</table>

Calculated nutrient composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME. kcal/kg</td>
<td>2850</td>
</tr>
<tr>
<td>CP. %</td>
<td>28.5</td>
</tr>
<tr>
<td>TSAA. %</td>
<td>1.05</td>
</tr>
<tr>
<td>Lysine. %</td>
<td>1.69</td>
</tr>
<tr>
<td>Calcium. %</td>
<td>1.20</td>
</tr>
<tr>
<td>Available phosphorus. %</td>
<td>0.60</td>
</tr>
<tr>
<td>K1. mg/kg</td>
<td>less than 0.02³</td>
</tr>
</tbody>
</table>

¹ Provided per kg diet: vitamin A (retinyl acetate), 8190 IU; dl-α-tocopherol acetate, 30 IU; vitamin D3 (cholecalciferol), 1,650 IU; vitamin B12, 15.9 µg; pantothenic acid, 13.2 mg; niacin, 75 mg; choline, 5090 mg; folic acid, 2.4; biotin 0.27 mg; pyridoxine HCl 6.0 mg; thiamine mononitrate 2.4

² Supplied per kg diet: Mn, 70 mg; Zn, 40 mg; Fe, 37; Cu, 6 mg; Se, 0.15 mg; NaCl (I), 2.60 g.

³ Bacitracin dimethylene salicylate.

³ The detection limit of phylloquinone analysis.
## TABLE 2. Effect of dietary K1 concentrations on body weight gain, feed intake and feed efficiency in young poults (Exp. 1)

<table>
<thead>
<tr>
<th>Supplemental K1 (mg/kg)</th>
<th>Body Weight 7 d (g)</th>
<th>Weight Gain 14 d</th>
<th>Feed Intake 7-14 d (g/poult)</th>
<th>Feed Efficiency 1-7 d, 7-14 d, 1-14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>177</td>
<td>120</td>
<td>138</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>181</td>
<td>123</td>
<td>144</td>
<td>1.16</td>
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<td>183</td>
<td>127</td>
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<td>1.17</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>126</td>
<td>147</td>
<td>1.17</td>
</tr>
<tr>
<td>SEM</td>
<td>3.6</td>
<td>3.5</td>
<td>3.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>0.27 0.38</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.33 0.92</td>
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</table>

1 Feed to gain ratio
TABLE 3. Effect of dietary K1 concentrations on bone ash and plasma 25-OH vitamin D3 levels in young poults (Exp. 1)

<table>
<thead>
<tr>
<th>Supplemental K1 (mg/kg)</th>
<th>Bone ash</th>
<th></th>
<th>25-OH vitamin D3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 d</td>
<td>14 d</td>
<td>7 d</td>
</tr>
<tr>
<td>0</td>
<td>30.4</td>
<td>34.3</td>
<td>6.56</td>
<td>6.65</td>
</tr>
<tr>
<td>0.5</td>
<td>31.2</td>
<td>ND</td>
<td>4.84</td>
<td>8.84</td>
</tr>
<tr>
<td>1.0</td>
<td>31.3</td>
<td>ND</td>
<td>7.06</td>
<td>7.59</td>
</tr>
<tr>
<td>2.0</td>
<td>31.0</td>
<td>36.9</td>
<td>8.92</td>
<td>8.06</td>
</tr>
<tr>
<td>0 (mash)</td>
<td>ND</td>
<td>37.9</td>
<td>ND</td>
<td>11.70</td>
</tr>
<tr>
<td>SEM</td>
<td>0.29</td>
<td>0.40</td>
<td>1.63</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Source of variation: Treatments 0.26 0.05 0.33 0.33

1. ND stands for not determined
2. The data was analyzed without inclusion of data from poults fed the basal diet in mash form from Day 1.
TABLE 4. Effect of dietary K1 concentrations on prothrombin time and plasma K1 and menaquinone-4 concentrations in young poults (Exp. 1)

<table>
<thead>
<tr>
<th>Supplemental K1 (mg/kg)</th>
<th>Prothrombin time (Seconds)</th>
<th>Plasma K1 (ng/mL)</th>
<th>Plasma MK-4 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 d</td>
<td>14 d</td>
<td>7 d</td>
</tr>
<tr>
<td>0</td>
<td>437</td>
<td>356</td>
<td>0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>155</td>
<td>156</td>
<td>4.25</td>
</tr>
<tr>
<td>1.0</td>
<td>147</td>
<td>116</td>
<td>4.86</td>
</tr>
<tr>
<td>2.0</td>
<td>121</td>
<td>114</td>
<td>2.95</td>
</tr>
<tr>
<td>SEM</td>
<td>36.4</td>
<td>34.7</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Sources of variation

<table>
<thead>
<tr>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>0.053</td>
</tr>
<tr>
<td>0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>0.295</td>
<td>0.0005</td>
</tr>
<tr>
<td>0.002</td>
<td>0.011</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0003</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Figure 1. Effect of dietary K1 concentrations on plasma phylloquinone and menaquinone-4 concentrations at 7 d, Experiment 1.
Figure 2. Effect of dietary phylloquinone on plasma phylloquinone and menaquinone-4 concentrations at 14 d. Experiment 1.
### TABLE 5. Effect of dietary concentrations of vitamin D3 (D3) and K1 on weight gain, feed intake and feed efficiency in poults (Exp. 2)

<table>
<thead>
<tr>
<th>Supplemental Vitamin</th>
<th>Body weight (g/poult)</th>
<th>Gain 7-14d (g/poult)</th>
<th>Feed Intake 7-14d (g/poult)</th>
<th>Feed efficiency</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 (IU)(mg/kg)</td>
<td>160 385</td>
<td>96 225 321</td>
<td>95 276 372</td>
<td>0.99 1.23 1.15</td>
<td></td>
</tr>
<tr>
<td>0.45 1650</td>
<td>160 385</td>
<td>96 225 321</td>
<td>95 273 368</td>
<td>0.99 1.21 1.14</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>163 388</td>
<td>100 224 324</td>
<td>97 275 371</td>
<td>0.98 1.23 1.14</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>162 386</td>
<td>98 224 322</td>
<td>96 274 369</td>
<td>0.98 1.22 1.14</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>161 389</td>
<td>97 228 324</td>
<td>96 278 374</td>
<td>0.96 1.22 1.15</td>
<td></td>
</tr>
<tr>
<td>0.45 550</td>
<td>150 366</td>
<td>86 215 302</td>
<td>95 282 377</td>
<td>1.10 1.31 1.25</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>160 376</td>
<td>96 216 312</td>
<td>96 271 367</td>
<td>1.00 1.26 1.17</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>163 381</td>
<td>99 218 316</td>
<td>100 272 372</td>
<td>1.02 1.25 1.17</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.6 5.1</td>
<td>2.4 3.5 5.5</td>
<td>1.8 4.9 6.4</td>
<td>0.01 0.01 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Sources of variation: D3, K1, D3 * K1

Probabilities: 0.0001 0.0001 0.0007 0.0001 0.0001

1Feed to gain ratio.
TABLE 6. Effect of dietary levels of vitamin D3 (D3) and K1 on prothrombin time, plasma K1 levels and bone ash (Exp. 2)

<table>
<thead>
<tr>
<th>Supplemental D3</th>
<th>Prothrombin time</th>
<th>Plasma K1</th>
<th>Bone ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 d</td>
<td>14 d</td>
<td>14 d</td>
</tr>
<tr>
<td>(Seconds)</td>
<td>(ng/ml)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>146</td>
<td>138</td>
<td>0.06</td>
</tr>
<tr>
<td>1650</td>
<td>0.45</td>
<td>135</td>
<td>142</td>
</tr>
<tr>
<td>1.0</td>
<td>132</td>
<td>115</td>
<td>3.79</td>
</tr>
<tr>
<td>2.0</td>
<td>130</td>
<td>113</td>
<td>7.87</td>
</tr>
<tr>
<td>0.1</td>
<td>149</td>
<td>105</td>
<td>0.18</td>
</tr>
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<td>0.45</td>
<td>140</td>
<td>94</td>
<td>1.75</td>
</tr>
<tr>
<td>550</td>
<td>1.0</td>
<td>124</td>
<td>91</td>
</tr>
<tr>
<td>2.0</td>
<td>122</td>
<td>94</td>
<td>8.03</td>
</tr>
<tr>
<td>SEM</td>
<td>13.5</td>
<td>11.5</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Source of variation | Probabilities |
---------------------|---------------|
D3                   | 0.99          | 0.07          | 0.92 | 0.96 |
K1                   | 0.59          | 0.20          | 0.002 | 0.61 |
D3 * K1              | 0.99          | 0.57          | 0.96 | 0.10 |
TABLE 7. Effect of dietary supplementation of K1 on body weight gain, feed intake and feed efficiency in poult (Exp. 3)

<table>
<thead>
<tr>
<th>Supplemental</th>
<th>Body weight</th>
<th>Weight Gain</th>
<th>Feed intake</th>
<th>Feed efficiency¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 6 d</td>
<td>13 d 19 d</td>
<td>1-6 d 7-13 d 1-19 d</td>
<td>1-6 d 7-13 d 1-19 d</td>
<td>1-6 d 7-13 d 1-19 d</td>
</tr>
<tr>
<td>Control²</td>
<td>140 330 545</td>
<td>78 190 483</td>
<td>82 233 627</td>
<td>1.06 1.29 1.30</td>
</tr>
<tr>
<td>0</td>
<td>136 289 469</td>
<td>75 153 408</td>
<td>76 204 540</td>
<td>1.02 1.34 1.32</td>
</tr>
<tr>
<td>0.37³</td>
<td>133 310 500</td>
<td>73 177 440</td>
<td>74 220 576</td>
<td>1.02 1.25 1.31</td>
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<tr>
<td>2.28</td>
<td>138 307 513</td>
<td>77 169 453</td>
<td>78 213 582</td>
<td>1.02 1.26 1.29</td>
</tr>
<tr>
<td>5.37</td>
<td>141 304 483</td>
<td>78 164 420</td>
<td>75 213 551</td>
<td>0.96 1.30 1.31</td>
</tr>
<tr>
<td>SEM</td>
<td>2.8 5.8 10.7</td>
<td>3.8 4.2 10.5</td>
<td>2.2 3.9 12.6</td>
<td>0.03 0.03 0.02</td>
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</tbody>
</table>

Source of variation ----------------- Probabilities -----------------

| Treatment | 0.35 0.03 0.002 | 0.64 0.0002 0.001 | 0.16 0.002 0.002 | 0.37 0.05 0.57 |

¹ Feed to gain ration
² The diet contained 1650 IU vitamin D3 and 2.0 mg K1 per kilogram.
³ Milligrams of K1 per kilogram of diets that contained 275 IU of vitamin D3
**TABLE 8. Effect of dietary supplemental K1 on plasma parameters related to bone metabolism and bone ash in poult s fed on low vitamin D3 diets (Exp. 3)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Alkaline phosphatase</th>
<th>Ca</th>
<th>P</th>
<th>Bone ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 6</td>
<td>Day 13</td>
<td>Day 19</td>
<td>Day 13</td>
</tr>
<tr>
<td>Control</td>
<td>79.3</td>
<td>70.0</td>
<td>54.6</td>
<td>7.2</td>
</tr>
<tr>
<td>0</td>
<td>75.3</td>
<td>103.9</td>
<td>91.5</td>
<td>8.6</td>
</tr>
<tr>
<td>0.37</td>
<td>84.1</td>
<td>74.3</td>
<td>67.7</td>
<td>8.9</td>
</tr>
<tr>
<td>2.28</td>
<td>78.6</td>
<td>74.9</td>
<td>81.4</td>
<td>8.9</td>
</tr>
<tr>
<td>5.33</td>
<td>84.7</td>
<td>80.6</td>
<td>69.8</td>
<td>8.5</td>
</tr>
<tr>
<td>SEM</td>
<td>5.68</td>
<td>6.56</td>
<td>8.36</td>
<td>1.11</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.77 0.018 0.063 0.77 0.07 0.06 0.08 0.002</td>
</tr>
</tbody>
</table>

1. The diet contained 1650 IU vitamin D3 and 2.0 mg K1 per kg.
2. Milligram of K1 in diets containing 275 IU vitamin D3 per kg.
DIETARY VITAMIN K1 REQUIREMENT OF YOUNG TURKEY POUILTS

A paper to be submitted to Poultry Science

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1Department of Animal Science, Iowa State University, Ames, IA

2Department of Biochemistry, University of Wisconsin, Madison,

ABSTRACT

In a preliminary experiment (Exp.), the inclusion of vitamin K1 (K1) at a dietary level of 0.1 mg/kg was as effective as 1 or 2 mg/kg in reducing plasma prothrombin time (PT). To obtain an estimate of the dietary K1 requirement of poults, three additional Exp. were conducted. In Exp. 1, a 2x3 factorial arrangement was used with two concentrations of neomycin (0 or 75 mg/L) in the drinking water and three concentrations of K1 (0, 0.1 or 0.5 mg/kg) in diets. Each treatment was fed to two pens with eight poults per pen, and individual poults were used as experimental units for the determination of PT and prothrombin concentration (PC) in plasma. PT and PC in plasma were not influenced by inclusion of neomycin in drinking water. However, PT was reduced to a nadir and PC in plasma increased to a plateau when diets contained 0.1 mg of K1/kg. Dietary K1 concentrations tested in Exp. 2 were 0, 0.08, 0.31 or 0.44 mg/kg and in Exp. 3 were 0, 0.1, 0.25, 0.5 or 2.0 mg/kg. A similar protocol as that of Exp. 1 was used in these two Exp. The results of Exp. 2 indicated that the dietary K1 requirement was 0.079 mg, based on the influence of dietary K1 on PT. The K1 requirement was estimated, on the basis of PT and PC, to be 0.099 and 0.13 mg/kg, respectively, in Exp. 3. Results of this research show that the dietary K1 requirement of
young turkeys is in the range of 0.079 to 0.13 mg/kg, and ingestion of neomycin did not affect estimates of the requirement.

Keywords: vitamin K1 requirement, prothrombin time, prothrombin concentration, menaquinone-4, turkey.

INTRODUCTION

Vitamin K is a general term used for a series of compounds with a structure of polyisoprenoid-substituted naphthoquinone. It is traditionally classified into K1 (phytolquinone) and K2 (menaquinones), according to their origination from plants or bacteria, respectively. A group of synthetic menadione derivatives such as menadione sodium bisulfite, etc. also have biological activity of vitamin K. The K vitamins are essential cofactors for microsomal vitamin K-dependent carboxylases, which catalyze a post-translational conversion of glutamyl residues in the nascent precursors to γ-carboxyglutamyl residues (Gla) in their respective mature proteins (Suttie, 1985). Gla proteins have been shown to be actively synthesized in various tissues such as liver, kidneys (Booth, 1997), bone (Hauschka and Reid, 1978) and cartilage (Luo et al., 1995). The Gla residues in these Gla proteins are essential for their full and wide variety of biological functions (Will et al., 1992).

The vitamin K requirement of animals, such as turkeys, is met by a combination supply from the diet and from microbial synthesis in the gastrointestinal tract. Intake of broad-spectrum antibiotics, such as neomycin, has been shown to reduce the production of menaquinones in the intestinal tract by microbial flora. Frick et al. (1967) reported that starvation combined with an intake of broad-spectrum antibiotic resulted in vitamin K
deficiency in humans whereas starvation alone did not. This observation was confirmed by Udall (1965).

Existence of a dietary requirement of vitamin K of chicks has been recognized for a long time (Almquist, 1939). However, there is very little information on the vitamin K requirement of turkeys. On the basis of prothrombin times of young turkeys, Griminger (1957) reported that, when menadione sodium bisulfite or menadione was used as sources of vitamin K, the requirements were 1.1 and 1.76 mg/kg diet, respectively. It should be noted that plasma prothrombin time is a non-specific index of vitamin K status. Quick (1959) stated “when the test was developed, thromboplastin and prothrombin were accepted as the two basic agents needed for the generation of thrombin ... .” However, it has been documented that there are many factors other than thromboplastin and prothrombin (Voet and Voet, 1995) involved in the conversion of prothrombin to thrombin. Kindberg and Suttie (1989) stated that one-stage prothrombin time was a less sensitive index of vitamin K status than some other methods, such as plasma prothrombin level.

The dietary vitamin K requirement of young turkeys listed by National Research Council (1994) is actually a menadione sodium bisulfite requirement based on the data of Griminger (1957) and no requirement for vitamin K1 is given. Griminger and Donis (1960) reported that the K1 requirement of chicks fed a purified diet was 1 mg/kg of diet. Nelson and Norris (1958) estimated the K1 requirement for chicks varied from 0.4 to 0.6 mg/kg diet. This latter observation was confirmed by Nelson and Norris (1960). Results of previous research in our laboratory, which involved use of different dietary concentrations of K1, indicated that young turkeys might require less that 0.1 mg of K1/kg. The objective of the
research reported here was to determine the K1 requirement of young turkeys by using
plasma prothrombin time and plasma prothrombin concentration as primary criteria.

MATERIALS AND METHOD

Diets and Treatments

In Experiment 1, a 2 × 3 factorial arrangement was used with two concentrations of
neomycin (0 or 75 mg/L) in the drinking water and three concentrations of K1 (0, 0.1, 0.5
mg/kg) supplemented to the basal diet shown in Table 1. Each treatment was fed to two pens
with eight poults per pen from Days 1 to 7. Diets were fed in mash form. Dietary K1
concentrations tested in Experiment 2 were 0, 0.08, 0.31, 0.44 or 1.8 mg/kg (by analysis)
based on the diet shown in Table 1. Each treatment was fed to two pens with ten poults per
pen from Days 1 to 7. In Experiment 3, dietary treatments consisted of 0, 0.1, 0.25, 0.5 or 2.0
mg of K1/kg supplemented to the basal diet shown in Table 1. Each treatment was fed to two
pens with eight poults per pen from Days 1 to 7.

Animals

One-day-old Nicholas male poults were obtained from a commercial hatchery, and
were randomly assigned to each treatment pen. The poults were kept in heated,
thermostatically controlled batteries with raised wire floors. Water and feed were provided
for ad libitum consumption. Water troughs were cleaned daily to reduce the possibility of
production of menaquinones by microbial fermentation. Excreta in feeders also were removed
daily. Droppings under the battery pens were removed frequently to minimize coprophagy. In
Exp 1, body weight and feed consumption data were recorded when poults were 7 d of age,
then, two blood samples were obtained from each poult of every pen. Citrate was used as
anticoagulant for the blood samples from which plasmas were used to determine prothrombin times. Heparin was used in blood samples from which plasmas were used for determination of plasma prothrombin concentrations. In Exp 2, body weight and feed consumption were recorded when poults were 7 d of age, then, one blood sample was obtained from each poult of every pen. A similar protocol as described in Exp 1 was used for plasma obtainment to determine prothrombin time. In Exp 3, body weight and feed consumption were recorded when poults were 7 d of age, then, two blood samples were obtained from each poult of every pen. A similar protocol as described in Exp 1 was used to obtain plasma and to determine prothrombin time and plasma prothrombin concentration. Plasma obtained using heparin as anticoagulant also was analyzed to determine plasma K1 and menaquinone-4 (MK-4) concentrations.

Analytical Procedures

Plasma K1 and MK-4 levels were determined by colleagues at University of Wisconsin1 using the high performance liquid chromatography (HPLC) method of Haroon et al. (1986). The following is a brief description of the method. Plasma samples were extracted through a liquid-phase with a ratio of plasma, water, ethanol, hexane at 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. K1 and MK-4 were analyzed on a 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

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1 Dr. Suttie's group, Department of Biochemistry, University of Wisconsin.
fluorescent hydroquinones. The mobile solvent used was 15% methylene chloride in methanol with 5mL/L reductive solvent additive. The rate of solvent flow was set to 1.0 mL/min, and pressure was 7757 cm mercury. Excitation was performed at 244 nm and emission was monitored at 418 nm. The internal standard used was di-hydrophylloquinone.

One-stage prothrombin time was determined manually using a commercial thromboplastin preparation, Simplastin® Excel®. The plasma prothrombin concentration was determined by an amidolysis assay (Shah et al., 1984). The principle of the method was briefly: plasma prothrombin was activated to thrombin with a commercial preparation of thromboplastin, Simplastin® Excel®. The thrombin produced catalyzed an amidolysis reaction of a specific chromogenic peptide substrate, Pefachrome TH®, to release p-nitroaniline. Concentration of p-nitroaniline was determined spectrophotometrically. A standard curve was developed with thrombin (EC 3.4.21.5)⁴

**Statistical Analysis**

Data were analyzed using the General Linear Model (SAS Institute, 1996). When appropriate, a regression analysis (the one-slope or two-slope model, broken line method) of Robbins (1986), was used to estimate the K1 requirement. Single degree of freedom contrasts were used to determine differences among treatment means. Significance was declared when the probability was less than 5%, and a trend was announced when the probability was less than 10%.

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² Organon Teknika Corp., Box 15969 Durham, NC 27704-0969
³ Centerchem, Inc., 225 High Ridge Road, Stamford, CT 06905.
⁴ Sigma Diagnostics Inc., St. Louis, MO 63178.
RESULTS

Experiment 1

Inclusion of neomycin in the drinking water significantly increased body weight gain. However, inclusion of neomycin in the drinking water had no effect on feed intake and feed efficiency (Table 2). Plasma prothrombin time and prothrombin concentration were also not influenced by the inclusion of neomycin in water (Table 3). Dietary K1 had no effect on body weight gain, feed intake and efficiency of feed utilization (Table 2). However, dietary supplemental K1 at the level of 0.1 mg/kg significantly reduced prothrombin time and increased prothrombin concentration compared with those of poults fed the basal diet (Table 3). Feeding the diet that contained 0.5 mg of K1/kg did not decrease plasma prothrombin time, nor increase in prothrombin concentration beyond values obtained with 0.1 mg of K1/kg. No interaction between neomycin and dietary K1 was detected for any parameters measured.

Experiment 2

Dietary K1 had no effect on body weight gain, feed intake and efficiency of feed utilization (Table 4). However, plasma prothrombin time was decreased by dietary K1 supplementation (Table 5). Supplementation of 0.08 mg of K1/kg in the basal diet was as effective as supplementation with 0.31, 0.44 or 1.8 mg of K1/kg in reduction of prothrombin time. The estimated dietary K1 requirement of poults using the broken-line method was 0.079 mg/kg, based on prothrombin time.
Experiment 3

Body weight gain, feed intake and feed efficiency were not influenced by dietary supplemental of K1 (Table 6). Dietary K1, however, significantly affected plasma prothrombin time and plasma prothrombin concentration. Supplementation with 0.1 mg of K1/kg tended to increase plasma prothrombin concentration and decrease plasma prothrombin time significantly compared with poults fed the basal diet (Table 7). Plasma prothrombin time was further decreased by supplementation of K1 at the level of 0.5 or more mg of K1/kg compared to that of poults fed a diet supplemented with 0.1 mg of K1/kg (Table 7). Supplementation of diets with 0.25, 0.5 or 2.0 mg of K1/kg, however, had no additional effect on plasma prothrombin concentration (Table 7 and Figure 3). The K1 requirements estimated using the broken line method were 0.099 mg and 0.13 mg/kg, based on plasma prothrombin time and prothrombin concentration, respectively. The concentrations of plasma K1 increased linearly as the dietary K1 level increase. The concentration of plasma menaquinone-4 increased in a similar fashion as described above for the plasma K1 concentration as the dietary supplemental K1 level increased.

DISCUSSION

It was observed in the research reported here that inclusion of neomycin in drinking water for 7 days had no effect on plasma prothrombin time and prothrombin concentration. Frick et al. (1967), however, reported that an intake of broad-spectrum antibiotic induced vitamin K deficiency in human beings when there was no dietary vitamin K source ingested. This discrepancy between the effect of ingestion of broad-spectrum antibiotics on the status of vitamin K observed in the research reported here and those observed by Frick et al. (1967)
might indicate a difference in the importance of the bacterially synthesized menaquinones in
the GI tract of turkeys and human beings as a source of vitamin K to meet their requirements.
This discrepancy might be a result of the length difference of broad-spectrum antibiotics
intake. Frick et al. (1967) reported that vitamin K deficiency developed in human being after
21-28 days of intake of broad-spectrum antibiotics. A long period of broad-spectrum
antibiotic intake also might result in substantial depletion of vitamin K stores of experimental
animals whereas a short period of administration as done in the study reported here might not
achieve sufficient depletion. Conly (1992) stated that bacterially synthesized menaquinones,
particularly those synthesized in the ileum, can and do contribute toward vitamin K
requirements in humans in maintaining coagulation homeostasis, especially during periods of
episodic dietary lack of the vitamin.

In the research reported here, the estimated K1 requirement was 0.099 mg/kg based
on the influence of dietary K1 on plasma prothrombin concentration, which is a more
specific and sensitive indicator of vitamin K status than prothrombin time (Kindberg and
Suttie, 1989). This estimation of K1 requirement based on the plasma prothrombin
concentration was close to the K1 requirements obtained by the using of the broken line
method to analyze data of prothrombin times in Exp. 2 and 3. The estimated K1 requirements
using the latter method were 0.079 and 0.13 mg of K1/kg, respectively. Griminger (1957)
reported that the requirements of young turkeys were 1.1 or 1.75 mg/kg, respectively, when
menadione sodium bisulfite or menadione requirement were used as vitamin K source. There
are no data available on the potency ratio between menadione sodium bisulfite and K1 or
menadione and K1 for turkeys. Thus it is impossible to estimate the K1 requirement of
turkeys based on the data reported by Griminger (1957). Griminger and Donis (1960)
reported that the K1 requirement of chicks fed a purified diet was 1 mg of K1/kg diet. Nelson and Norris (1958) estimated the K1 requirement for chicks varied from 0.4 to 0.6 mg of K1/kg diet. This latter observation was confirmed by Nelson and Norris (1960), who reported that the K1 requirement of chicks were 0.524 and 0.528 mg of K1/kg diets, respectively. In all the research on the vitamin K1 requirement of chicks mentioned above, prothrombin time was used as the only criteria. It should be noted that plasma prothrombin time is a non-specific index of vitamin K status and also is not a very sensitive index of vitamin K status (Kindberg and Suttie 1989). Using more sensitive and specific indicators of vitamin K, Suttie et al. (1988) reported that the K1 requirement of a human was approximately 1 μg/kg body wt/d. If it assumed that the K1 requirement per unit of metabolic body weight was same for human beings and young turkeys, the calculated K1 requirement of 7- to 14-d-old turkeys should be less than 0.01 mg K1/kg diet.

Based on the influence of dietary K1 on plasma prothrombin concentration and prothrombin time, the estimated dietary K1 requirement of young turkeys is in the range of 0.079 to 0.13 mg/kg. and ingestion of neomycin did not affect estimates of the requirement.

REFERENCES


TABLE 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal (48%CP)</td>
<td>567.03</td>
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<tr>
<td>Corn starch</td>
<td>267.70</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>80.00</td>
</tr>
<tr>
<td>Stripped corn oil</td>
<td>39.02</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>23.83</td>
</tr>
<tr>
<td>Lime stone</td>
<td>12.67</td>
</tr>
<tr>
<td>Vitamin premix ( ^1 )</td>
<td>3.00</td>
</tr>
<tr>
<td>Mineral premix ( ^2 )</td>
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</tr>
<tr>
<td>DL-Methionine</td>
<td>2.00</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.50</td>
</tr>
<tr>
<td>BMD ( ^3 )</td>
<td>0.25</td>
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Calculated nutrient composition

<table>
<thead>
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<th>Nutrient</th>
<th>Content</th>
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</thead>
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<tr>
<td>ME. kcal/kg</td>
<td>2850</td>
</tr>
<tr>
<td>CP. %</td>
<td>28.5</td>
</tr>
<tr>
<td>TSAA. %</td>
<td>1.05</td>
</tr>
<tr>
<td>Lysine. %</td>
<td>1.69</td>
</tr>
<tr>
<td>Calcium. %</td>
<td>1.20</td>
</tr>
<tr>
<td>Available phosphorus. %</td>
<td>0.60</td>
</tr>
<tr>
<td>K1. mg/kg</td>
<td>less than 0.02( ^4 )</td>
</tr>
</tbody>
</table>

\( ^1 \) Provided per kg diet: vitamin A (retinyl acetate), 8190 IU; dl-\( ^\alpha \)-tocopheryl acetate, 30 IU; vitamin D3 (cholecalciferol), 1.650 IU, vitamin B12, 15.9 \( ^\mu \)g; pantothenic acid, 13.2 mg; niacin, 75 mg; choline, 5090 mg; folic acid, 2.4; biotin 0.27 mg; pyridoxine HCl 6.0 mg; thiamine mononitrate 2.4

\( ^2 \) Supplied per kg diet: Mn, 70 mg; Zn, 40 mg; Fe, 37 mg; Cu, 6mg; Se, 0.15 mg; NaCl (I), 2.60 g.

\( ^3 \) Bacitracin dimethylene salicylate.

\( ^4 \) Detection limit for vitamin K1.
TABLE 2. Effect of neomycin and dietary supplemental K1 on body weight gain, feed Intake and feed utilization in young turkey poults (Experiment 1)

<table>
<thead>
<tr>
<th>Neomycin (mg/L)</th>
<th>Supplemental K1 (mg/kg)</th>
<th>Day 7</th>
<th>Day 1-7</th>
<th>Day 1-7</th>
<th>Day 1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>143.3</td>
<td>81.9</td>
<td>73.4</td>
<td>0.869</td>
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<tr>
<td>0</td>
<td>0.1</td>
<td>149.8</td>
<td>91.6</td>
<td>91.2</td>
<td>0.998</td>
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<tr>
<td>0.5</td>
<td>15.4</td>
<td>91.7</td>
<td>89.9</td>
<td>0.980</td>
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<tr>
<td>0</td>
<td>0.0</td>
<td>133.3</td>
<td>74.1</td>
<td>76.2</td>
<td>1.027</td>
</tr>
<tr>
<td>75</td>
<td>0.1</td>
<td>135.1</td>
<td>76.5</td>
<td>80.4</td>
<td>1.051</td>
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<tr>
<td>0.5</td>
<td>138.5</td>
<td>79.9</td>
<td>86.1</td>
<td>1.079</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>5.23</td>
<td>5.44</td>
<td>0.064</td>
<td>5.68</td>
<td></td>
</tr>
</tbody>
</table>

Means of main effect

<table>
<thead>
<tr>
<th>Neomycin</th>
<th>Day 7</th>
<th>Day 1-7</th>
<th>Day 1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>135.6</td>
<td>76.9</td>
<td>80.9</td>
</tr>
<tr>
<td>75</td>
<td>147.8</td>
<td>89.4</td>
<td>84.8</td>
</tr>
<tr>
<td>0</td>
<td>138.3</td>
<td>79.6</td>
<td>74.8</td>
</tr>
<tr>
<td>K1 0.1</td>
<td>142.5</td>
<td>84.1</td>
<td>85.8</td>
</tr>
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<td>0.5</td>
<td>144.5</td>
<td>85.9</td>
<td>88.0</td>
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Sources of variation

<table>
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<th>Source</th>
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<th>Day 1-7</th>
<th>Day 1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin</td>
<td>0.03</td>
<td>0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>K1</td>
<td>0.52</td>
<td>0.53</td>
<td>0.44</td>
</tr>
<tr>
<td>Neomycin \times K1</td>
<td>0.90</td>
<td>0.92</td>
<td>0.53</td>
</tr>
</tbody>
</table>

\(^{1}\)Feed to gain ratio.
TABLE 3. Effect of neomycin and dietary supplemental K1 on plasma prothrombin time and prothrombin concentration (Experiment I)

<table>
<thead>
<tr>
<th>Neomycin</th>
<th>Supplemental K1</th>
<th>Prothrombin time (Seconds)</th>
<th>Prothrombin concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/L)</td>
<td>(mg/kg)</td>
<td></td>
<td>(NIH)^2</td>
</tr>
<tr>
<td>0</td>
<td>124.6</td>
<td>44.3</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.1</td>
<td>112.5</td>
<td>104.0</td>
</tr>
<tr>
<td>0.5</td>
<td>93.6</td>
<td>102.6</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>129.7</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.1</td>
<td>10.68</td>
<td>103.8</td>
</tr>
<tr>
<td>0.5</td>
<td>101.2</td>
<td>120.8</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>7.75</td>
<td>17.2</td>
<td></td>
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Means of main effect

<table>
<thead>
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<th>Neomycin</th>
<th>Prothrombin time</th>
<th>Prothrombin concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>109.7</td>
<td>83.6</td>
</tr>
<tr>
<td>75</td>
<td>112.1</td>
<td>85.3</td>
</tr>
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</table>

<table>
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<tr>
<th>K1</th>
<th>Prothrombin time</th>
<th>Prothrombin concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>126.9^a</td>
<td>137.8^a</td>
</tr>
<tr>
<td>0.1</td>
<td>109.2^b</td>
<td>103.9^b</td>
</tr>
<tr>
<td>0.5</td>
<td>96.9^b</td>
<td>111.7^b</td>
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</table>

Sources of variation

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Neomycin</th>
<th>K1</th>
<th>Neomycin x K1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.71</td>
<td>0.90</td>
<td>0.67</td>
</tr>
</tbody>
</table>

^a,b Means within a column with no common superscript differ significantly (P < 0.05).
1Prothrombin concentration is expressed in the terms of the activity of thrombin activity in NIH units. 2NIH unit is obtained by direct comparison to a NIH Thrombin Reference Standard, Lot J. Sigma, St Louis, MO 63178


TABLE 4 Effect of dietary K1 on body weight gain, feed intake and efficiency of feed utilization in young turkey poults (Experiment 2)

<table>
<thead>
<tr>
<th>Supplemental K1 (mg/kg)</th>
<th>Body weight Day 7</th>
<th>Weight gain Day 1-7</th>
<th>Feed intake Day 1-7</th>
<th>Feed efficiency Day 1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>129.7</td>
<td>72.9</td>
<td>80.8</td>
<td>1.116</td>
</tr>
<tr>
<td>0.08</td>
<td>130.8</td>
<td>74.2</td>
<td>82.1</td>
<td>1.107</td>
</tr>
<tr>
<td>0.31</td>
<td>136.6</td>
<td>79.8</td>
<td>86.2</td>
<td>1.080</td>
</tr>
<tr>
<td>0.44</td>
<td>136.5</td>
<td>78.6</td>
<td>83.1</td>
<td>1.058</td>
</tr>
<tr>
<td>1.8</td>
<td>138.2</td>
<td>80.7</td>
<td>85.9</td>
<td>1.065</td>
</tr>
<tr>
<td>SEM</td>
<td>4.11</td>
<td>4.06</td>
<td>2.46</td>
<td>0.035</td>
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</table>

Sources of variation

| K1          | 0.54 | 0.60 | 0.52 | 0.72 |

¹ Feed to gain ratio.
### TABLE 5. Effect of dietary K1 on plasma prothrombin time (Experiment 2)

<table>
<thead>
<tr>
<th>K1 (mg/kg)</th>
<th>Prothrombin time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>130.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.08</td>
<td>88.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.31</td>
<td>98.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.44</td>
<td>100.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.80</td>
<td>92.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>7.59</td>
</tr>
</tbody>
</table>

Sources of variation

| K1 | 0.0011 |

<sup>ab</sup> Means within a column with no common superscript differ significantly (P < 0.05).
TABLE 6. Effect of dietary supplemental K1 on body weight gain, feed intake
and efficiency of feed utilization in young turkey poults (Experiment 3)

<table>
<thead>
<tr>
<th>Supplemental K1 (mg/kg)</th>
<th>Body weight Day 7</th>
<th>Weight gain Days 1-7</th>
<th>Feed intake Days 1-7</th>
<th>Feed efficiency¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>133.3</td>
<td>74.2</td>
<td>76.2</td>
<td>1.027</td>
</tr>
<tr>
<td>0.1</td>
<td>135.1</td>
<td>76.6</td>
<td>80.5</td>
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<td>78.3</td>
<td>82.1</td>
<td>1.049</td>
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<tr>
<td>2.0</td>
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<tr>
<td>SEM</td>
<td>3.62</td>
<td>3.49</td>
<td>3.45</td>
<td>0.028</td>
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</table>

Source of variation

| K1     | 0.34 | 0.30 | 0.18 | 0.78 |

¹ Feed to gain ratio
TABLE 7. Effect of dietary supplemental K1 on plasma prothrombin time, and prothrombin concentration, and plasma K1, menaquinone-4 (MK-4) concentrations in young turkey poults (Experiment 3).

<table>
<thead>
<tr>
<th>K1 (mg/kg)</th>
<th>Prothrombin time (Seconds)</th>
<th>Prothrombin concentration(^1) (NIH)(^2)</th>
<th>Plasma K1 (ng/mL)</th>
<th>MK-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>124.6(^a)</td>
<td>44.3(^a)</td>
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<td>0.1</td>
<td>112.5(^{ab})</td>
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<td>0.25</td>
<td>116.1(^b)</td>
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<td>16.3</td>
<td>0.883</td>
<td>0.432</td>
</tr>
</tbody>
</table>

Source of variation

| K1        | 0.0001 | 0.078  | 0.0001 | 0.0001 |

\(^a-c^\) Means within a column with no common superscript differ significantly (P < 0.05).

\(^1\) prothrombin concentration is expressed by the activity of thrombin activity in NIH units.

\(^2\) NIH unit is obtained by direct comparison to a NIH Thrombin Reference Standard, Lot J. Sigma. St Louis. MO 63178.
Figure 1. Effect of dietary supplemental K1 on plasma K1 and MK-4 concentrations in 7-d-old turkeys. Experiment 3.
GENERAL CONCLUSIONS

The research reported herein had two objectives 1) to determine the K1 requirement of turkey pouls. 2) to determine the influence of dietary K1 on bone mineralization and related plasma parameters in young turkeys fed diets that were adequate or deficient in D3.

The results of this research showed that the dietary K1 requirement of 7- to 14-d-old turkeys is in the range of 0.079 to 0.13 mg/kg, based on the effect of dietary supplemental K1 on plasma prothrombin concentration and plasma prothrombin time. Administration of neomycin to minimize microbial synthesis of vitamin K in the intestine did not affect estimates of the K1 requirement. The concentrations of plasma K1 increased linearly as the dietary supplemental K1 level increased. The concentration of plasma menaquinone-4 increased in a similar fashion as that of plasma K1 as the dietary supplemental K1 level increase. Dietary supplemental K1 had little effect on bone development in pouls fed diets either adequate or deficient in D3, even though K1 supplementation of D3 deficient diets increased plasma inorganic phosphorus and decreased plasma alkaline phosphatase. An increase in feed intake in response to K1 supplementation to the low D3 diet probably accounted for some of the changes in plasma alkaline phosphatase and inorganic phosphorus in pouls fed a low vitamin D3 diet with supplemental K1 at a level of 0.37 or 2.88 mg of K1/kg. The increase in bone ash caused by supplementation with a high level of K1 during a recovery from a rickets-like condition observed in Experiment 1 was not related to vitamin D status of the pouls. The mechanism by which K1 had this effect is not evident from data obtained in the current study.