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Effects of Additional Dietary Levels of Folic Acid, Biotin, Vitamin K, and Selenium on Egg Production, Fertility, Hatchability, Early and Late Embryonic Mortality in White Pekin Duck Breeders

presented by

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EFFECTS OF ADDITIONAL DIETARY LEVELS OF FOLIC ACID, BIOTIN, VITAMIN K AND SELENIUM ON EGG PRODUCTION, FERTILITY, HATCHABILITY, EARLY AND LATE EMBRYONIC MORTALITY IN WHITE PEKIN DUCK BREEDERS

By

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ABSTRACT

EFFECTS OF ADDITIONAL DIETARY LEVELS OF FOLIC ACID, BIOTIN, VITAMIN K AND SELENIUM ON EGG PRODUCTION, FERTILITY, HATCHABILITY, EARLY AND LATE EMBRYONIC MORTALITY IN WHITE PEKIN DUCK BREEDERS

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This study was conducted to determine the effect of the addition of folic acid, biotin, vitamin K or selenium (above requirements and/or above the amount already used in commercial diets) on production, fertility and hatchability of White Pekin duck eggs.

Beginning on October 4, 1982 through March 31, 1983 (a 24 week period) a total of 435 (four hundred and thirty five) White Pekin duck breeders was used for this experiment. Ducks were housed in three pens per treatment with 29 ducks in each pen. Daily egg production data and mortality records were maintained. During the last three consecutive days of each 28 day period eggs were collected three times per day for fertility and hatchability tests.

The results indicated that none of the treatment groups had any significant improvement ($P < 0.01$) over the control group. However, mortality was significantly higher in the control group when compared to the group supplemented with vitamin K ($P < 0.01$).

To my beloved and loving mother and father

who are no more between us, but

their presence is always felt

in the utmost of my being, I

dedicate this thesis.

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I. INTRODUCTION

Although nutrition alone is not responsible for decreased performance in avian species, it has been established that nutritional deficiences play a major role and cause abnormal embryonic development. B vitamins and fat-soluble vitamin E, zinc and manganese among trace minerals have been reported by Couch and Ferguson (1972) to contribute to these deficiences. According to Couch and Ferguson (1972) the poultry industry is still losing 5-10% of all eggs set due to embryonic mortality at different stages of the incubation period. Hatchability for total eggs set in commercial broiler breeder flocks ranges between 80-85% and approximately 10% of all eggs set are infertile. Apart from nutrition, other factors such as genetic lethals and physiological derangements are also responsible for this loss. It has been estimated that a 1% increase in the hatchability of duck eggs could be worth $50,000 profit annually to a company hatching 5,000,000 ducks (Flegal, 1983; personal communication).

Intensive investigation in the past three decades has been conducted on chickens and turkeys to determine their nutritional requirements, but there are very few studies available on the nutritional requirements of duck. Except for the few nutrients listed by the NRC (1977) most of the requirement are estimated values determined for chicken breeders. Available evidence suggests that these values are applicable to ducks. The lack of information

can be due to the low commercial demand for ducks compared to the higher production of chickens and turkeys. According to USDA (1984) the total number of chickens and turkeys produced in 1983 was 4,500,000,000; and 170,000,000, respectively. In the same period of time, only 15,000,000 ducks were produced (Flegal, 1984, personal communication).

This study was initiated due to a variation in hatchability reported by Maple Leaf Duck Farms, the largest duck producer nationwide. Hatchability of duck eggs from several different farms in several states owned by this company was reported to range from 40% to 85%. These variations were thought to be due to nutritional deficiences, management and/or egg handling problems. Analysis of the diets revealed that in some cases the level of certain nutrients, such as biotin and iron, were below NRC values (1977) and/or the recommendations of Scott et al. (1982). It should be noted that Scott et al. (1982) have not listed any recommendations for duck breeders, thus the comparison was mostly made with hen breeder requirements. Poultry World (1981) has listed a complete set of duck nutritional requirements at different stages of life.

II. OBJECTIVES

The objectives of this research were:

1. What, if any, is the effect of additional folic acid, biotin, vitamin K and selenium on egg production, fertility, hatchability and embryonic mortality in White Pekin duck breeders?
2. Is there any significant difference between any of the treatment group versus control group in any of the parameters measured?

III. REVIEW OF LITERATURE

A. Folic Acid

Folic acid and vitamin B_{12} are two B-complex vitamins which are closely related in certain aspects of their biological function. In general, a deficiency of folic acid or vitamin B_{12} can result in what is clinically known as macrocytic anemia and megaloblastic bone marrow. This anemia is caused by a halt in DNA synthesis and cell division in erythrocyte precursors in bone marrow.

Wells first reported this disease in 1931 in a woman patient in Bombay, India, and called it Tropical Macrocytic anemia. This disease was mostly seen in pregnant women and the administration of concentrated extract of autolyzed yeast, four grams two to four times daily, relieved the patient. Day and associates of the Arkansas Medical School (see Scott et al., 1982) found similar results when dealing with anemic monkeys and called the active ingredient in the yeast vitamin "M". Other names such as factor 'U', factor 'R' and vitamin B_c were also given to this vitamin which prevented anemia (Scott et al., 1982).

Folic acid, folacin and folate are general terms used to describe a group of closely related compounds containing the basic structure, pteroylglutamic acid (PGA) (Figure 1). In addition, the name folic acid is used specifically for pteroylglutamic acid. This chemically synthesized vitamin form, pteroylglutamic acid, is composed of pteridine, para amino benzoic acid, and L-glutamic acid, and is fully active and available to living organism for reduction and conversion to the co-enzyme form required in metabolism (Stokstad and Thenen, 1972).

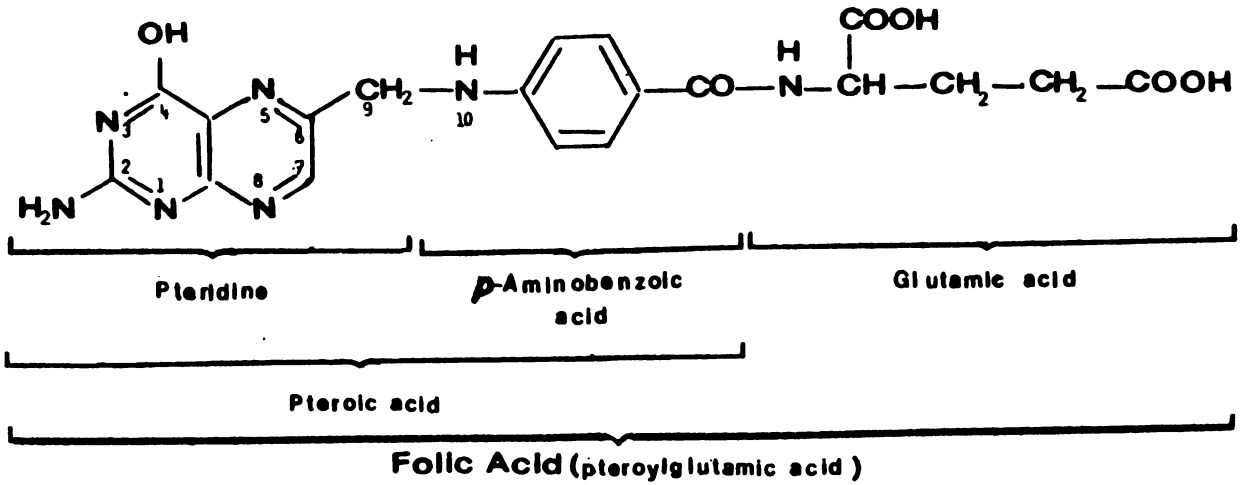


Figure 1. Structure of folic acid compound.

Folic acid derivaties function biochemically as coenzymes in several metabolic pathways involving the transfer of single carbon units at various levels of oxidation. Specific reactions involving single carbon transfer by folic acid compounds are: a) serine and glycine interconversion; b) as a source of C_1 units of glycine α-carbon in synthesis of different compounds, c) degradation of histidine; d) purine synthesis; and e) methyl groups synthesis for compounds such as methionine, thymine and choline.

Dietary sources for folic acid can be found in meat by-products, soybeans, wheat germ and yeast (Toepfer et al., 1951). Recent evidence suggests that many feedstuffs may contain less folic acid than previously reported. Scott et al. (1982) reported that free acid is in limited quantity in natural products and the major portion of folic acid is in the conjugated form with two or more glutamic acid residues joined in α-glutamyl linkages to the single glutamic acid moeity of the vitamin.

The need for folic acid in the diet of the poult for growth and prevention of cervical paralysis has been noted by Richardson et al. (1945); Jukes et al. (1947); Russell et al. (1947); and Lance and Hogan (1948). Schweigert et al (1948) fed 0.12 mg/kg and 0.42 mg folic acid per kg of ration in turkey breeder rations. There was no difference in egg production or hatchability, however the poults from the hens fed the ration low in folic acid needed more of the vitamin for growth than did the control poults. Titus and Fritz (1971) have reported that hatchability of fertile eggs may sometimes be increased by addition of folic acid to the breeder diet. Some investigations have reported on mortality and the incidence of malformations in chicken embryos (Cravens and Halpin, 1949). The deficiencies were

induced either by feeding folic acid deficient rations or natural feedstuffs (Taylor, 1947), or purified ingredients (Sunde et al., 1950), or were induced artificially in the egg by the injection of a folic acid antagonist (Karnofsky et al., 1949). Taylor (1949) demonstrated that folic acid is essential for hatchability. His investigations revealed that a high proportion of embryos from deficient hens died during the latter stage of development, usually about the seventeenth day of incubation. The embryo may also die soon after pipping the air cell (Sunde et al., 1950). These researchers also showed that injection of five micrograms or more of folic acid into the egg prior to incubation resulted in a marked increase in hatchability. Hatchability was also markedly improved by injecting folic acid as late as the seventeenth day of incubation. When turkey hens were fed a diet deficient in folic acid, embryos in eggs died at 26-28 days of development and the embryos were reduced in size (Wilgus et al., 1939). NRC (1977) have reported that folic acid deficiency reduces egg production and hatchability. Findings of Kratzer et al. (1956) confirmed the studies made by Wilgus et al. (1939) and they concluded that a folic acid deficient diet had no effect on egg production. They too, had high mortality in turkey embryos just prior to hatching time.

Requirments:

There seem to be different opinions among researchers regarding folic acid requirements. Taylor (1947) reported that 0.12 mg of folic acid/kg diet was essential to support normal egg production in Single Comb White Leghorn (SCWL) yearling hens and a somewhat larger quantity was necessary for satisfactory hatchability. In that experiment

the greatest increase in embryonic death rate occurred in the last four days of incubation. Jukes et al. (1947) and Scott et al. (1948) showed that the requirement for the poult was 0.8 mg/kg diet. Schweigert et al. (1948) fed a basal diet containing 0.42 mg PGA (folic acid)/kg diet to turkey and chicken hens and observed no detrimental effects on egg production, hatchability, hemoglobin level or general appearance as compared to a level of 2.0 mg/kg diet. However, the group receiving the basal diet had a markedly decreased PGA content in the eggs. Craven and Halpin (1949), in their studies, have shown that the folic acid requirement for normal hatchability in SCWL pullets was not over 0.25 mg/kg diet for the laying hen and 0.5-1.0 mg/kg diet for breeding hens. Further, they concluded that pteroylglutamic acid synthesis in the intestinal tract of the breeding hen is favoured by diets containing dextrin. The requirement for folic acid indicated by Craven and Halpin (1949) is in agreement with the finding of Sunde et al. (1950). Further, Sunde et al. (1950) observed high embryonic mortality at two critical periods (before the fifth day of incubation and 18-21 days of incubation) with a basal diet containing 0.08 mg folic acid/kg diet. A high percentage of the embryos pipped the air cell but were unable to break the shell. They suggested that there was an increased synthesis of folic acid in the intestinal tract of hens fed diets containing dextrin, and that dextrin basal diets allowed the deposition of more folic acid in the eggs than a diet that contained sucrose. This has been questioned by Harwood et al. (1976) who found no differences in the folic acid requirements of chicks fed sugar or starch. Bird et al. (1954) concluded that the requirement of folic acid for chicks should be 0.55 mg/kg diet and for breeders 0.35 mg/kg diet. In their experiment they had good egg production but very

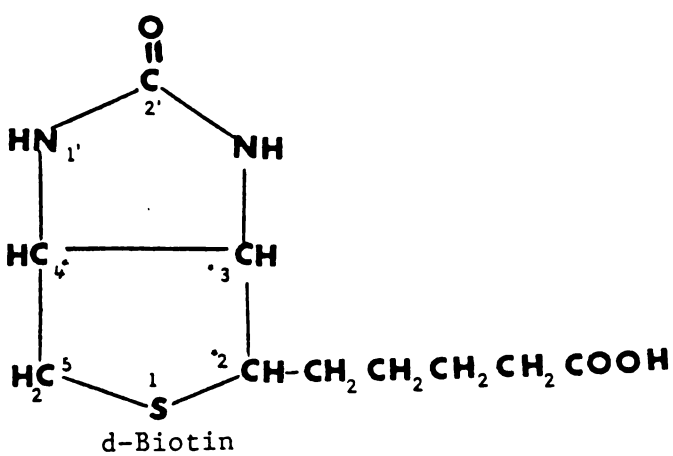
low hatchability and this indicates that the requirement for hatchability exceeds that for egg production. Kratzer et al. (1956) suggested that the requirement for hatchability in turkey breeder hens is 0.7 mg/kg diet. Wong et al. (1977) have suggested that folic acid requirements for the broiler chick is based on dietary composition. With 0.7% glycine in a casein diet, the folic acid requirement is between 0.34 and 0.49 mg/kg. But with a casein-gelatin basal diet, the folic acid requirement was found to be between 0.12 and 0.27 mg/kg diet.

The NRC (1977) lists 0.35 mg of folic acid/kg diet for chicken breeding hens and 1.0 mg folic acid/kg diet for breeding turkeys which is somewhat higher than the folic acid requirement listed for chickens. No requirement is listed by the NRC (1977) for breeder ducks. Poultry World (1981) recommended 1.0 mg/kg diet for duck breeders.

B. Biotin

Biotin, a water-soluble B-complex vitamin is one of the most interesting vitamins. At first it was known as one of the unidentified factors required for life in yeast and other microorganisms. These unidentified factors were also known as "Bios". Initially, these mysterious factors were characterized by several people in several different ways. Allison et al. (1933) found this factor to be necessary for respiration in Rhizobia (legume nodule bacteria) and named it co-enzyme R. Kögl and Tonnis (1936) were able to extract a crystalline substance from the boiled yolk of duck eggs and found it to be an extremely potent source of "Bios 2" necessary for the growth of yeast. Gyorgy et al. (1940) studied a toxic condition which

occurred in animals fed raw egg white. They called the factor which prevented this condition vitamin H. They were also able to show that vitamin H, co-enzyme "R" and "Bios 2" were all the same substance. In 1942, Du Vegneaud et al. announced the structure and properties of this substance and called it biotin. It was synthesized by Harris and Associates of Merek in the following year. The structure of d-Biotin (Figure 2) is the active form of the compound. It is monocarboxylic acid containing a cyclic urea structure with sulfur in a thio ether linkage. Biotin is important in numerous vital mechanisms involving carboxylation and decarboxylation reactions. Therefore, it is important in carbohydrate metabolism, fatty acid synthesis, protein synthesis, amino acid deamination, purine synthesis and nucleic acid metabolism. As a result, it affects thyroid and adrenal function, reproductive performance, the nervous system and cutaneous system.



d-Biotin

Figure 2. Structure of biotin.

Sources of biotin:

Eggs, liver, yeast and peanuts are the richest sources of natural biotin. Green leafy plants, oil seed meals and alfalfa meal are good to excellent sources. Animal protein supplements are better sources of biotin than the oil seed meals. All grains are relatively poor sources of biotin although a two fold difference was found among the different grains tested (Anderson and Warnick, 1970). Biotin is found as bound and in free-forms, but much of the bound form is unavailable to animals. Patrick et al. (1942) stated that it is possible for practical poult diets to be deficient in biotin as most of the biotin is not biologically available.

In chickens, biotin deficiency can be induced simply by feeding biotin-low diets but in mammals such as rats, it is difficult to produce biotin deficiency because the major portion of this vitamin is provided by intestinal synthesis. Patrick et al. (1942) showed that a deficiency resulted in perosis and a dermatitis that appeared first on the foot pads. Jensen (1967) reported that egg production increased as biotin was added to the chicken diet. However, Arends et al. (1971) found no significant effects in egg production at different levels of biotin. Titus and Fritz (1971) concluded that biotin is required for hatchability and any deficiency in adult laying and breeding hens will cause a reduction in hatchability without affecting egg production. Brewer and Edwards (1972) added 0, 0.02, 0.04, 0.08, 0.160 and 0.320 mg biotin/kg of basal diet (containing 0.02 mg biotin by analysis) and compared these diets to a practical corn-soy diet (containing 0.185 mg biotin/kg diet) fed to laying hens. There was a significant difference in egg production between the semi-purified diets and the practical type diet, with the practical diet producing

better results. Fertility was not significantly affected. Biotin deficiency in hens caused a sharp decrease in hatchability which is in agreement with findings of Cravens et al. (1942). Cravens et al. (1944), in their experiment, candled the eggs on the sixth, thirteenth and seventeenth day at which time the infertile eggs and dead embryos were removed. In their findings, they associated biotin deficiency with an increase in the embryonic mortality during the first week of incubation with a peak about the third day. A second peak of mortality due to biotin deficiency occurred during the last three days of incubation. Maternal diet should be supplied with adequate biotin and any deficiency will result in a peak of embryonic mortality at approximately 72 hours of incubation (Cravens et al., 1944; Couch et al., 1948). Couch and Ferguson (1972) have indicated that in chicken fed a biotin deficiency diet, the highest percentage of embryonic death occurred during the third period of embryonic development (18th through 21st day). Titus and Fritz (1971) have concluded that biotin content of the eggs is another factor in determining the rate of embryonic mortality. In eggs that contain as little as 50 nanograms of biotin per gram egg, there will be a failure of embryonic development. Eggs which contain 150 nanograms or more of biotin per gram egg will support normal embryomic development.

Requirements:

Titus and Fritz (1971), NRC (1977) and Scott et al. (1982) recommended the level of 0.15 mg biotin/kg diet for chicken breeders. Poultry World (1981) recommended 0.08 mg biotin/kg diet for duck breeders.

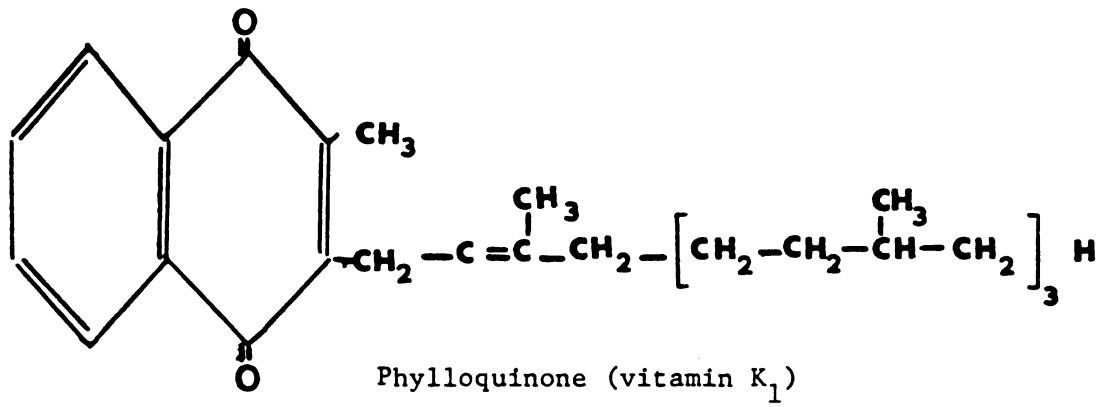
C. Vitamin K

Of the four fat soluble vitamins, vitamin K was the last one to be discovered (Suttie, 1978). Initially this vitamin was identified by Henrik Dam in 1935 as a blood clotting factor needed by chicks fed a fat-free diet during studies on cholestrol metabolism. It was due to this discovery that Dam received the Nobel Prize and this vitamin was named after the first letter of the German word Koagulation.

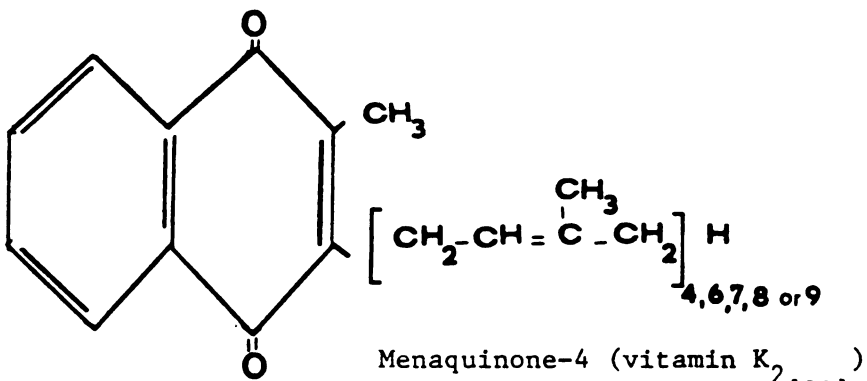
Vitamin K is now recognized as an integral factor involved in clotting, including prothrombin or factor II. However, as the gut microflora in most species can synthesize much of the required vitamin K, deficiency is not a major problem in species other than poultry and sometimes in cattle (the latter suffering from sweet clover poisoning). Originally vitamin K deficiency was confused with ascorbic acid deficiency, but continued research and effort isolated this compound from alfalfa and decaying fish meal. This led to the discovery that the active compound was a quinone. Three commonly recognized forms of vitamin K are shown in Figure 3.

Sources of Vitamin K:

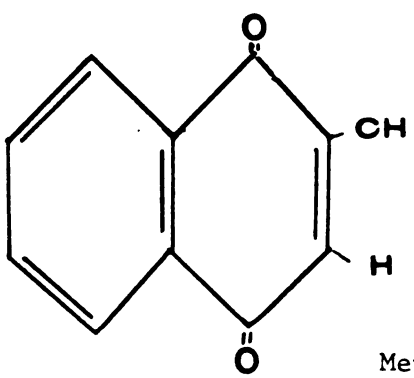
Plant food sources, such as green leafy vegetables (alfalfa and spinach), are major natural sources of phylloquinone (vitamin K_1). Vitamin K_2 (prenyl-minaquinones) is produced by the bacterial flora in animals. This form is important in human and most other mammals in providing the vitamin K requirements. However, the chick does not benefit much from intestinal microbial synthesis for its requirements for vitamin K. Biologically active synthetic vitamin K is produced commercially on a large scale. Most of the vitamin K is converted to vitamin K_2 in the liver, vitamin K_2 may be the metabolically active form of vitamin K in animals (Scott et al., 1982).



Phylloquinone (vitamin K_1)



Menaquinone-4 (vitamin $K_{2[20]}$)



Menadione (vitamin K_3)

Figure 3. Chemical structures of the vitamin K compounds.

Vitamin K is lipid-soluble and depends on the presence of dietary fat and bile salts for optimum absorption and utilization by the body. Whiteside et al. (1965) in their studies fed cholestyramine, a bile-acid sequestrant, to the chicks and reduced absorption of vitamin K. They concluded that formation of lipid bile salt micelles is needed for optimum absorption. The simplest form of the vitamin, menadione, is passively absorbed in the distal portion of the small intestine and to a limited degree in the colon. Once absorbed, vitamin K is carried in chylomicrons and transported via the lymph and peripheral circulation to the liver. Griminger and Brubacher (1966) showed that when they fed phylloquinone to chicks, the major portion of it was absorbed and deposited intact in the liver intact. There are two ways to measure vitamin K deficiency; blood clotting time and prothrombin time. Prothrombin time is a more accurate measure of vitamin K deficiency. Normal prothrombin time is 17-20 seconds in chick blood (Scott et al., 1982) and in severely deficient chicks this time extends to 5-6 minutes or longer. Dean (1972) showed that when no vitamin K was added to a vitamin K deficient basal diet the prothrombin time was 83.7 seconds in ducklings. Minimum prothrombin times were obtained when vitamin K was supplemented at a level of 0.4 mg/kg diet.

Requirements:

The requirement for vitamin K in chicken diets depends on the presence or absence of stress producing agents such as sulfaquinoxaline or other drugs. Dean (1972) added 0.05% sulfaquinoxaline to a basal diet in ducklings and the mortality increased to 67% and the prothrombin time was shifted to 94.2 seconds. Adding 0.5 mg MPB (menadine dimethylpyrimidinol)/kg diet reduced the mortality to 9% and

the prothrombin time to 41.5 seconds. Addition of 5.0 mg MPB/kg diet to the same diet caused the mortality and prothrombin time to return to a near normal value. There was no significant difference between prothrombin time of ducklings fed vitamin K, and those fed MPB.

The requirement for vitamin K in the absence of stress agents for chicks is 0.8 mg/kg diet (Griminger, 1964) or 0.6 mg/kg (Scott et al., 1982). Griminger (1965) has shown that in a vitamin K deficient diet, the requirement increased to 2 mg/kg diet with 0.2% sulfaquinxoaline, and according to studies by Scott et al (1982) the requirement increased ten fold in the presence of these agents or drugs. Further, Scott et al. (1982) suggested that adding a dietary source of phylloquione such as alfalfa meal is desirable. Disease, too, has a similar effect on the vitamin K requirement of chickens. Squibb (1964) obtained increased prothrombin times indicating a higher vitamin K requirement when chickens were infected with the early stage of Newcastle disease.

The degree of carry-over of vitamin K from the hen through the egg to the newly hatched chick is the most important factor which affects the vitamin K requirement of the chicken (Scott et al., 1982). Hemorrhagic syndrome in day-old chicks can be due to a vitamin K deficient diet fed to the parent flocks (Kohane et al., 1960). The symptoms of the deficiency appear after two to three weeks in hens fed a vitamin K deficient diet. In areas such as the breast, legs and wings hemorrhages can occur and in severe cases the bird may bleed to death from the slightest bruise or other injury. Griminger (1964) showed that there was little embryonic mortality before the 18th day of incubation, but most embryos died between the 18th and 21st day of the incubation period from the dams not receiving supplemental vitamin K.

Shelton et al. (1956) reported that the chicks requirement for menadione was 1.20 and 1.39 mg/kg diet in diets containing 5.7% and 2.7% of fat, respectively, and 0.20 mg/kg diet for menadione sodium regardless of fat level; however, Perdue et al. (1957) observed that 0.40 mg of vitamin K_1/kg diet was insufficient. Nelson and Norris (1960) concluded that the requirement for vitamin K_1 in a chick diet at 2 and 4 weeks of age was 0.524 $\pm$ 0.46 and 0.528 $\pm$ 0.01 mg/kg of diet, 0.0588 $\pm$ 0.284 and 0.479 $\pm$ 0.140 mg/kg diet for menadione, and 0.305 $\pm$ 0.051 and 0.357 $\pm$ 0.036 mg/kg diet for menadione sodium bisulfite complex (MSBC). The NRC (1977) recommended 0.5 mg vitamin K/kg diet for chicken breeders. For turkeys breeders the amount listed is 1.0 mg/kg diet. Poultry World (1981) recommended vitamin K at a level of 2 mg/kg diet for duck breeder.

D. Selenium

There are currently available good reviews on selenium (Underwood, 1977; Burk, 1978). This trace element is a divalent cation found in the periodic table as a member of the family which includes oxygen and sulfur. This element was discovered over one hundred years ago by Berzilius (Scott et al., 1982). However, it was more well known for the toxic effects it produced which were characterized by malformation in chicks and mammalian embryos. Although selenium is required in the smallest amount of any of the generally accepted trace elements, it is also the most toxic. In 1957, it was discovered that selenium at levels of 0.05 to 0.2 was essential in poult nutrition. Selenium protects poultry against exudative diathesis and pancreatic fibrosis (Thompson and Scott, 1969; Scott, 1978) and gizzard and heart myopathies in turkeys (Scott and

Cantor, 1972). A nutritional need for selenium has been demonstrated in ducks by several investigators (Jager, 1972; Yarrington et al., 1973; Moran et al., 1974).

Sources of Selenium:

Since the Food and Drug Administration restricts the supplementation of commercial feeds with selenium compounds, it is of great importance to identify the selenium content in the common feedstuffs used for formulation of poultry diets. The best sources for selenium among the common poultry feedstuffs are tuna, herring fish meal and dried sardine solubles although the biological availability is poor (Scott et al., 1982). Scott and Cantor (1972), in their studies have shown that selenium in tuna meal is readily digested and absorbed but it is only about one third effective in comparison to sodium selenite. The selenium content of the feeds is dependent to a large extent on the selenium content of the soil in which the plants are grown. Measuring the biological availability is another way to evaluate selenium content in the feedstuffs. Cantor et al. (1975) showed that selenium in most feedstuffs of plant origin was highly available and this availability ranged from 60 to 90% (Table 1) but the value is less than 25% in animal products. Cantor et al. (1975) have shown that average biological availability of selenium in alfalfa is 210% compared to that of the selenium in sodium selenite and is highly digestible and available. According to Gries and Scott (1972) the requirements of the chick for selenium and vitamin E are interrelated. These researchers showed that the two nutrients spare each other. Selenium compounds appear to be involved in some unknown way in a carrier system for vitamin E retention (Desai and Scott, 1965). Schwarz et al. (1957) reported that by adding a small amount of

Table 1. Biological availability of selenium in products of plant and animal origin.[a]

Source	Biological Availability %
Plant	
Wheat	70.7 ± 14.0
Brewer's yeast	88.6 ± 5.5
Brewer's grain	79.8 ± 14.9
Corn	86.3 ± 14.6
Soybean meal	59.8 ± 13.9
Cotton seed meal	86.4 ± 18.6
Dehydrated alfalfa meal	210.0 ± 14.7
Distiller's dried grain plus solubles	65.4 ± 13.5
Animal	
Tuna meal	22.4 ± 7.0
Poultry by-product meal	18.4 ± 4.6
Menhaden meal	15.6 ± 4.4
Fish solubles	8.5 ± 1.5
Herring meal	24.9 ± 6.7
Meat and bone meal	15.1 ± 4.2

[a]Taken from Journal of Nutrition 105:96-105, 1975.

selenium in the diet there was some improvement in vitamin E-deficient animals. Supplementing large amounts of vitamin E could only reduce a little the need for selenium requirements (Thompson and Scott, 1969). Further Thompson and Scott (1969) have shown that selenium deficiency interferes with the utilization of vitamin E. Arnold et al. (1973) reported that Se content of eggs and tissues was most consistently increased with the 1.0 ppm supplement of sodium selenite. Although selenium can spare the vitamin E requirements for prevention of muscular dystrophy (M.D.) in chicks, it cannot prevent M.D. in absence of a low dietary level of vitamin E (Scott et al., 1982).

Requirements:

In the presence of 100 ppm vitamin E, as little as 0.01 ppm of dietary selenium prevents deficiency signs (Thompson and Scott, 1969). Arnold et al. (1973), and the NRC (1977) have recommended 0.1 ppm Se for chickens and 0.2 ppm for turkey breeders. Scott et al. (1982) have listed 0.15 ppm Se for laying and breeder hens. The requirement for duck breeders is 0.15 ppm (Dean and Comb, 1981; Poultry World, 1981).

Deficiency:

Most studies on the role of Se in adult chickens have been concerned with toxicity rather than deficiency. Fertility and hatchability are affected by a Se-deficient diet (Arnold et al., 1973). Cantor and Scott (1974) in their work, found that selenium had an effect on egg production, hatchability and selenium content of eggs. In a practical corn-soybean meal layer diet with no Se added (the amount of Se was 0.02 ppm of naturally occurring selenium), the production fell to 55% after 46 days. When the diet of these birds

was supplemented with 0.1 ppm Se, the production increased and was maintained above 75%. Hatchability of the eggs produced by the bird fed the unsupplemented diet dropped to zero by the 17th week but adding Se in the diet increased hatchability to over 90% of fertile eggs. Most of the embryos from eggs produced by hens fed the Se deficient diet died between day eighteen and day twenty one of incubation, and 25% of them showed symptoms resembling signs of exudative diathesis in growing chicks. Se content of eggs was also affected and was increased from 0.035 to 0.138 ppm when the basal diet had an addition of 0.1 ppm selenium. Latshaw et al. (1977) observed that in the non-supplemented group (containing 0.04 ppm naturally occuring selenium) egg production, early embryonic dead and hatchability of fertile eggs were significantly lower compared to the group where Se was added in the diet.

An excess of Se in the diet (5-20 ppm and above) results in a highly toxic effect (NRC, 1977). Poley and Maxon (1938) showed that hatchability of fertile eggs was not affected when the laying hen ration had 2.5 ppm Se but a ration with 5 ppm selenium slightly reduced the hatchability and 10 ppm Se reduced the hatchability to zero. Small increases in tissue selenium concentration can result from the addition of Se in excess of the nutritional requirement (Dean and Combs, 1981).

IV. Methods and Materials

A. Experimental Procedure

The experiment was conducted at the Michigan State University Poultry Science Research and Teaching Center from October 4, 1982 to March 16, 1983.

Experimental Design:

Four hundred and thirty five, 24-week old White Peking Duck (WPD) were used in this experiment. They were randomly selected and housed in 15 pens. A chart of random numbers was used so that each treatment was distributed randomly (Figure 4). This was done to reduce the chance of having a treatment group in a particular pen be more favorably treated than those placed elsewhere.

Treatments:

The experiment consisted of five treatments with three replicates each. There were 24 females and five males within each replicate with a single tube-type feeder. Feed and water were provided ad libitum. The composition and analysis of the feed used throughout the duration of the experiment are given in table 2. The treatment consisted of five rations as follows:

Group one, diet A (which served as a control since this diet was used commercially)

Group two, diet B (with folic acid added at a level calculated to be 50% above diet A)

Group three, diet C (with biotin added at the level calculated to be 50% above diet A)

Group four, diet D (with vitamin K added at the level calculated to be 50% above diet A)

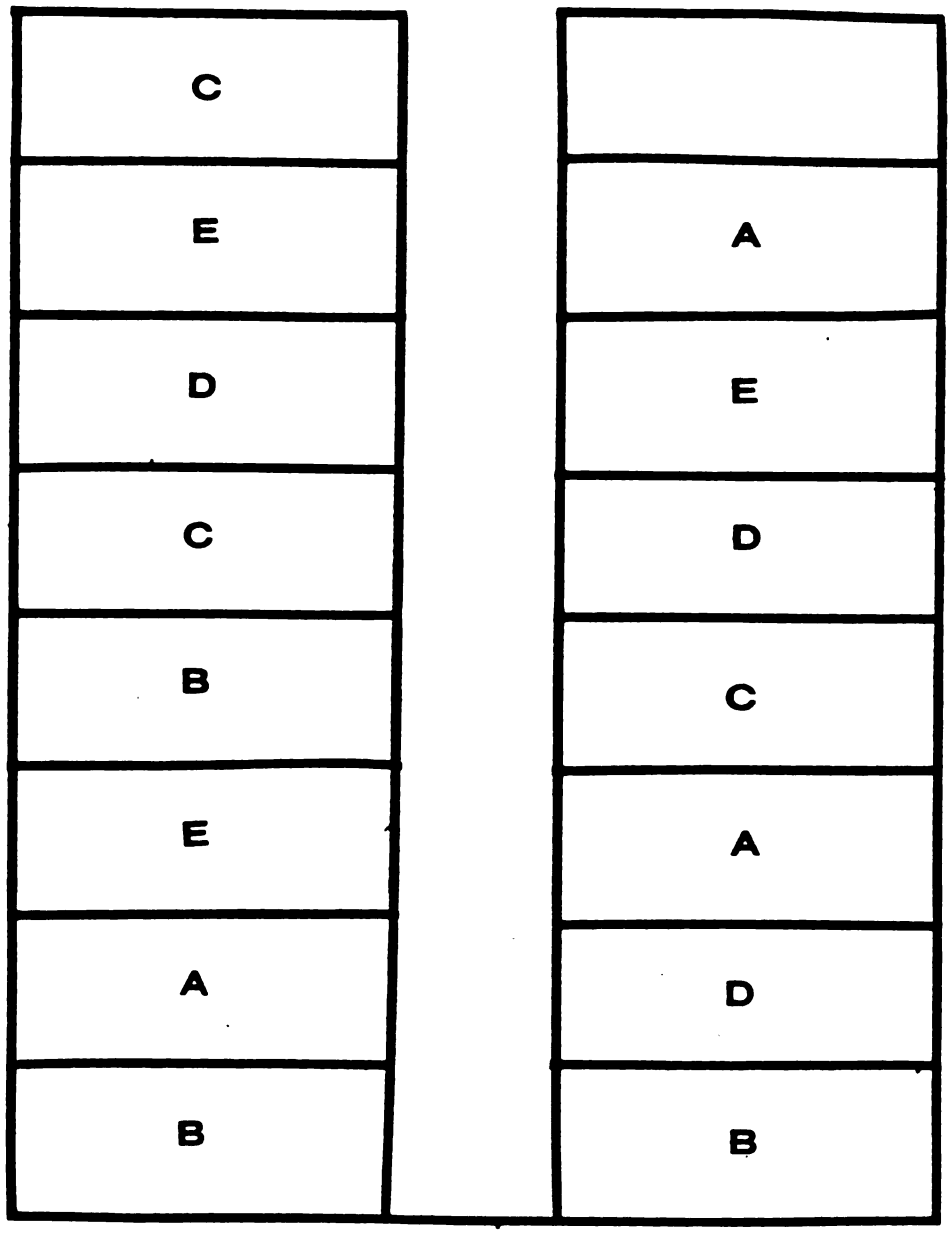


Figure 4. Experimental design and allocation.

Group five, diet E (with selenium added at the level calculated to be 50% above diet A)

The control diet used for this experiment (Table 2) was basically a corn-soybean type diet satisfying all the nutrient requirements of the breeder duck as far as is known. All rations were isocaloric and isonitrogenous.

Management and Feeding Program:

The house was mechanically ventilated and incandescent lights were used to provide fourteen hours of light per day. Ducks were in pens with cement floors which were covered by wood shavings. These shavings were changed every three days.

Rations were prepared and pelleted at the Michigan State University Swine Barn. All ducks were given the control diets for a period of four weeks to allow an adaptation period. At the end of the four week period ducks were given the test diets. Daily records for egg production (Table 5) and mortality (Table 26) were maintained for each pen. During the last three consecutive days of each period (28 days), eggs were collected and washed for the hatchability test. Eggs were submerged in a solution containing 67 ml L.O.C. (Liquid Organic Cleaner of Amway) per 15 gallons of water at 42°C for two minutes. In this experiment several criteria related to hatchability and fertility were measured.

These criteria could be divided into three periods:

1. Pre-incubation period

    1.1 Production: was recorded daily and calculated on hen/day basis.

Table 2. Composition of the control diet used for the experiment.

Ingredients	Percentage of ration[a]
Corn, No. 2 yellow	69.323
Soybean meal (48%)	15.115
Alfalfa leaf meal (17%)	1.250
Wheat Middling	1.666
Fish meal (Menhaden)	1.700
Meat & Bone meal (50%)	1.550
DL-Methionine	0.077
Limestone	5.733
Phosphorus[b]	1.333
Salt	0.250
Premix (vitamins[c] & minerals[d])	2.003
Total	100.000

Theoretical composition[e]:
ME/KCal/kg: 2852; Protein, Nx6.25: 15.87%; ME:protein ratio = 179.7:1

[a] as fed basis

[b] Dynafos

[c] Supplied the following per kg of diet: Vitamin A, 11,000 I.U.; Vitamin D_3, 3,300 I.C.U.; Vitamin E, 22 I.U.; Vitamin K, 2.2 mg; Thiamine, 2.2 mg; Riboflavin, 6.6 mg; Panthothenic acid, 17.6 mg; Niacin, 66 mg; Pyridoxine, 3.3 mg; Biotin, 0.083 mg; Folic acid, 0.55 mg; Vitamin B_{12}, 0.015 mg; Choline Chloride 70%, 551 mg.

[d] Supplied the following per kg of diet: Zinc, 75 mg; Manganese, 75 mg; Iron, 37.5 mg; Copper, 7.5 mg; Iodine, 0.75 mg; Cobalt, 0.25 mg; Selenium, 01. mg.

[e] Based on feed ingredient compositional data presented by Scott *et al*. (1982).

1.2 Egg washing: After eggs were collected on the last three days of each period (28 days) they were transferred to the laboratory. As previously mentioned they were dip-washed. Eggs were then marked individually and set in Jamesway 252 incubators.

2. During incubation period

Temperature and humidity were adjusted according to the following chart published by Jamesway (Table 3).

During incubation, eggs were periodically checked for putrefied eggs and any putrefied eggs were removed and recorded. Before eggs were transferred to the hatcher, they were candled (day 24) in order to determine fertility and embryo mortality. All eggs failing to hatch were broken to determine fertility and to make gross examinations of the embryos for developmental abnormalities. Eggs showing cracked shells were removed before being placed in the incubator.

2.1 Fertile or infertile: clear eggs showing no embryonic development were considered to be infertile. The percent fertility was the number of fertile eggs divided by the number of eggs set.

2.2 Dead embryos in different stages were classified as follows:

2.2.1 Early dead (E.D.) These embryos died in very early stage of development (0-9 days).

2.2.2 Middle dead (M.D.) Embryos in this stage were dead when half developed and had distinguishable parts, such as a beak, legs, etc. (day 10 through day 18).

Table 3. Temperature, humidity and ventilation guide for model 252 (Jamesway) single stage incubator-hatcher:chickens.

	Dry Bulb Temperature	Wet Bulb Temperature	Exhaust Setting
		Ducks	
1st to 3rd day	99-3/4°	88° -	Closed
3rd to 16th day	99-1/2°	84° - 82°	1/4" Opening
16th day to transfer	99°	83° - 80°	1/4" Opening
Hatching	98-1/4°	86° - 90°	1/2" Opening to full open as required to control temp. and humidity

Allow humidity to increase from 86° to 90° as hatching progresses.

2.2.3 Late dead (L.D.) In this group, embryos were fully developed and were in their final stage of development when death occurred (Day 19-28).

2.3 At hatching time (day 28) unhatched but pipped eggs were divided into two groups:

2.3.1 Live in shell (L.S.) Ducklings in this group were unable to hatch on day 28.

2.3.2 Dead in shell (D.S.) In this group the fully developed ducklings died soon after they had pipped the egg shell.

2.3.3 Unhatched eggs were left in the incubator for a period of 24 hours and then removed, however, these ducklings were not considered in the calculations.

3. Post incubation period: Hatch and Hatchability

3.1 Percent hatch: Hatched ducklings were counted on the 28th day of incubation and the number of ducklings was divided by the number of eggs initially set.

3.2 Percent hatchability: To determine hatchability the number of ducklings hatched was divided by the number of fertile eggs.

Throughout the incubation period eggs were sprayed and fumigated. The schedule for spraying and fumigation is shown in Table 4.

B. Statistical Analysis

In this experiment all data were analyzed by analysis of variance, using a M.S.U. Hustler Computer. Significance differences between treatment means (control and supplemented groups) were tested at the level of 1 and 5 percent, by the use of split-plot measurement procedures (Gill, 1981).

Table 4. Schedule for spraying duck eggs during incubation.

Day	Description of work to be done
1	Fumigate 12 mls Russells* disinfectant
2 through 5	Spray with 30 mls Bio-Shield per one gallon water
5 and 6	No spray
7 through 10	Spray as day 2-5
14	Fumigate 12 mls Russells disinfectant
15 through 18	Spray as day 2-5
19 and 20	No spray
21 through 23	Spray as day 2-5
24	Transfer the eggs--stop spray
25	-----
26	Fumigate 4 mls Russells, increase humidity 93-94°
27 through 28	-----
29	Take off hatch

*Russells disinfectant=Russell incubation fumigant L.D. Russell Co., Laboratories, Kansas City, MO 64162.

All data were transformed by the arcsin procedure (Steel and Torrie, 1960) before being analyzed by analysis of variance.

C. Chemical Analysis

Six samples from diets A, B, C were sent to Rosner/Runyon Laboratories, Inc., Chicago, Illinois to determine chemically the amount of folic acid and biotin. Samples from diet E were sent to Michigan State Animal Health Diagnostic Laboratory for measurement of the amount of selenium.

D. Prothrombin Time

Thirty blood samples from the control group (diet A) and thirty blood samples from the vitamin K supplemented group (diet D) were used for determination of prothrombin time. The procedures used were as published by Dade Diagnostic, Inc. (1979).

V. RESULTS

Regarding egg production, the analysis of variance (ANOVA) showed that none of the treatments was significantly different at the 5.0% level. The statistical analysis by ANOVA showed only the expected ($P < 0.01$) decline in egg production due to period. This decline started during the second period of the experiment (Figure 5, Table 5, 6). Regarding fertility, none of the treatment differences approached significance ($P > 0.05$). Only period had a significant effect on fertility ($P < 0.01$, Figure 6, Table 7, 8).

As evident from Tables 9 and 10, there was no differences in percent hatch of all eggs set (Figure 7), and percent hatchability (Tables 11, 12 and Figure 8) of fertile eggs due to dietary treatment. However, (Figure 8) as the experiment progressed, hatchability decreased ($P < 0.01$). This trend was consistent for all treatment groups.

The stage of embryo mortality was not influenced by dietary treatment. Period of production did not significantly alter the number of dead embryos, classified as early or late dead (Figures 9 and 11, Tables 13, 14, 17 and 18). However, the number of dead embryos classified as middle dead were significantly different (Figure 10, Tables 15 and 16) due to the period of production ($P < 0.01$). Figure 10 shows that as the periods proceeded the percentage of embryos classified as middle dead increased significantly. The increase in percentage middle dead embryos was very high for group A during the third period whereas group E increased more rapidly during the sixth period. In the other groups the increase was gradual. Groups A, C, E showed a moderate decrease in the number of middle dead

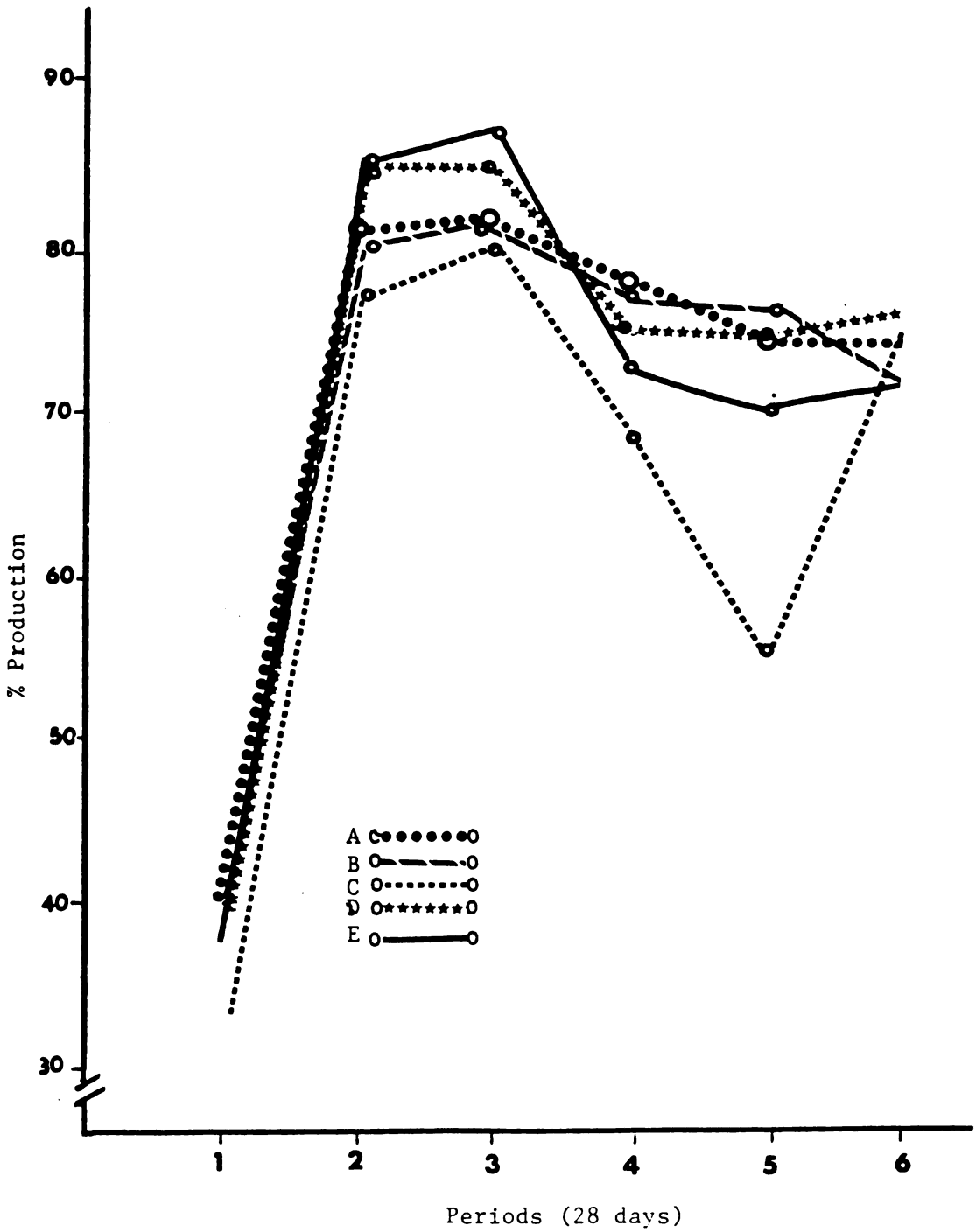


Figure 5. Percent egg production by period for control and supplemented groups.

Table 5. The average percent production in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	40.90	80.82	81.66	78.23	74.25	74.16
B	40.01	80.53	81.70	77.30	76.63	72.06
C	33.60	77.10	80.37	68.90	55.27	74.68
D	41.17	87.78	85.50	75.20	74.47	76.97
E	38.00	85.97	87.49	73.57	71.01	72.60

SE_{Da}=3.40 difference between two treatments at the same period.
SE_{Db}=2.97 difference between two periods for the same treatment.

Table 6. Analysis of period and treatment effect on percent production in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	292.41	73.10	1.935
Rep/trt (C/A)	10	377.59	37.76	
Period (B)	5	7620.12	1524.02	114.760**
A.B	20	350.33	17.52	1.319
Error B	50	663.95	13.28	
Total	89	9304.41	104.54	

**$P < 0.01$

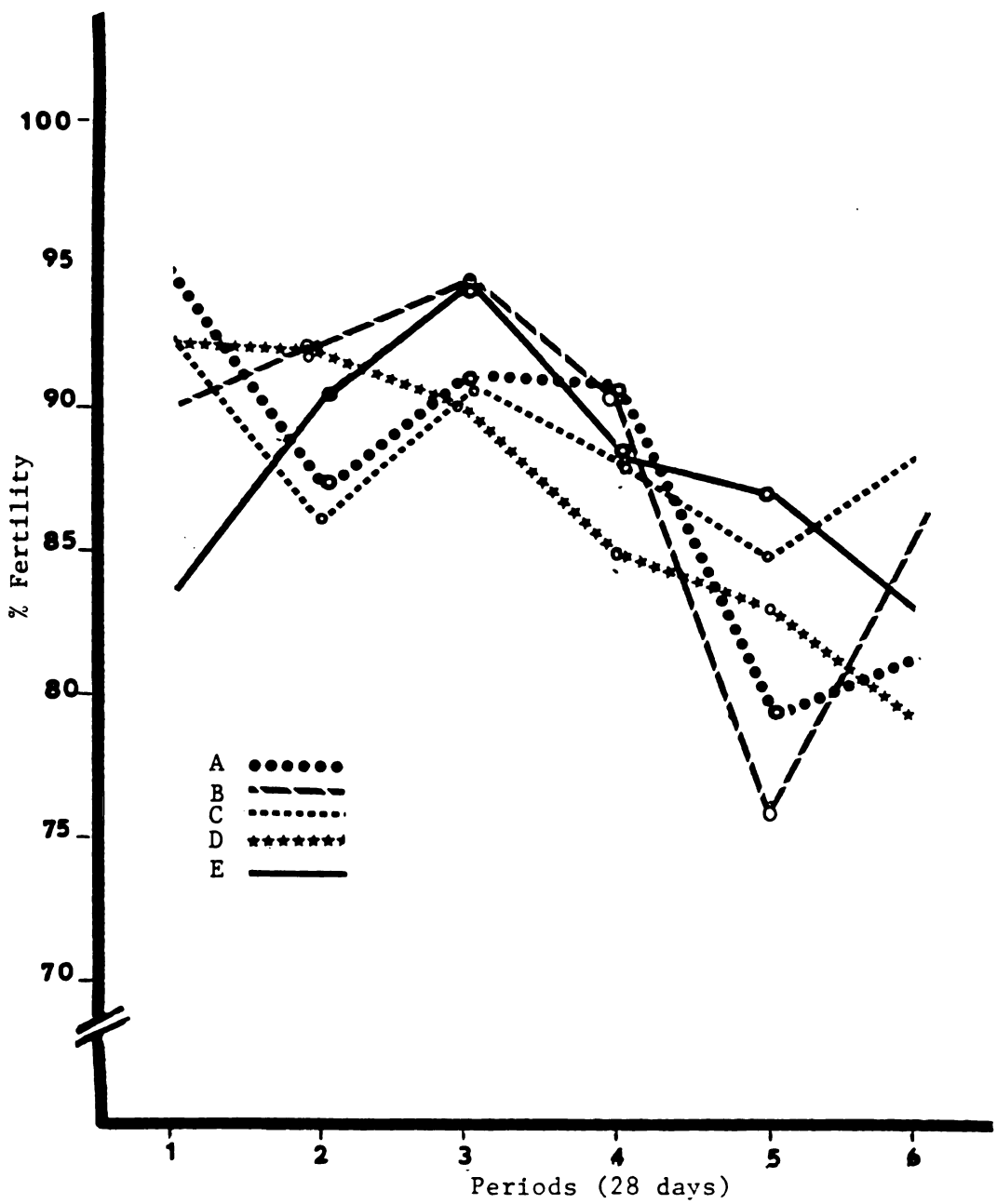


Figure 6. Percent fertility by period for control and supplemented groups.

Table 7. The average percent fertility in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	96.40	87.27	91.33	91.20	79.20	81.23
B	87.50	90.23	94.06	90.36	75.70	85.67
C	93.60	86.33	92.13	88.03	84.73	88.67
D	91.30	92.17	89.97	84.87	83.27	78.67
E	88.86	92.27	94.57	88.47	87.40	83.07

SE_{Da}=3.81 difference between two treatments at the same period.
SE_{Db}=3.83 difference between two periods for the same treatment.

Table 8. Analysis of period and treatment effect on percent fertility in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	34.48	8.62	0.401
Rep/trt (C/A)	10	214.79	21.48	
Period (B)	5	1017.14	203.43	9.268**
A.B	20	652.72	32.64	1.487
Error B	50	1097.40	21.95	
Total	89	3016.54	33.89	

**$P < 0.01$

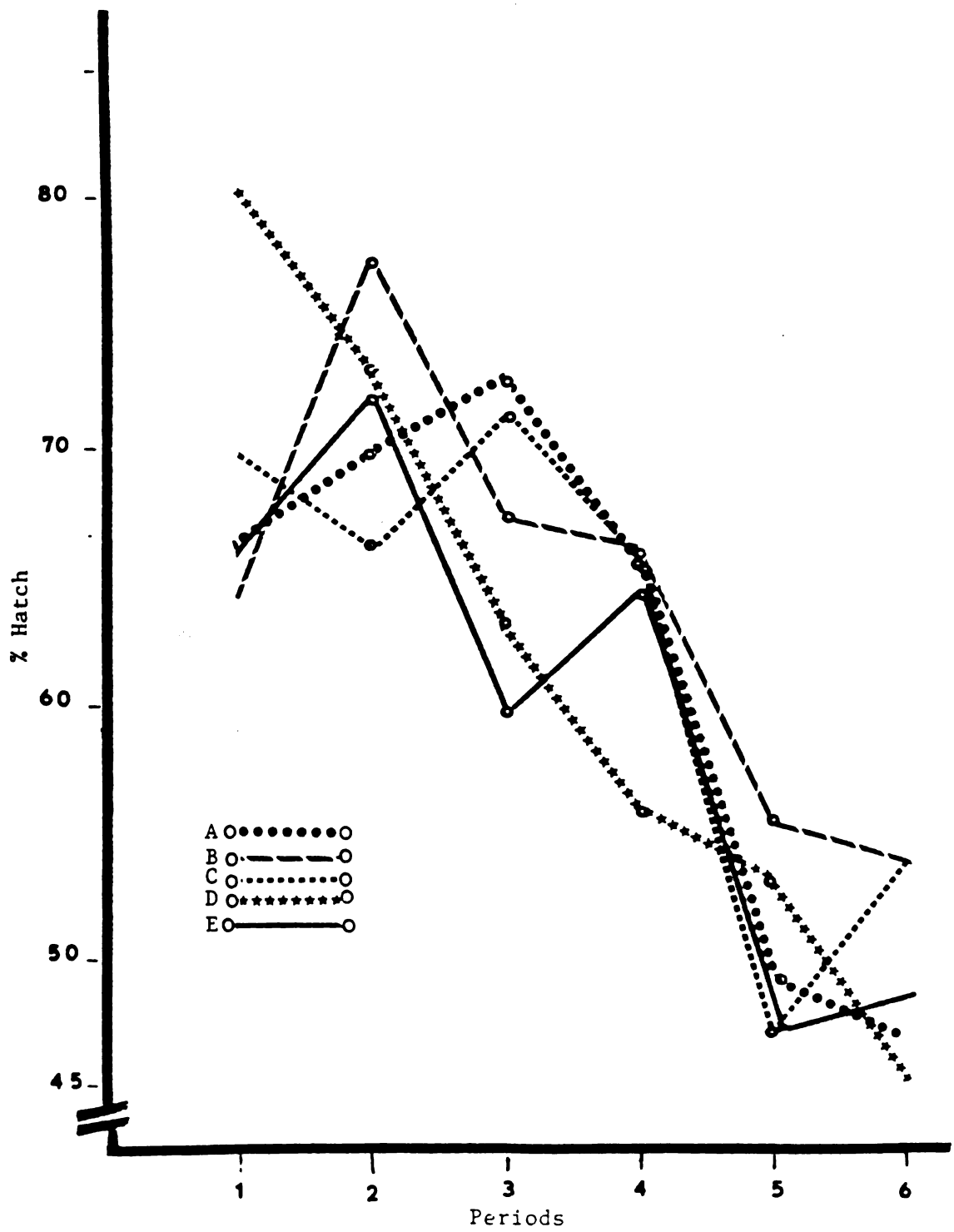


Figure 7. Percent hatch by period for control and supplemented groups.

Table 9. The average percent hatch in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	66.20	72.33	59.63	64.42	49.96	46.26
B	64.30	77.66	67.43	66.50	55.26	54.26
C	69.92	66.43	71.76	65.60	47.03	53.83
D	80.65[a]	73.26	63.53	55.87	53.33	45.36
E	66.93	69.86	72.93	65.60	47.50	48.76

[a]The value is for two replicates instead of three replicates due to missing value.

SE_{Da}=3.93 difference between two treatments at the same period.

SE_{Db}=3.81 difference between two periods for the same treatment.

Table 10. Analysis of period and treatment effect on percent hatch in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	72.50	18.13	0.608
Rep/trt (C/A)	10	298.04	29.80	
Period (B)	5	2539.68	507.94	23.257**
A.B	20	597.86	29.89	1.369
Error B	49	1070.01	21.84	
Total	88	4578.08	52.02	

**$P < 0.01$

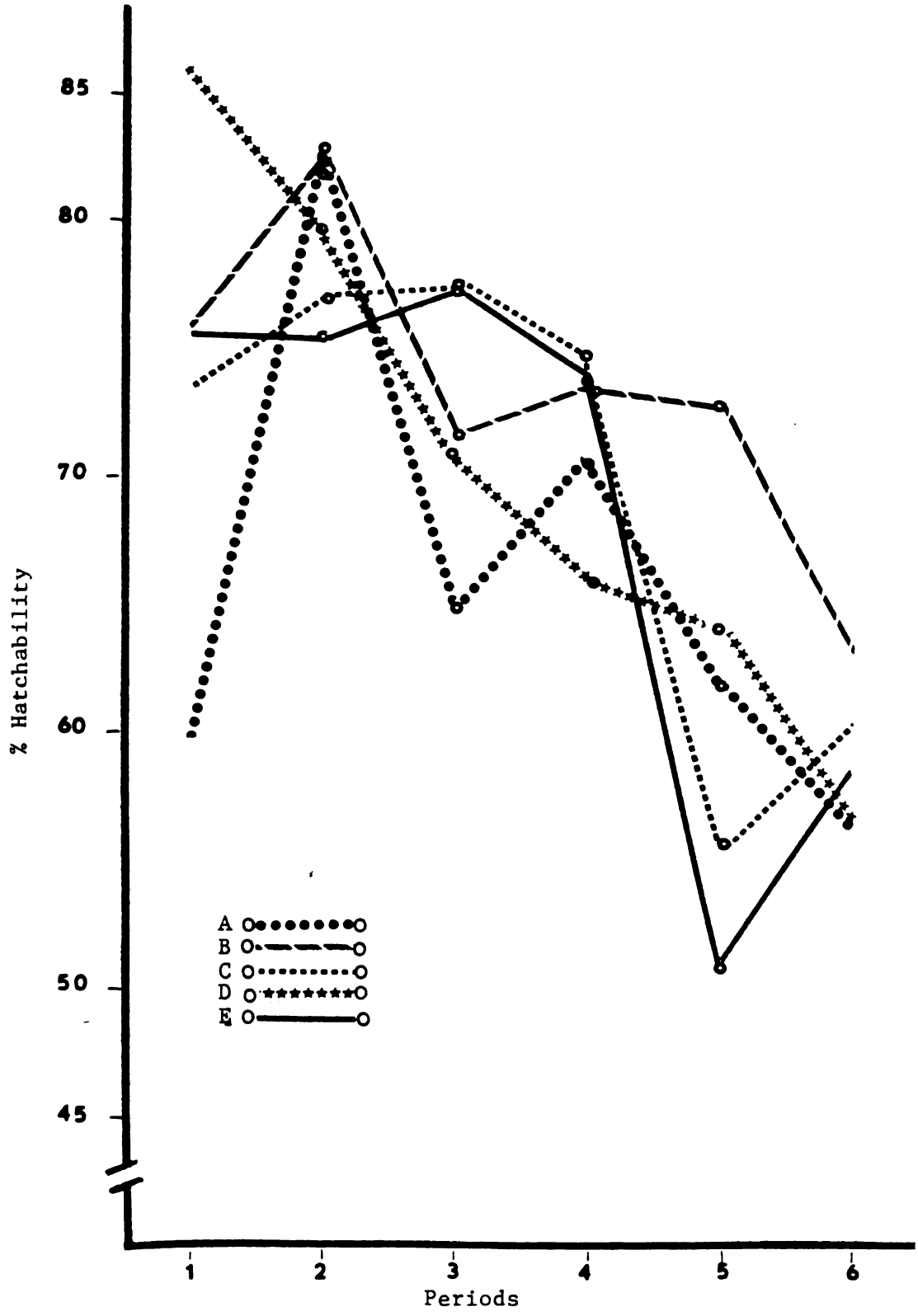


Figure 8. Percent hatchability by period for control and supplemented groups.

Table 11. The average percent hatchability in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	59.97	83.07	64.83	70.66	61.86	56.43
B	76.03	82.76	71.63	73.76	72.76	63.23
C	74.66	77.20	77.56	74.73	55.63	60.43
D	86.05[a]	79.43	70.73	65.86	64.90	57.00
E	75.96	75.63	77.23	73.76	53.23	58.63

[a]The value is from two replicates instead of three replicates due to missing value.

SE_{Da}=4.69 difference between two treatments at the same period.

SE_{Db}=4.59 difference between two periods for the same treatment.

Table 12. Analysis of period and treatment on percent hatchability in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	231.49	57.87	1.447
Rep/trt (C/A)	10	399.80	39.98	
Period (B)	5	1978.18	395.64	12.518**
A.B	20	880.03	44.00	1.392
Error B	49	1548.67	31.61	
Total	88	5038.17	57.25	

**$P < 0.01$

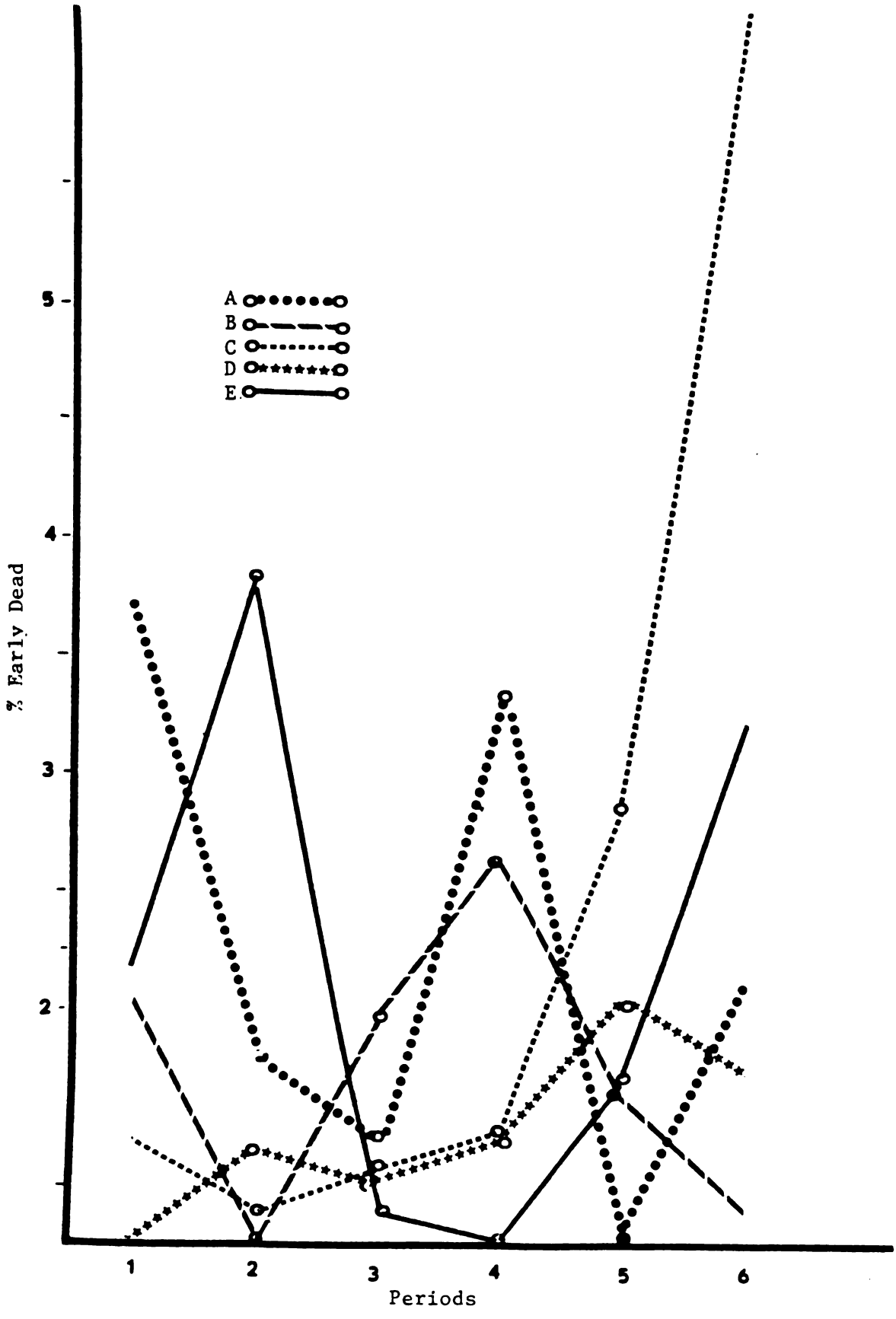


Figure 9. Percent early dead (ED) by period for control and supplemented groups.

Table 13. The average percent early dead (ED) in the control and supplemented groups.

	28 Day Periods					
Treatments	1	2	3	4	5	6
A	3.73	1.53	1.23	3.36	0	2.16
B	2.03	0	1.73	2.63	1.36	0.76
C	1.23	0.53	1.06	1.23	2.80	6.42
D	0[a]	1.13	1.03	1.16	2.02	1.23
E	2.20	3.86	0.50	0	1.43	3.26

[a]The value is for two replicates instead of three replicates due to missing data.

SE_{Da}=4.15 difference between two treatments at the same period.

SE_{Db}=4.17 difference between two periods for the same treatment.

Table 14. Analysis of period and treatment effect on percent early dead (ED) in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	56.72	14.18	0.580
Rep/trt (C/A)	10	244.50	24.45	
Period (B)	5	118.06	23.61	0.904
A.B	20	880.22	44.01	1.686
Error B	49	1279.13	26.10	
Total	88	2578.62	29.30	

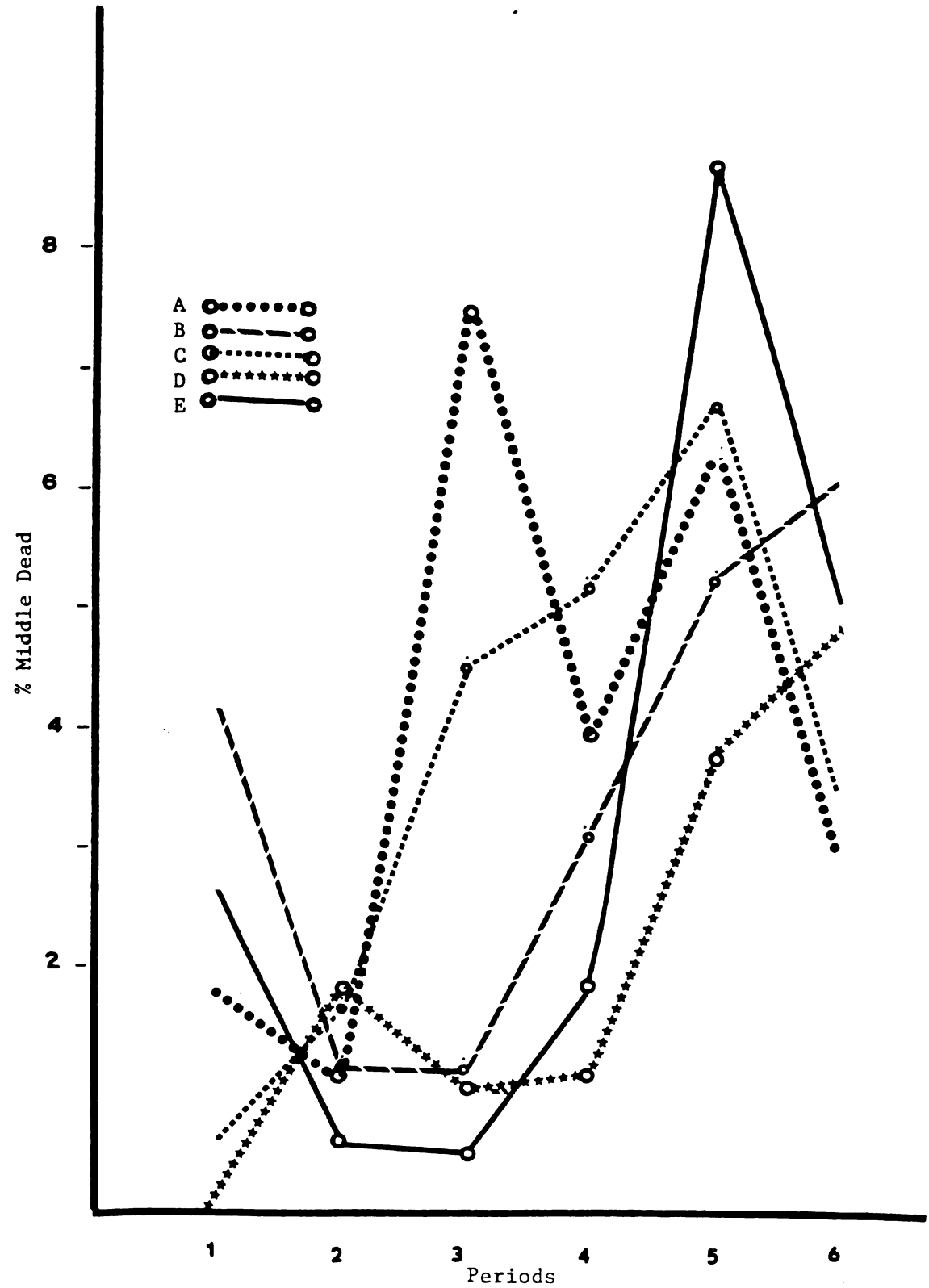


Figure 10. Percent middle dead (MD) by period for control and supplemented groups.

Table 15. The average percent middle dead (MD) in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	1.83	1.10	7.53	3.96	6.33	3.03
B	4.20	1.13	1.13	3.10	5.26	6.16
C	0.63	1.66	4.53	5.20	6.76	3.56
D	0[a]	1.83	1.56	1.16	3.83	4.86
E	2.66	1.06	2.66	1.86	8.74	5.13

[a]The value is for two replicates instead of three replicates due to missing data.

SE_{Da}=4.38 difference between two treatments at the same period.

SE_{Db}=3.84 difference between two periods for the same treatment.

Table 16. Analysis of period and treatment effect on percent middle dead (MD) in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	149.08	37.29	0.602
Rep/trt (C/A)	10	619.21	61.92	
Period (B)	5	884.90	176.98	8.011**
A.B	20	508.91	25.45	1.152
Error B	49	1082.57	22.09	
Total	88	3244.66	36.87	

**$P < 0.01$

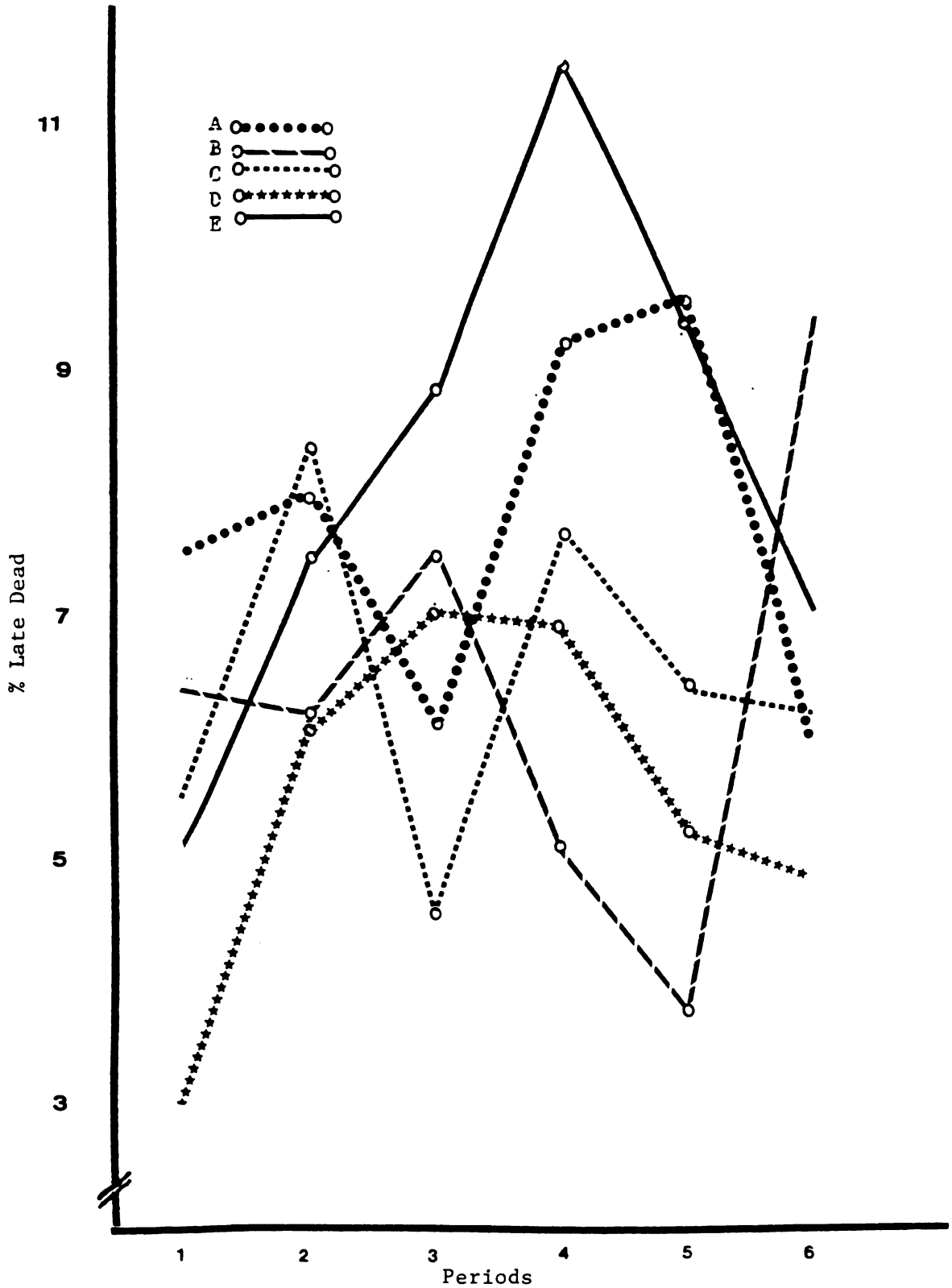


Figure 11. Percent late dead (LD) by period for control and supplemented groups.

Table 17. The average percent late dead (LD) in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	7.50	8.13	6.26	9.26	9.60	6.03
B	6.40	6.26	7.56	5.16	3.76	9.49
C	5.55	8.46	4.50	7.70	6.46	6.23
D	3.12[a]	6.10	7.06	6.96	5.23	4.90
E	5.16	7.50	8.80	11.49	9.41	7.05

[a]The value is for two replicates instead of three replicates due to missing data.

SE_{Da}=4.58 difference between two treatments at the same period.

SE_{Db}=4.22 difference between two periods for the same treatment.

Table 18. Analysis of period and treatment effect on percent late dead (LD) in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	84.41	21.10	0.382
Rep/trt (C/A)	10	551.89	55.19	
Period (B)	5	53.24	10.65	0.397
A.B	20	336.44	16.82	0.628
Error B	49	1313.58	26.81	
Total	88	2339.56	26.59	

embryos during the sixth period, but group B and D continued to increase in the number of middle dead embryos.

Embryos that were able to pip the eggshell were also classified into two groups: Those that were live (live in shell or L.S.) or dead (dead in shell or D.S.). Dietary treatment had no significant effect on live in shell embryos (Figure 12, Tables 19 and 20). Period of production had a significant effect on live in shell embryos ($P < 0.01$). There was an increase in percentage of L.S. for all treatment groups during the third period (Figure 12, Tables 19 and 20). After that the increase in L.S. embryos was rather sharp with the highest increase for treatment D and the smallest increase in L.S. embryos in treatment C. During the sixth period, group C and D showed some decrease but group A, B and E had an increase in the percentage of L.S. embryos.

Period had a significant effect on the percentage of dead in shell embryos (D.S.) (Figure 13, Tables 21 and 22, $P < 0.01$). A sharp increase in dead in shell embryos was observed in every dietary treatment except treatment B during the fifth period (Figure 13 and Tables 21 and 22).

Putrefied eggs:

Eggs which were either putrefied or exploded during the incubation period were classified as putrefied eggs (Figure 14, Tables 23 and 24). Although there was no difference in percentage of putrefied eggs due to dietary treatment, a significant difference was observed as the periods proceeded (Tables 23 and 24, $P < 0.01$). There was a sharp increase in the putrefied eggs for all groups in the fifth period but after that (during sixth period) all treatments except group E had a decrease in the putrefied eggs (Figure 14).

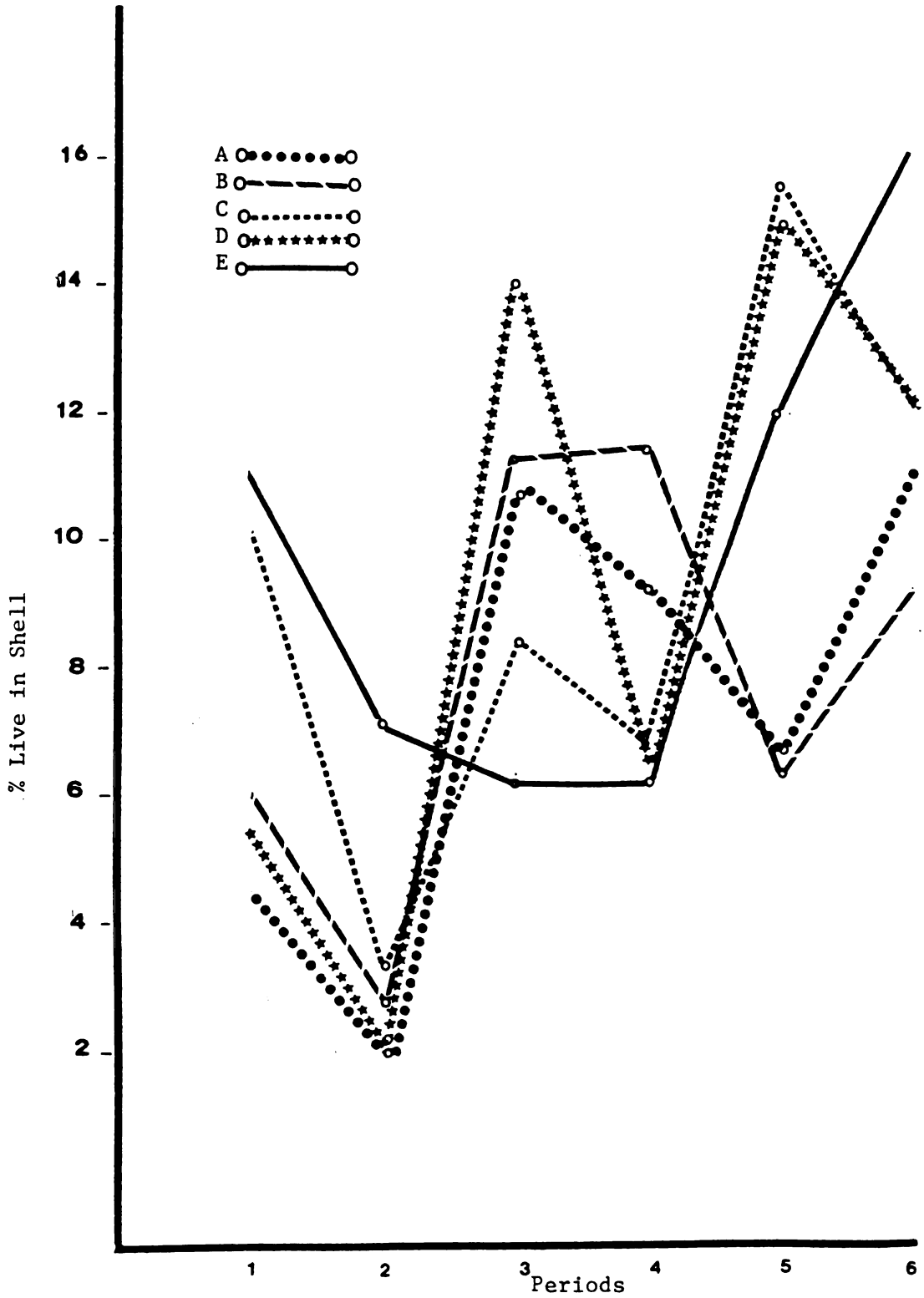


Figure 12. Percent live in shell (L.S.) by period for control and supplemented groups.

Table 19. The average percent live bird in shell (LS) after incubation period (and pipped) in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	4.40	1.90	10.90	9.26	6.76	10.95
B	6.23	2.80	11.30	11.50	5.25	9.20
C	10.23	3.39	8.46	6.69	15.53	12.02
D	5.56[a]	2.13	14.16	6.50	12.00	16.23
E	11.06	7.18	6.26	6.26	15.20	12.12

[a]The value is for two replicates instead of three replicates due to missing data.

SE_{Da}=5.76 difference between two treatments at the same period.

SE_{Db}=5.29 difference between two periods for the same treatment.

Table 20. Analysis of period and treatment effect on percent live in shell (LS) in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	107.91	26.98	0.303
Rep/trt (C/A)	10	889.87	88.99	
Period (B)	5	1178.08	235.62	5.607**
A.B	20	584.88	29.24	0.696
Error B	49	2058.99	42.02	
Total	88	4819.73	54.77	

**$P < 0.01$

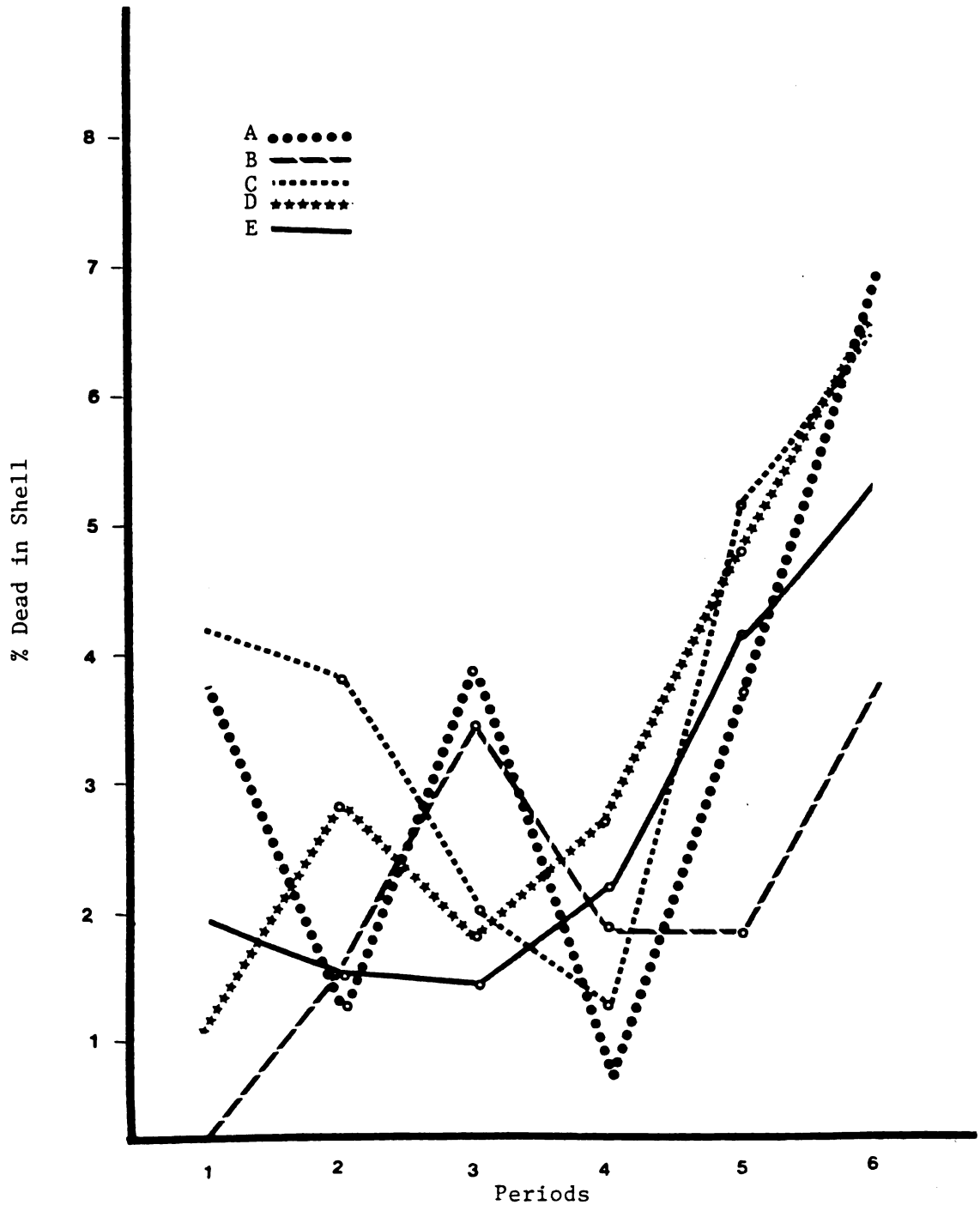


Figure 13. Percent dead in shell (D.S.) by period for control and supplemented groups.

Table 21. The average percent dead in shell (DS) after incubation period (and pipped) in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	3.76	1.26	3.93	0.70	3.70	7.02
B	0	1.63	3.53	1.93	1.92	3.87
C	4.28	3.93	2.13	1.36	5.20	6.45
D	1.10[a]	2.86	2.30	2.76	4.80	6.67
E	1.96	1.63	1.53	2.25	4.28	5.36

[a]The value is for two replicates instead of three replicates due to missing value.

SE_{Da}=4.00 difference between two treatments at the same period.

SE_{Db}=3.46 difference between two periods for the same treatment.

Table 22. Analysis of period and treatment effect on percent dead in shell (D.S.) in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	68.00	17.00	0.314
Rep/trt (C/A)	10	540.26	54.03	
Period (B)	5	638.18	127.64	7.087**
A.B	20	541.73	27.09	1.504
Error B	49	882.65	18.01	
Total	88	2670.81	30.35	

**$P < 0.01$

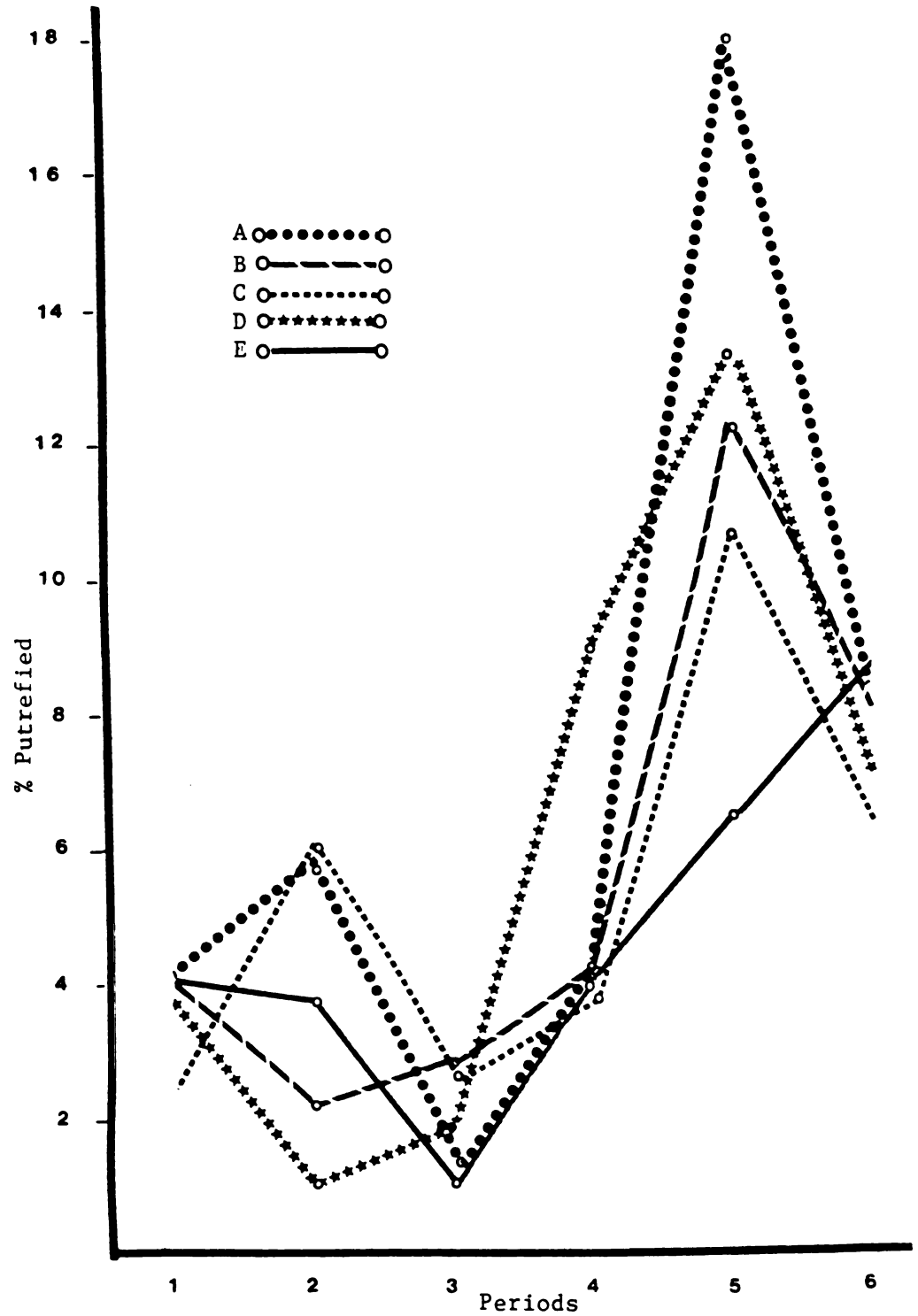


Figure 14. Percent putrefied eggs by period for control and supplemented groups.

Table 23. The average percent putrefied eggs in the control and supplemented groups.

Treatments	28 Day Periods					
	1	2	3	4	5	6
A	4.17	6.35	1.23	4.60	17.90	8.44
B	4.09	2.33	2.90	4.43	12.30	8.09
C	2.52	6.16	2.66	3.80	10.73	6.40
D	2.52[a]	1.13	1.80	8.00	13.33	10.00
E	2.74	3.80	1.03	4.20	6.70	8.70

[a]The average is for two replicates instead of three replicates due to missing value.

SE_{Da}=4.00 difference between two treatments at the same period.

SE_{Db}=3.53 difference between two periods for the same treatment.

Table 24. Analysis of period and treatment effect on percent putrefied eggs in five experimental groups.

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	f-Ratio
Treatment (A)	4	72.59	18.15	0.360
Rep/trt (C/A)	10	507.27	50.73	
Period (B)	5	1883.90	376.78	20.159**
A.B	20	555.07	27.75	1.485
Error B	49	915.93	18.69	
Total	88	3934.76	44.71	

**$P < 0.01$

Table 25. A summary of the percent production, fertility, hatch, hatchability, early dead, middle dead, late dead, live in shell, dead in shell and putrefied in the five experimental groups.

	Production[1]	Fertility	Hatch	Hatch-ability	Early Dead	Middle Dead	Late Dead	Live in Shell	Dead in Shell	Putrefied Eggs
A	71.67	87.77	59.80	66.15	2.00	3.96	7.79	7.36	3.39	7.11
B	71.37	87.25	64.24	73.36	1.41	3.49	6.44	7.71	2.14	5.69
C	64.98	88.91	62.43	70.03	2.21	3.72	6.48	9.38	3.81	5.37
D	73.18	86.71	62.00	70.66	1.09	2.11	5.56	9.42	3.41	6.13
E	71.44	89.11	61.93	69.07	1.86	3.24	8.24	9.68	2.83	4.53

[1] Each datum is average of six months from three replicates for five treatments.

Table 26. The effect of five treatment groups on mortality of breeder ducks.

Treatments[a]	Period 1	2	3	4	5	6	Total
A	--	4	1	5	1	--	11
B	--	2	1	1	2	1	7
C	1	2	--	2	2	2	9
D	--	--	--	1	1	--	2**
E	--	--	1	3	2	2	8
Total	1	8	3	12	8	5	37

**$P < 0.01$

[a]Each treatment group had a total of 87 males and females at the beginning of the experiment.

A summary of the data for the whole experiment is given in Table 25. A summary of the mortality of duck breeders was maintained and can be seen in Table 26. Diet A (control group) had the highest mortality and diet D had the lowest mortality. This difference was significant ($P < 0.01$), however, there were no other significant differences in mortality between treatment groups.

VI. DISCUSSION

The majority of information available for dietary recommendations for duck breeders relates to recommendations based on experiments done by researchers dealing with chicken hens and to some extent with turkeys. This has occurred because much more research has been conducted on chickens, therefore the determined requirements have been applied to ducks. NRC (1977) has listed requirements for chickens and turkeys but there is only a short list of nutrient requirements for duck breeders and even some of the listed requirements are only estimates. Poultry World (1981) published a complete list of requirements for ducks.

As discussed earlier the calculated amount of folic acid supplied in group A (control group) and group B (additional folic acid supplemented group) were 0.55 mg and 0.84 mg/kg diet, respectively. However, the chemical analysis of diet B showed that the mean amount of folic acid was 1.29 mg/kg which is much higher than the recommendation made by NRC (1977) and Scott et al. (1982). The results in our experiment indicated that there were no significant effects on any of the measured criteria (i.e. percent egg production, fertility, hatchability and embryonic mortality) due to supplemental folic acid.

Most researchers have shown that the range for requirements of folic acid can vary and some (Taylor, 1947) have given a very low level without any adverse effect on egg production. But most reseachers have concluded that amounts from 0.5 mg/kg diet (Sunde et al., 1950) to 1.0 mg/kg diet (Craven and Halpin, 1949) will result in high production and good hatchability. Another factor that must be taken into consideration is the type of diet and biological

availability of folic acid. Cravens and Halpin (1949) have shown that the dietary requirement increases (0.5 - 1.0 mg/kg diet) when the diet does not favour the intestinal synthesis of folic acid. NRC (1977) has also suggested a requirement for the chick of 0.35 mg/kg and 1.2 mg/kg with sucrose diet. Some authors produced better results when they used dextrin instead of sucrose in the diet (Sunde et al., 1950). Another method to estimate the dietary folic acid can be done by analyzing the folic acid content of the eggs as Schweigert et al., (1948) have shown that although low amounts of folic acid (0.42 mg/kg diet) did not affect egg production, hatchability, hemoglobin level or general appearance, there was a marked decrease in the folic acid content of the eggs. However, verification of this measurement was not within the scope of our experiment.

Scott et al. (1982), NRC (1977), and Arends et al. (1971) have recommended that the level of biotin in the diet of breeder hens and turkeys should be 0.15 mg/kg diet; however, Poultry World (1981) recommended that the level of biotin in the duck's diet is 0.08 mg/kg. In our experiment the amount added to diet A (control group) and diet C (additional biotin supplemented group) was 0.082 and 0.124 mg/kg, respectively. The chemical analysis has shown that the total amount of the biotin was much higher than the recommended levels but it should also be noted that the total amount is not all biologically available (Patrick et al., 1942). Some authors have also shown that similar to folic acid, the diet should favour the intestinal synthesis of biotin. Low levels of biotin in the diet do not adversely affect egg prodution (Cravens et al, 1942; Brewer and Edwards, 1972), but higher amounts are needed to increase hatchability (Cravens et al, 1942). Brewer and Edwards (1972) also showed that excess biotin can

be deposited in the liver or eggs. Embryonic mortality can be affected by low biotin in the diet and the peak mortality can be observed at two critical periods (1-4 days and 18-21 days of incubation periods) in chickens (Cravens et al., 1944). In this experiment, none of the measured criteria were affected by treatment C (additional biotin supplemented group) when compared to the control (Diet A) group. Therefore, it can be concluded that either the diet had a sufficient amount of biotin or the diet favoured the synthesis of this vitamin.

Menadione Sodium Bisulfite Complex (MBSC) was used in our experiment to supply vitamin K. This form of vitamin K is made commercially and has 50% activity. Group A (control group) had 4.4 mg/kg of MBSC which yielded 2.2 mg/kg of vitamin K and group D (additional supplemented vitamin K) had 6.6 mg/kg of MBSC which yielded 3.3 mg/kg vitamin K. These amounts are not in agreement with the recommendation made by NRC (1977). NRC recommended a level of 0.5 mg/kg diet for chicken breeder and a level of 1.0 mg/kg diet for turkey breeders. Scott et al. (1982) have recommended that this level should be 2.2 mg/kg diet and Poultry World (1981) has recommended that the level of vitamin K in the duck diet should be 2 mg/kg diet.

Dean (1972) did not find any significant differences between groups with supplemented vitamin K at a level of 0.4 mg/kg on prothrombin time, which has been proven to be an accurate way to measure vitamin K deficiency. The result of prothrombin time in our experiment revealed no significant difference between the two groups from which it was concluded that vitamin K was provided in adequate amount in these diets. Studies by Dean (1972), with ducks, have shown that absence of vitamin K or presence of stress producing agents can

increase the rate of mortality and that the addition of up to 5.0 mg MPB (Menadione dimethylpyrimidinol bisulfite)/kg reduced the mortality. Mortality of the breeder ducks during the experimental period proved to be significant ($P < 0.01$, Table 26) as the control group had a higher rate of mortality in comparison to group D (group with additional supplemented vitamin K). There is no solid evidence to confirm whether this difference was solely due to a low vitamin K content of diet A, as the laboratory tests could not determine specific reasons for the mortality.

Combs and Scott (1979) have reported that a calculated level of 0.05 ppm selenium was adequate for egg production but a calculated level of .10 ppm selenium is required for optimum hatchability (NRC, 1977; Combs and Scott, 1979) in breeding chickens. Cantor and Scott (1974) have observed that amounts below 0.03 ppm total selenium (without supplemental vitamin E) caused a significant reduction in egg production and hatchability in chickens. NRC (1977) has recommended 0.1 ppm selenium for layer breeders and 0.2 ppm for turkey breeders. Poultry World (1981) listed 0.1 ppm selenium for duck breeders. The calculated amount of selenium in experimental diet A was 0.1 ppm and in diet E (additional supplemented group) 0.15 ppm selenium. The analysis of variance showed no significant difference in any of the parameters measured between these two groups. Chemical analysis of diet E showed that the mean total amount of the selenium in the diet was 0.308 ± 0.017 ppm. This amount is higher than the recommendation made by different authors (NRC, 1977, Scott *et al*, 1982) but it is probable that not all of the selenium was biologically available (Cantor *et al*., 1975). Poley *et al*., (1941) improved hatchability

in chickens with as high a level of selenium as 2 ppm, but Arnold et al. (1973) could only improve egg production without any effect on hatchability. In our experiment the egg production was above 72% in both group A and E all throughout the experimental periods (24 weeks), except during the first period, and this is in agreement with findings of Cantor and Scott (1974) where they maintained egg production above 75% with chickens by supplementing 0.1 ppm selenium in the diet. In our studies, hatchability was significantly different due to period only, and ranged from 57% to 84% in group A and 51% to 78% in group E. This is not in agreement with the findings of Cantor and Scott (1974) where they had 90% hatchability when they added 0.1 ppm selenium in a chicken diet. According to Gilbert (1980), the hatchability of fertile eggs in meat type ducks ranges betweeen 75-80% which is in agreement with our findings. Further, Gilbert (1980) has indicated that fertility of duck eggs is 90%, which is in agreement with our findings.

VII. SUMMARY AND CONCLUSION

An experiment was conducted to evaluate the effects of the addition of any one of three vitamins or a mineral on egg production, fertility, embryonic mortality and hatchability of duck eggs.

White Pekin Duck Breeders, twenty-four weeks of age, were used in this experiment. These ducks were randomized into treatment groups with three replications per treatment. In each of these replications there were twenty-nine ducks. The total number of ducks in this experiment was 435. The experiment lasted 24 weeks.

Five diets were prepared. A control diet, which was similar to a commercial preparation, and four other diets exactly like the control diet with a 50% increase in the amount of folic acid (Diet B), biotin (Diet C), vitamin K (Diet D) or selenium (Diet E). All the diets were isocaloric and isonitrogeneous. There were no significant differences on egg production, fertility, embryonic mortality or hatchability due to diet ($P < 0.01$).

The results of this trial would indicate that factors other than the four nutrients added to the control diet may be responsible for the erratic production and hatchability of duck eggs produced on different commercial duck breeder farms.

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