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The relationship of vitamin K to cecal coccidiosis of chickens

James Fred Stephens

To the Graduate Council:

I am submitting herewith a thesis written by James Fred Stephens entitled "The relationship of vitamin K to cecal coccidiosis of chickens." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

J. K. Bletner, Major Professor

We have read this thesis and recommend its acceptance:

O. E. Goff, R. L. Tugwell, O. G. Hall

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 20, 1959

To the Graduate Council:

I am submitting herewith a thesis written by James Fred Stephens entitled "The Relationship of Vitamin K to Cecal Coccidiosis of Chickens." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Poultry.

J. K. B. Olney
Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

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Dean of the Graduate School

THE RELATIONSHIP OF VITAMIN K TO CECAL COCCIDIOSIS OF CHICKENS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

James Fred Stephens

June 1959

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INTRODUCTION

The fact that severe cecal hemorrhage accompanies severe Eimeria tenella infections in young chickens suggested that mortality from cecal coccidiosis might be reduced by the administration of vitamin K, the anti-hemorrhagic vitamin.

For almost thirty years, vitamin K has been recognized as an essential nutrient for the normal clotting of blood in young chickens. At the time of the discovery and chemical characterization of vitamin K, the possibility of hypovitaminosis K occurring in chickens fed practical diets was considered unlikely due to the high vitamin K content of many commonly used feed ingredients. During this thirty year period, the composition of practical chick diets has been changed tremendously. Diets high in protein and energy have replaced the more bulky-type diets. Consequently, the quantity of alfalfa meal and other naturally occurring materials high in vitamin K content has been greatly reduced. Furthermore, methods have been developed for a more complete extraction of the oil from soybean meal, one of the principle ingredients of most poultry diets, resulting in a meal with a lower vitamin K content. Reducing the alfalfa meal and soybean oil content of the diets resulted in markedly lowered vitamin K levels.

Outbreaks of the classical hemorrhagic syndrome of young chickens provided evidence that practical chick diets were often low in vitamin K content. Many cases of this condition were attributed to the feeding of high energy, low vitamin K diets and the concurrent administration of excessive quantities of drugs capable of acting as vitamin K antagonists.

Studies at the Tennessee Station illustrated that mortality from cecal coccidiosis could be increased by feeding a diet low in vitamin K. It appeared that this mortality might be due to excessive blood loss resulting from failure of the blood to clot rapidly. These data suggested that mortality from cecal coccidiosis might be related to blood clotting time and, therefore, might be reduced by supplying additional dietary sources of vitamin K. Considering these possibilities and relationships, a series of experiments were initiated to study the effects of vitamin K on chickens artificially infected with Eimeria tenella.

OBJECTIVES

The fact that hypovitaminosis K does occur in young chickens fed currently available rations is evidenced by the recent occurrence of a hemorrhagic syndrome of poultry. It has also been demonstrated that mortality from cecal coccidiosis can be increased by feeding the vitamin K antagonistic compound Dicumarol.[1]

The objectives of the experiments reported in this thesis were to determine (1) the effects of vitamin K on blood clotting time, weight gain, and mortality from cecal coccidiosis in experimentally inoculated chickens, (2) the effects of various levels of vitamin K on blood clotting time, mortality, and weight gain of chicks inoculated with varying numbers of sporulated Eimeria tenella oocysts, and (3) the relative efficacies of Nicarbazin and four potential sources of vitamin K in the prevention of morbidity and mortality in birds fed diets low in vitamin K and experimentally infected with cecal coccidiosis.

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[1]Trade name of 3,3' - Methylenebis (4-hydroxycoumarin). A product of the Wisconsin Alumni Research Foundation.

LITERATURE REVIEW

Many scientists have contributed to the present knowledge of vitamin K and its relation to hemorrhage in chickens. The literature pertaining to vitamin K and cecal coccidiosis is extensive and of special interest to a study of the relationship of vitamin K to cecal coccidiosis.

Dam and Schonheyder (1934) observed prolonged blood clotting times in young chickens suffering from a hemorrhagic condition of unknown etiology. Pathological observations of affected chicks revealed large subcutaneous and intramuscular hemorrhages and changes in the horny stratum of the gizzard.

The existence of a fat soluble factor necessary for the prevention of hemorrhages and maintenance of normal blood clotting times in young chicks was reported by Dam (1935). After determining that this factor was not identical with any previously discovered nutrient, Dam proposed the name vitamin K. It was further observed that a deficiency of vitamin K was not likely to occur since alfalfa meal and other green vegetable materials high in vitamin K content were normally included in "practical-type" poultry rations at that time.

At approximately the same time, Almquist and Stokstad (1935) investigated a hemorrhagic condition in chicks fed a diet consisting primarily of polished rice, ether extracted fish meal, and ether extracted dried brewer's yeast. This condition was prevented when the basal diet was supplemented with 0.5 percent alfalfa meal, ethyl ether extract of alfalfa meal equivalent to 10 percent alfalfa meal, or the unsaponifiable fraction of ether extract equivalent to 10 percent alfalfa

meal. Fish meal used in the basal diet, after being washed with water and dried slowly, also prevented the hemorrhagic condition. These investigators concluded that the development of an antihemorrhagic factor in the water washed fish meal was due to microbial activity.

Many other materials were subsequently investigated for vitamin K content.

Almquist and Stokstad (1936) reported that chicken feces possessed vitamin K activity. This vitamin K activity was believed to have resulted primarily from microbial action, partially in the intestine of the chicken, and partially after passage of the feces.

Soybean oil was first reported to contain a relatively large quantity of vitamin K by Almquist and Stokstad (1937).

Recently, Tugwell and Harms (1957) found that autoclaved chicken litter supplied at a level of five percent to an otherwise vitamin K-low diet resulted in the maintenance of normal blood clotting times in young chickens.

McKee, et al. (1939) crystallized a substance possessing vitamin K activity from putrefied fish meal. This substance was found to differ both in chemical structure and biological activity from the form of vitamin K found in alfalfa. Consequently, the terms vitamin K_1 and vitamin K_2 were adopted for the forms derived from alfalfa and fish meal respectively.

A number of synthetic compounds have been observed to possess vitamin K activity. Some have been reported to be more potent sources of vitamin K activity than the natural occurring forms.

Almquist and Klose (1939) reported the biological activity of

vitamin K_1 to be only 38 percent as great as that of the synthetic compound menadione, chemically, 2-methyl-1, 4-napthaquinone.

More recently, Frost and Spruth (1955) reported that on a total weight basis menadione sodium bisulfite,[1] another synthetic material, possessed four times the vitamin K activity of menadione. This material was observed to be from six to eight times as effective as menadione for maintenance of normal blood clotting times in studies conducted by Shelton, et al. (1956).

Perdue, Spruth, and Frost (1957) reported the vitamin K activity of Klotogen F to be 1.5 times that of vitamin K_1 as determined by the reduction of prothrombin times of young chicks fed a practical broiler diet modified to contain no menadione or alfalfa meal.

Klotogen F was described by Frost and Spruth (1955) as a water soluble compound containing 63 percent menadione sodium bisulfite. These workers reported that as little as 90 to 180 milligrams of this material added per ton of vitamin K-low diet was adequate for maintenance of normal blood clotting times in young chickens.

Several methods, all involving the use of young chickens, have been developed for determining the relative vitamin K content of various feedstuffs. A whole blood clotting time method was employed by Dam and Schonheyder (1934) and Schonheyder (1935). Plasma prothrombin times formed the basis for vitamin K assay in studies conducted by Dam, Schonheyder, and Tage-Hasen (1936) and Schonheyder (1936). Whole blood prothrombin times, determined by a method developed by Almquist and

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[1]Trademark Klotogen F. A product of Abbott Laboratories. Hereafter referred to as Klotogen F.

Klose (1939b), were used as a basis for vitamin K assay by Otto, et al. (1958).

A method for determining blood clotting times of chicks involving the use of capillary tubes was described by Levinson and MacFate (1951).

Frost and Spruth (1955) compared the accuracy of two techniques, similar to two of the above, for determining blood clotting times. Duplicate blood-filled capillary tubes were periodically broken to determine blood clotting times of individual birds. Blood samples obtained from the same birds were agitated in test tubes in a constant temperature water bath. The blood clotting times arrived at by the two methods were found to compare favorably except in cases of chicks having very long blood clotting times. In these cases, more error was observed to occur when the capillary tube method was employed.

Contrary to the observation of Dam (1935), vitamin K deficiencies have occurred in chickens fed practical diets. Bletner, et al., (1953), Baker and Jaquett (1953), Goldhaft (1953), Bornstein and Samberg (1954), Cover, Mellen, and Gill (1955), Frost and Spruth (1955), and Norris and Nelson (1955) reported the occurrance of a hemorrhagic condition resembling hypovitaminosis K in young chickens fed commercial rations and maintained under field conditions. Cases reported by Bornstein and Samberg (1954), Frost and Spruth (1955), and Norris and Nelson (1955) responded to vitamin K administrations. Cover, Mellen, and Gill (1955) and Frost, Perdue, and Spruth (1957) reported that some of the field cases of the hemorrhagic condition were not curable by vitamin K therapy.

Anderson et al.,(1954) produced a hemorrhagic syndrome in the laboratory similar to the field hemorrhagic condition by feeding young chickens

a diet consisting of corn, soybean oil meal, vitamins, and minerals. Prolonged blood clotting times were observed independently of the severity of the hemorrhagic condition. This condition was cured by the administration of vitamin K. Growth rate was not affected by vitamin K administration.

Recent changes in the composition of commercial poultry rations which may have led to hypovitaminosis K and, consequently, the hemorrhagic condition, were described by Norris and Nelson (1955). These workers concluded that the decrease or elimination of dehydrated alfalfa meal and the change from expeller to solvent extracted soybean oil meal were changes most significant in the reduction of the quantity of vitamin K in poultry diets.

Yacowitz et al.,(1955), Norris and Nelson (1955), Frost and Spruth (1955) and Frost, Perdue, and Spruth (1957) observed the use of high levels of drugs, particularly sulfaquinoxaline, in the diets of birds affected with the hemorrhagic condition. Frost, Perdue, and Spruth (1957) suggested that the excessive use of sulfa drugs in the diets of chickens already somewhat deficient in vitamin K "triggered" many outbreaks of the hemorrhagic condition. These workers also suggested that the recommendation of the National Research Council (1954) of 0.18 milligrams of vitamin K_1 per pound of diet was probably too low for modern-day broiler rations.

Foster (1949) reported that approximately ten percent of the total disease mortality in poultry resulted from coccidiosis, the major portion of this percentage being caused by the cecal type. It was also observed that the total economic loss was greater than that indicated by mortality alone since the loss resulting from morbidity and the cost of medicants

for prevention and treatment of coccidiosis must be taken into account.

Tyzzer (1929) credited the naming of the protozoan parasite causing acute coccidiosis, Eimeria tenella, to Railliet and Lucet in 1891, but suggested that Revolta observed this species as early as 1878.

The most striking external manifestation of an acute form of coccidiosis observed by Charles (1921) was bloody droppings. Hemorrhages in the ceca and bloody droppings also characterized the acute form of coccidiosis as reported by Tyzzer (1927, 1929).

Studies on the morphological and pathological nature of Eimeria tenella were conducted by Tyzzer (1929), Tyzzer, Theiler, and Jones (1932), and Herrick, Ott, and Holmes (1936).

The nature and course of cecal coccidiosis in young chickens were described by Tyzzer (1929), Herrick, Ott, and Holmes (1936), Herrick (1936), Pratt (1937), Seeger (1949), and Cuckler and Malanga (1956). Hemorrhaging was reported by Tyzzer (1929) to occur concurrently with the release of merozoites from the second generation schizonts.

Numerous chemical compounds have been tested for anticoccidial activity, two of the common being sulfaquinoxaline and Nicarbazin.[2]

Grumbles, Delaplane, and Higgins (1948), Jungherr and Winn (1949), and Grumbles, et al. (1949) reported that low levels of sulfaquinoxaline were effective in reducing mortality from cecal coccidiosis.

Cuckler and Malanga (1956) reported that young chickens were able, not only to survive outbreaks of cecal coccidiosis, but also to develop

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[2]Trade name of the complex 4,4'-dinitrocarbanilid-2hydroxy-4,6-dimethylpyrimidine. A product of Merck and Company, Incorporated.

immunity to further infection with Eimeria tenella when fed a diet containing 0.0125 percent Nicarbazin. Both sulfaquinoxaline and Nicarbazin have been used commercially for the prevention of mortality from cecal coccidiosis.

A relationship between vitamin K and cecal coccidiosis was first reported in 1941; however, the research leading to this observation was limited. Baldwin, Wiswell, and Jankiewicz (1941) employed a total of thirty chicks in an experiment designed to study the effect of reduction of hemorrhage on mortality from cecal coccidiosis. These chicks were divided into infected and noninfected control groups and an infected group treated with vitamin K. The groups consisted of ten birds each. Only one bird in the vitamin K treated group died, as compared to seven birds in the inoculated control group. Since all groups were fed the same commercial diet, the authors attributed the reduced mortality to the reduction of hemorrhage resulting from treatment with vitamin K.

Harms and Tugwell (1956) fed a diet containing Dicumarol to birds experimentally infected with Eimeria tenella. The resulting increase in mortality from cecal coccidiosis was attributed to excessive cecal hemorrhage resulting from the vitamin K antagonistic action of Dicumarol. Mortality was decreased in the presence or absence of Dicumarol when one gram of Klotogen F per ton of vitamin K-low diet was supplied to infected chicks from one day of age until the end of the test. When the feeding of Klotogen F was delayed until the day of inoculation, mortality from cecal coccidiosis was not reduced even though twenty grams of Klotogen F per ton of diet was supplied. Otto, et al. (1958) interpreted results of this trial as providing support to the theory

that the beneficial effects of Klotogen F in cecal coccidiosis result from the reduction of hemorrhage and not from any anticoccidial properties of this product.

Weight gains of chicks were unaffected when either Dicumarol or Klotogen F was added to the vitamin K-low diet.

PROCEDURE

Experimental Animals

A total of 1560 chicks were employed in a series of four trials. All chicks were obtained from commercial hatcheries a few hours after hatching. With the exception of those used in the second trial, all were crossbred broiler-type chickens. Chicks employed in the second trial were Single Comb White Leghorn males.

Care of Experimental Animals

At one day of age, all chicks were sexed, vaccinated with a combination bronchitis-Newcastle disease vaccine utilizing the intraocular method, randomized within each sex, and wing banded. They were then placed in electrically heated battery brooders with raised woven-wire floors where they were maintained until termination of the trial.

Temperatures beneath the heating elements of the battery pens were adjusted at approximately 95 degrees Farenheit during the first week of each trial, 90 degrees during the second week, 85 degrees during the third week, 80 degrees during the fourth week, and 75 degrees for the remaining part of the trial.

Feed and water were supplied ad libitum. Water troughs were washed and fresh water supplied daily in an effort to limit the synthesis of vitamin K in the water troughs.

Diets

A diet composed of yellow corn and solvent extracted soybean oil meal, calculated to meet the recommendations of the National Research Council for all known minerals and vitamins except vitamin K, was

employed as the basal diet in each trial. This diet was known to be sufficiently low in vitamin K content to produce prolonged blood clotting time in young chicks. The composition of this diet, as shown in Table I, was altered to the necessary extent to facilitate the testing of additional feed ingredients for vitamin K activity.

Supplemental ingredients added to the basal diet in one or more of the trials included Klotogen F, alfalfa meal, alfalfa leaf meal, fish meal, autoclaved chicken litter, Nicarbazin, and a combination of Nicarbazin and Klotogen F.

Inoculations

Eimeria tenella oocysts were obtained for experimentally infecting birds in these trials as follows: Small quantities (approximately 1500) of sporulated oocysts of Eimeria tenella were given orally to four week old chicks. At nine days post inoculation, the chicks were sacrificed, the ceca removed, and the contents washed into a two percent potassium dichromate solution. Oocysts collected in this manner were sporulated at 30 degrees Centigrade for 48 hours and then retained in a refrigerator at five degrees Centigrade until used.

The number of oocysts per unit volume was determined with the aid of a hemocytometer. When necessary, tap water was added to reduce the concentration of oocysts to the desired level.

A volume of the inoculum calculated to contain the desired number of oocysts was deposited directly into the crop of each bird by means of a calibrated pipette.

TABLE I

COMPOSITION OF BASAL DIET

Ingredient	Amount per 100 pounds
Yellow Corn	65.7
Soybean Oil Meal (50% protein)	31.0
Ground Limestone	1.2
Salt	0.5
Defluorinated Phosphate[a]	1.0
Vitamin Mix[b]	0.6

[a]Contains 34 percent calcium and 17 percent phosphorus.

[b]Calculated to contain per pound of feed: 0.9 mg. riboflavin, 2 mg. calcium pantothenate, 5 mg. niacin, 3 mcg. vitamin B_{12}, 250 mg. choline chloride, 1737 I. U. vitamin A, 1050 I. C. U. vitamin D_3, 0.08 gms. $MnSO_4$, 2 mg. procaine penicillin, and 45 mg. arsanilic acid.

Criteria of Comparisons

Weight gains, blood clotting times, and mortality from cecal coccidiosis formed the bases for comparing the effectiveness of the experimental diets.

Each bird was individually weighed on the day of inoculation and 14 days post inoculation at which time each trial was terminated.

Blood clotting times, when taken, were determined on the day of inoculation by the following capillary tube method.

Blood was obtained by puncturing the brachial vein with the point of a scalpell blade or an 18 gauge hypodermic needle. Duplicate capillary tubes were filled with blood, care being exercised to avoid touching the tissue of the chick with the capillary tubes. Capillary tubes representing individual chicks were broken at time intervals of approximately 30 seconds. Blood clotting time was recorded as the time interval between the appearance of blood following the puncture of the brachial vein and the observation of a firm clot upon breaking either of the duplicate capillary tubes.

Mortality records were maintained throughout each trial. Dead birds were removed from the battery pens twice daily during the experimentally induced outbreaks of cecal coccidiosis. A necropsy was performed whenever the cause of death was not apparent.

All pertinent data were statistically analyzed using analysis of variance according to Snedecor (1956) with significant treatment differences determined by Duncan's multiple range test (1955).

Trial 1

The first trial was designed to study the influence of a diet varying in vitamin K content upon weight gains of both Eimeria tenella inoculated and noninoculated chicks and upon mortality from cecal coccidiosis.

Ten pens of male and ten pens of female day-old Lancaster x New Hampshire chicks were used in this trial. Twenty birds per pen were utilized. Five experimental treatments were used resulting in two pens of males and two pens of females per treatment.

The positive control pens were fed the vitamin K-low basal diet shown in Table I. The negative control pens were fed the same diet, but were inoculated with Eimeria tenella sporulated oocysts. The diets of the other three experimental groups were formed by adding one gram of Klotogen F per ton of feed, twenty grams of Klotogen F per ton of feed, and three percent of alfalfa leaf meal respectively to the basal diet.

When the birds were 23 days of age, each of those in the three experimental groups and in the negative control group was inoculated with 50,000 sporulated oocysts of Eimeria tenella.

Trial 2

The second trial of the series was designed to duplicate the preceding experiment and, in addition, to study the relationship of the chicks' blood clotting times to the other variables under observation.

With the exception of the following changes, the procedures for this trial were identical with those of the first trial:

(1) the experimental chicks were Single Comb White Leghorn males

(2) the experimentally infected birds were inoculated with 60,000 sporulated oocysts of Eimeria tenella at 28 days of age

(3) blood clotting times of 30 birds selected at random from each treatment group were determined on the day of inoculation

Trial 3

The effects of four levels of vitamin K on blood clotting times, weight gains, and mortality from cecal coccidiosis in chickens experimentally inoculated with 50,000 and 500,000 sporulated oocysts of Eimeria tenella were studied in the third trial.

A total of 480 day-old broiler-type chicks (Lancaster x New Hampshire) were used in this trial. Twenty-four battery pens, each containing ten males and ten females, were employed.

Four experimental diets were fed resulting in six pens of chicks being fed each diet. The basal diet was supplemented with 0, 0.36, 1.08, and 9.72 grams of Klotogen F per ton of feed, respectively.

When the chicks were 21 days of age, four of the six pens receiving each diet were inoculated with sporulated oocysts of Eimeria tenella. Two of these pens received 50,000 oocysts per bird, and the other two pens received 500,000 oocysts per bird. The remaining two pens fed each diet were not inoculated and served as positive controls for the corresponding inoculated pens.

Blood clotting times were determined on the day of inoculation using five males and five females selected at random from each pen.

Trial 4

The fourth trial was designed to compare the efficacy of four potential sources of vitamin K in the presence and absence of Eimeria tenella infections. The effects of the coccidiostatic drug, Nicarbazin, both alone and in combination with Klotogen F, were also compared to those of the four potential vitamin K sources employed in this trial.

A total of 280 day-old broiler-type chicks (Vantress x Nichols' 108) were used. Twenty-eight pens, each containing five males and five females, were employed. Four pens of chicks were fed each of seven experimental diets shown in Table II.

Adjustments were made in order to maintain a crude protein percentage of from 21.4 to 21.5 in all diets, however, no attempt was made to equalize energy levels of these diets.

The blood clotting times of all chicks were determined when 21 days of age. Each chick in two pens fed each diet was individually inoculated with 50,000 sporulated oocysts of Eimeria tenella.

Feed consumption data were collected both at time of inoculation and 14 days post inoculation. Feed efficiency was subsequently calculated and statistically analyzed to further evaluate the experimental treatments.

TABLE II

COMPOSITION OF DIETS - TRIAL 4[a]

Ingredient	Basal Diet	Fish Meal 3 Percent	Alfalfa Meal 3 Percent	Poultry Litter 5 Percent
Yellow Corn	65.7	66.2	63.2	62.2
Soybean Oil Meal	31.0	27.5	30.5	29.5
Alfalfa Meal			3.0	
Fish Meal		3.0		
Poultry Litter				5.0
Defluorinated Phosphate[b]	1.0	1.0	1.0	1.0
Ground Limestone	1.2	1.2	1.2	1.2
Salt	0.5	0.5	0.5	0.5
Vitamin Mix[c]	0.6	0.6	0.6	0.6

[a]Three additional diets employed were formed by adding to the basal diet: (1) 50 mg. Klotogen F., (2) 5.7 gm. Nicarbazin, and (3) 50 mg. Klotogen F and 5.7 gm. Nicarbazin.

[b]Contains 34 percent calcium and 17 percent phosphorus.

[c]Calculated to contain per pound of feed: 0.9 mg. riboflavin, 2 mg. calcium pantothenate, 5 mg. niacin, 3 mcg. vitamin B_{12}, 250 mg. choline chloride, 1737 I. U. vitamin A, 1050 I. C. U. vitamin D_3, 0.08 gms. $MnSO_4$, 2 mg. procaine penicillin, and 45 mg. arsanilic acid.

RESULTS AND DISCUSSION

Trials 1 and 2

The purpose, procedure, and results of the first two trials of this series were almost identical and formed the bases upon which the other trials were conducted; therefore, these results are discussed concurrently. Blood clotting times were determined for randomly selected chicks fed each experimental diet in the second trial but not in the first trial.

In each trial, mortality of chicks inoculated with Eimeria tenella was significantly[1] reduced by addition of a source of vitamin K to the basal diet. These results are compatable with results of the limited trials conducted by Baldwin, Wiswell, and Jankiewiez (1941). Alfalfa leaf meal at a level of 3 percent of the basal diet was as effective in preventing excessive mortality from cecal coccidiosis as either one or twenty grams of Klotogen F per ton of diet. In the first trial, mortality from cecal coccidiosis of birds receiving twenty grams of Klotogen F per ton of feed was significantly less than that of chicks receiving one gram of Klotogen F per ton of feed. However, no significant difference in mortality could be detected between these two groups in the second experiment.

Blood clotting time was reduced by the addition of a source of vitamin K to the basal diet in the second experiment. When a significant

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[1]"Significant " refers to statistical differences at the 5 percent level of probability. "Highly Significant" refers to statistical differences at the 1 percent level of probability.

difference occurred in blood clotting time, a corresponding difference in mortality was observed (Table III). This suggests that vitamin K affected mortality from cecal coccidiosis through its role in the blood clotting mechanism, and not by coccidiostatic or coccidiocidal effect.

Body weights, taken on the day of inoculation and 14 days post inoculation, indicate that growth rate was unaffected by including a source of vitamin K in the diets of chicks in the presence or absence of cecal coccidiosis.

In the first trial, the noninoculated group fed the basal diet were significantly heavier at 14 days post inoculation than any of the other groups. This indicates that growth rate was retarded by the Eimeria tenella infection regardless of the diet fed. No significant weight differences between treatments were observed in the second trial.

No significant weight differences between groups were observed at the time of inoculation in either test, indicating that vitamin K alone has no effect upon growth rate of chicks. These results compare favorably with those reported by Anderson et al. (1954) and Harms and Tugwell (1956). Weight data relating to both trials are summarized in Table IV.

Trial 3

Blood clotting times of chicks employed in this trial were not further reduced by supplying quantities of Klotogen F in excess of 0.36 grams per ton of basal diet (Table V), the level of vitamin K_1 suggested by the National Research Council (1954).

Prolonged blood clotting time and excessive mortality from cecal

TABLE III

THE EFFECTS OF VITAMIN K ON BLOOD CLOTTING TIME AND MORTALITY FROM COCCIDIOSIS - TRIALS 1 AND 2

Treatment	Trial 1	Trial 2	
	Mortality[a]	Mortality[a]	Blood Clotting Time[a]
	Percent	Percent	Seconds
Basal Inoc.	36.7	44.6	751
Basal Non-Inoc.	0.0	0.0	824
1 gm. Klotogen F/ton-Inoc.	19.2	2.5	468
3% Alfalfa Leaf Meal-Inoc.	13.2	6.3	545
20 gms. Klotogen F/ton-Inoc.	11.7	8.2	428

[a]Means not grouped by line are significantly different at the 5 percent level of probability.

TABLE IV

THE EFFECTS OF VITAMIN K AND CECAL COCCIDIOSIS ON WEIGHT GAIN - TRIALS 1 AND 2

Treatment	Average Weight[a]			
	Day of Inoculation		14 Days Post Inoculation	
	Trial 1	Trial 2	Trial 1	Trial 2
	Grams			
Basal Noninoculated	338.37	284.52	601.80	449.19
Basal Inoculated	338.51	268.71	571.56	470.67
1 gm. Klotogen F/ton Inoc.	335.64	288.25	571.58	475.49
3 percent Alfalfa Leaf Meal/Inoc.	347.37	275.26	572.37	436.70
20 gms. Klotogen F/ton Inoc.	335.02	284.07	582.20	419.75

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

TABLE V

THE EFFECT OF QUANTITY OF KLOTOGEN F
ON BLOOD CLOTTING TIME - TRIAL 3

Klotogen F Per Ton of Basal Diet	Average Blood Clotting Time[a]
Grams	Seconds
0.36	675
1.08	721
9.72	731
0.00	1,386

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

coccidiosis in chicks inoculated with 50,000 sporulated oocysts of Eimeria tenella were prevented when the basal diet was supplemented with as little as 0.36 grams of Klotogen F per ton of diet. A level of 0.36 grams of Klotogen F per ton of diet also reduced mortality in groups inoculated with 500,000 oocysts per bird; however, this reduction was significantly less than that resulting from the addition of either 1.08 or 9.72 grams of Klotogen F to the diets of groups of chicks inoculated with either 50,000 or 500,000 oocysts of Eimeria tenella (Table VI).

Since the blood clotting times of chicks supplied 0.36 grams of Klotogen F per ton of diet were as short as those of chicks fed diets containing either 1.08 or 9.72 grams of Klotogen F per ton, the greater mortality of the first group, when inoculated with 500,000 oocysts, is believed to indicate that 0.36 grams per ton supplied a marginal level of vitamin K activity.

The existence of relatively short blood clotting times in chicks supplied marginal levels of vitamin K may be explained on the basis of work reported by Tidrick, Joyce, and Smith (1939). These workers found that normal blood clotting times occurred when prothrombin levels were only 30 to 40 percent of normal. Since the degree of morbidity resulting from inoculation with 500,000 oocysts undoubtedly resulted in reduced feed consumption, chicks having subnormal prothrombin levels originally probably developed more severe hypoprothrombinemia as a failure to ingest sufficient vitamin K and, consequently, suffered excessive hemorrhage during the course of the cecal coccidiosis infection.

Weight gains were not affected by the quantity of Klotogen F

TABLE VI

THE EFFECTS OF NUMBER OF OOCYSTS AND QUANTITY OF VITAMIN K UPON MORTALITY OF EXPERIMENTALLY INOCULATED CHICKS - TRIAL 3

Klotogen F/ton	Oocysts/Bird	Mortality[a]
Grams	Number	Percent
0.0	0	0.0
9.72	50,000	0.0
1.08	50,000	0.0
0.36	50,000	2.5
1.08	500,000	5.2
9.72	500,000	7.5
0.36	500,000	22.5
0.0	50,000	57.2
0.0	500,000	95.0

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

supplied in the ration. This is in agreement with results of the preceeding experiments.

Weight gains during the fourteen day post inoculation period were affected by the number of oocysts in the inoculating dose. Average weight gains of the chicks inoculated with 500,000 sporulated oocysts of Eimeria tenella per bird were significantly less than those of all other treatment groups regardless of the quantity of Klotogen F supplied. It is apparent that the decreased growth rates of these chicks resulted primarily from the severe coccidial infection.

The average weight gain of birds in the group supplied no supplemental vitamin K and inoculated with 500,000 oocysts per bird was significantly less than that of any other treatment group. However, since only two birds in this group survived until 14 days post inoculation, no conclusion is drawn from the weight gain data of these survivors.

Data pertaining to weight gains of birds supplied four levels of Klotogen F and inoculated with 0; 50,000; or 500,000 sporulated oocysts of Eimeria tenella are summarized in Table VII.

Trial 4

Of the materials employed as potential vitamin K sources in the fourth trial, only menhadden fish meal failed to exhibit an effect upon blood clotting time and mortality from cecal coccidiosis.

When compared with the basal diet, the feed containing 3 percent menhadden fish meal failed to reduce blood clotting time and mortality from cecal coccidiosis. These data indicate the absence of vitamin K in menhadden fish meal.

TABLE VII

THE EFFECTS OF NUMBER OF OOCYSTS AND QUANTITY OF VITAMIN K ON WEIGHT GAINS DURING THE FOURTEEN DAY POST INOCULATION PERIOD - TRIAL 3

Klotogen F Per Ton	Oocysts Per Bird	Average Weight Gain[a]
Grams	Number	Grams
9.72	0	248.9
0.36	0	246.2
1.08	0	245.3
0.0	50,000	237.5
0.0	0	236.2
0.36	50,000	235.3
1.08	50,000	229.7
9.72	50,000	215.8
0.36	500,000	175.9
9.72	500,000	174.8
1.08	500,000	169.5
0.0	500,000	118.0

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

Mortality from cecal coccidiosis was reduced by supplementing the basal diet with 0.0125 percent Nicarbazin even though the blood clotting times of birds fed this diet were as prolonged as those of birds fed the unsupplemented basal diet.

Alfalfa meal at a level of 3 percent of the total ration, autoclaved chicken litter at the 5 percent level, one gram of Klotogen F per ton of basal diet, and a combination of 0.0125 percent Nicarbazin and one gram of Klotogen F per ton of diet were effective in the prevention of prolonged blood clotting times and excessive mortality from cecal coccidiosis. Although no statistically significant differences in mortality were found between the above groups, it should be noted that some mortality did occur in all inoculated groups with the exception of the group fed the basal diet supplemented with both 0.0125 percent Nicarbazin and one gram of Klotogen F per ton. These results indicate that the coccidiostatic drug, Nicarbazin, at the 0.0125 percent level is most effective in preventing losses from coccidiosis in the presence of quantities of vitamin K adequate for prevention of prolonged blood clotting time.

Average blood clotting time of all birds employed in this trial, and mortality percentages of the inoculated groups are shown in Table VIII. Data on weight gains secured in this trial indicate that Nicarbazin, when added to diets either low in vitamin K content or adequately supplemented with vitamin K significantly retards the growth rate of chickens when compared with that of chicks fed the unsupplemented basal diet. Of the other experimental groups, only the birds fed the 3 percent alfalfa meal diet were significantly lighter at 3 weeks of age than the basal fed group.

TABLE VIII

THE EFFECTS OF NICARBAZIN AND SOURCE OF VITAMIN K ON BLOOD CLOTTING TIME AND MORTALITY FROM CECAL COCCIDIOSIS - TRIAL 4

Diet	Average Blood[a] Clotting Time	Mortality
	Seconds	Percent
Basal + .0125% Nicarbazine + 1 gm. Klotogen F per ton	402.36	0.0
3% Alfalfa	437.97	10.5
5% Litter	445.18	5.3
1 gm. Klotogen F per ton	471.08	10.0
0.0125 % Nicarbazin	1,942.40	5.0
Basal Diet	2,227.67	89.5
3% Fish Meal	2,763.92	93.7

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

The average weight of all surviving inoculated birds at 14 days post inoculation was 508.98 grams, while the average weight of all non-inoculated birds was 545.49 grams. Statistically, this difference was highly significant.

The effects of Nicarbazin and the various sources of vitamin K activity on weight gain are summarized in Table IX.

No significant difference in feed conversion was observed between groups of birds fed the seven experimental diets. Feed conversion figures obtained at time of inoculation and 14 days post inoculation are presented in Table X.

TABLE IX

THE EFFECT OF NICARBAZIN AND VITAMIN K ON WEIGHT GAINS OF CHICKS - TRIAL 4

Diet	3 Week Weights[a]	5 Week Weights[a]
	Grams	Grams
3% Fish Meal	271.44	572.00
Basal	269.90	550.60
5% Chick Litter	264.77	547.32
Basal + 1 gm. Klotogen F/ton	257.74	535.46
3% Alfalfa Meal	253.85	536.00
Basal + 1 gm. Klotogen F/ton + 0.0125% Nicarbazin	248.72	501.75
Basal + 0.0125% Nicarbazin	240.90	500.84

[a]Means not grouped by the same line are significantly different at the 5 percent level of probability.

TABLE X

THE EFFECTS OF NICARBAZIN AND VITAMIN K
ON FEED CONVERSION - TRIAL 4

Diet	3 Weeks[a]	5 Weeks[a]
	Feed/wt.	Feed/wt.
Basal	1.41	1.98
Basal + 1 gm. Klotogen F/ton	1.45	1.99
3% Fish Meal	1.43	1.97
5% Litter	1.42	2.03
3% Alfalfa Meal	1.55	2.11
Basal + 0.0125% Nicarbazin	1.52	2.08
Basal + 0.0125% Nicarbazin + 1 gm. Klotogen F/ton	1.51	2.16

[a]Means grouped by the same line are not significantly different at the 5 percent level of probability.

SUMMARY

A total of 1560 chicks were employed in four trials designed to determine the effects of vitamin K on mortality, morbidity, and blood clotting time of chicks experimentally infected with cecal coccidiosis.

When vitamin K was added to a vitamin K-low diet, blood clotting time was significantly reduced. Inoculated chicks with a short blood clotting time suffered a lower percentage mortality from coccidiosis than did those with a prolonged blood clotting time.

Morbidity, as measured by decreased growth rate during cecal coccidiosis was unaffected by the addition of vitamin K supplements to the vitamin K-low basal diet.

Mortality from cecal coccidiosis in groups of birds inoculated with Eimeria tenella was significantly reduced by the addition of 0.36 grams of Klotogen F per ton of basal diet. Mortality was further reduced in groups inoculated with 500,000 oocysts by supplying either 1.08 or 9.72 grams of Klotogen F per ton of basal diet.

Blood clotting time was significantly reduced by supplying 0.36 grams of Klotogen F per ton of basal diet. Further reduction of blood clotting time was not attained by supplying either 1.08 or 9.72 grams of Klotogen F per ton of basal diet.

The fact that mortality in groups of birds inoculated with 500,000 oocysts was not minimized by supplying 0.36 grams of Klotogen F per ton of diet, even though blood clotting time was reduced to an apparent minimum, suggest that 0.36 grams of Klotogen F supplied a marginal level of vitamin K activity.

When compared with groups of birds fed the basal diet, those

fed comparable diets including one gram of Klotogen F per ton of diet, a combination of one gram of Klotogen F per ton and 0.0125 percent Nicarbazin, 3 percent alfalfa meal, or 5 percent autoclaved poultry litter, respectively, had significantly shorter blood clotting times and less mortality resulting from the experimentally induced cecal coccidiosis.

Mortality in inoculated groups was significantly less in groups receiving 0.0125 percent Nicarbazin in the basal diet, even though blood clotting time was as prolonged as that of the groups fed the basal diet. No mortality occurred in inoculated groups fed the basal diet supplemented with both Nicarbazin and Klotogen F.

Menhadden fish meal, supplied at a level of 3 percent of the total ration, was ineffective as a source of vitamin K for the reduction of blood clotting time and mortality from cecal coccidiosis.

Weight gain and feed efficiency were unaffected when sources of vitamin K were added to the diets of either cecal coccidiosis infected or noninfected birds. Diets containing Nicarbazin, both with and without supplemental vitamin K, significantly retarded growth rate when compared with the basal diet.

REFERENCES

REFERENCES

Almquist, H. J. and A. A. Klose, 1939a. Antihemorrhagic activity of 2-methyl-1, 4-napthaquinone. J. Biol. Chem. 130:787-789.

Almquist, H. J. and A. A. Klose, 1939b. Determination of the antihemorrhagic vitamin. Biochem. J. 33:1055-1060.

Almquist, H. J. and E. L. R. Stokstad, 1935. Hemorrhagic chick disease of dietary origin. J. Biol. Chem. 111:105-113.

Almquist, H. J. and E. L. R. Stokstad, 1936. Factors influencing the incidence of dietary hemorrhagic disease in chicks. J. Nutrition 12:329-335.

Almquist, H. J. and E. L. R. Stokstad, 1937. Assay procedure for vitamin K (antihemorrhagic vitamin). J. Nutrition 14:235-240.

Anderson, G. C., J. H. Hare, J. K. Bletner, C. E. Weakley, Jr., and J. A. Mason, 1954. A hemorrhagic condition in chicks fed simplified rations. Poultry Sci. 33:120-126.

Baker, H. R. and D. C. Jaquett, 1953. Observations concerning the hemorrhagic syndrome of poultry. Proc. N. E. Conf. Lab. Workers in Pullorum Disease 25:103-104.

Baldwin, F. M., O. B. Wiswell, and H. A. Jankiewicz, 1941. Hemorrhagic control of Eimeria tenella infected chicks when protected by antihemorrhagic factor, vitamin K. Proc. Soc. Exp. Biol. Med. 48:278-280.

Bletner, J. K., G. C. Anderson, C. E. Weakley, and J. H. Hare, 1953. A hemorrhagic condition in chicks. W. Va. Ag. Exp. Sta. Bull. 363, part 2 pp. 8ff.

Bornstein, S. and Y. Samberg, 1954. Field cases of vitamin K deficiency in Israel. Poultry Sci. 33:831-836.

Charles, T. B., 1921. Practical experience in the control of coccidiosis in young chicks. Poultry Sci. 1:51-55.

Cover, M. S., W. J. Mellen, and E. Gill, 1955. Studies of hemorrhagic syndrome in chickens. Cornell Vet. 45:366-386.

Cuckler, A. C. and C. M. Malanga, 1956. The effects of Nicarbazin on the development of immunity to avian coccidia. J. Parasitol. 42: 593-607.

Dam, H., 1935. The antihaemorrhagic vitamin of the chick. Occurrence and chemical nature. Nature 135:652-653.

Dam, H., and F. Schonheyder, 1934. A deficiency disease in chicks resembling scurvy. Biochem. J. 28:1355-1359.

Dam, H., F. Schonheyder, and E. Tage-Hansen, 1936. Studies on the mode of action on vitamin K. Biochem. J. 30:1075-1079.

Duncan, D. B., 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Foster, A. O., 1949. The economic losses due to coccidiosis. Ann. N. Y. Acad. Sci. 52:434-442.

Frost, D. V., H. S. Perdue, and H. C. Spruth, 1957. Vitamin K as related to hemorrhage in poultry. Feedstuffs 29, No. 14, pp. 18-28.

Frost, D. V. and H. C. Spruth, 1955. Control of hemorrhagic condition in chickens with menadione sodium bisulfite. Poultry Sci. 34:56-64.

Goldhaft, T. H., 1955. The pathology of hemorrhagic disease in poultry. Proc. Semi-Annual Meeting of Nutrition Council. Feed Mfgers. Assoc. Nov. 27-29, pp. 17-20.

Grumbles, L. C., J. P. Delaplane, and T. C. Higgens, 1948. Continuous feeding of low concentrations of sulfaquinoxaline for the control of coccidiosis in poultry. Poultry Sci. 27:605-608.

Grumbles, L. C., B. A. Tower, W. T. Oglesby, and C. W. Upp, 1949. Field observations on the use of sulfaquinoxaline for the control of coccidiosis in young chicks. Ann. N. Y. Acad. Sci. 52:558-561.

Harms, R. H. and R. L. Tugwell, 1956. The effect of experimentally induced prolonged blood clotting time on cecal coccidiosis of chicks. Poultry Sci. 35:937-938.

Herrick, C. A., 1936. Organ specificity of the parasite Eimeria tenella J. Parasitol. 22:226-227.

Herrick, C. A., G. L. Ott, and C. E. Holmes, 1936. The chicken as a carrier of the coccidia Eimeria tenella. Poultry Sci. 15:322-325.

Jungherr, E. L. and J. D. Winn, 1949. Continuous low level sulfaquinoxaline feeding in the practical control of coccidiosis in broilers. Ann. N. Y. Acad. Sci. 52:563-570.

Levinson, S. A. and R. P. MacFate, 1951. Clinical Laboratory Diagnosis, Fourth Edition. Lea and Febiger, Philadelphia. p. 511.

McKee, R. W., S. B. Binkley, D. W. MacCorquodale, S. A. Thayer, and E. A. Doisey, 1939. The isolation of vitamins K_1 and K_2. J. Am. Chem. Soc. 61:1295.

National Research Council Subcommittee on Poultry Nutrition, 1954. Recommended Nutrient Allowances for Poultry, Washington, D. C.

Norris, L. C. and T. S. Nelson, 1955. The hemorrhagic syndrome of the chick. Proc. Semi-Annual Meeting of Nutrition Council. Am. Feed Mfgers. Assoc., Nov. 27-29. pp. 17-20.

Otto, G. F., H. A. Jeske, D. V. Frost, and H. S. Perdue, 1958. Menadione sodium bisulfite complex (Klotogen F) in cecal coccidiosis. Poultry Sci. 38: 201-205.

Perdue, H. S., H. C. Spruth, and D. V. Frost, 1957. Comparison of vitamin K activity of vitamin K_1 and Klotogen (menadione sodium bisulfite complex). Poultry Sci. 36:633-635.

Pratt, I., 1937. Excystation of the coccidia, Eimeria tenella. J. Parasitol. 23:426-427.

Schonheyder, F., 1935. The antihaemorrhagic vitamin of the chick. Measurement and biological action. Nature 135:653.

Schonheyder, F., 1936. The quantitative determination of vitamin K. Biochem. J. 30:890-896.

Seeger, K. C., 1949. The course of caecal coccidiosis epidemics in broilers in Delaware. Ann. N. Y. Acad. Sci. 52:458-459.

Shelton, D. C., G. C. Anderson, T. A. Clark, and C. E. Weakley, Jr., 1956. Studies on the vitamin K requirement of the chick. Abstracts of papers, 45th Annual Meeting, Poultry Sci. Assoc., Aug. 7-10. p. 43.

Snedecor, G. W., 1956. Statistical Methods. Fifth Edition, The Iowa State College Press, Ames, Iowa.

Tidrick, R. T., F. T. Joyce, and H. P. Smith, 1939. Vitamin K deficiency and prothrombin levels. Effects of vitamin K administration. Proc. Soc. Exp. Biol. Med. 42:853-857.

Tugwell, R. L. and R. H. Harms, 1957. Unpublished Data. University of Tennessee.

Tyzzer, E. E., 1927. Species and strains of coccidia in poultry. J. Parasitol. 13:215.

Tyzzer, E. E., 1929. Coccidiosis in gallinaceous birds. Am. J. Hyg. 10:269-383.

Tyzzer, E. E., H. Theiler, and E. E. Jones, 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. Am. J. Hyg. 15:319-393.

Yacowitz, H., E. Ross, V. L. Sanger, E. N. Moore, and R. D. Carter, 1955. Hemorrhagic syndrome in chickens fed normal rations supplemented with sulfaquinoxaline. Proc. Soc. Exp. Biol. Med. 89:1-7.