AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF VITAMIN E, ETHOXYQUIN AND LINOLEIC ACID ON REPRODUCTION OF WHITE LEGHORN MALES

Abstract approved Redacted for Privacy
Dr. G. H. Arscott

Two lots of six White Leghorn male chickens were fed diets containing 7. 3% linoleic acid and either . 075% ethoxyquin (lot 1) or 32. 4 mg/kg of added vitamin E (lot II) for 47 weeks both of which were designated as positive controls. A third group (lot III) containing 30 males received the same level of linoleic acid but had no added vitamin E or ethoxyquin until the 38th week when the lot was divided into five sub-lots, equalized for fertility, based on the results of the 34th week. At this time the subdivided lots were supplemented with . 075 or . 3% ethoxyquin (lots IV and V) and 32. 4 or 162. 0 mg/kg of vitamin E (lots VI and VII) while one group was retained as the negative control (lot III).

During the depletion period fertility in lot III, the negative control group, decreased to 29. 2% at 38 weeks. Fertility in the lots supplemented throughout the trial with . 075% ethoxyquin (lot I) and 32. 4 mg/kg of vitamin E (lot II) was significantly greater than lot III

beginning at 25 and 30 weeks, respectively, and throughout the remainder of the depletion period. During the depletion period no meaningful differences were observed between the two positive control groups. At 47 weeks, fertility of the lot retained as the negative control decreased to 4.8% while fertility in lots supplemented with .075 or .3% ethoxyquin (lots IV and V) and 32.4 or 162.0 mg/kg of vitamin E (lots VI and VII) increased to levels comparable to the positive controls. At 47 weeks fertility in all supplemented lots was significantly greater than the negative control.

Semen density from males in lot III decreased with each measurement throughout the depletion period. Lot I had a significantly higher density than the negative control beginning at 20 weeks and throughout the remainder of the experiment, except during the 30th week while lot II was significantly greater during the 34th and 38th weeks of the depletion period and at 47 weeks. No significant differences were present between the positive control lots throughout the trial. At 47 weeks, density in all supplemented lots was significantly greater than the negative control with the exception of lot IV.

The pattern of significant differences for concentration was quite similar to that for semen density during the depletion period except lot I was significantly greater than lot II at 12 weeks and lot III at 30 weeks. With the exception of lot IV, semen concentration in all supplemented lots was significantly higher than that of lot III at the end of the trial.

No meaningful differences were noted for hatchability of fertile eggs, semen volume, sperm live-dead percent, feed consumption or body weight throughout the trial.

From these findings one may conclude the following:

1. Male chickens fed diets high in linoleic acid maintained their fertilizing capacity when supplemented with .075% ethoxyquin or 32.4 mg/kg of vitamin E when compared to the .3% and 162.0 mg/kg, respectively, reported by Arscott et al. (1965).

2. Sterility induced by linoleic acid and a deficiency of vitamin E and ethoxyquin in male chickens was equally reversible by supplementation with .075% ethoxyquin and 32.4 mg/kg of vitamin E as well as .3% ethoxyquin and 162.0 mg/kg of vitamin E. Thus, vitamin E appears to act similarly to an antioxidant in reversing sterility of male chickens fed diets high in linoleic acid.

3. The procedure for semen concentration outlined in this thesis gave results similar to the procedure for semen density (Kosin and Wheeler, 1956) in determining the percent of sperm per unit volume of semen.

Effect of Vitamin E, Ethoxyquin and Linoleic Acid on Reproduction of White Leghorn Males

by

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INTRODUCTION

Since Adamstone and Card (1934a) reported that vitamin E was essential for reproduction in male chickens, a considerable amount of research has been directed towards this subject.

It is known that a vitamin E deficiency may result in decreased egg production, hatchability and possibly fertility in the female fowl. In the male fowl it has been reported that the absence of vitamin E or antioxidants may result in reduced fertility, particularly if a high level of linoleic acid is present. It has further been shown that this low fertility is reversible by supplementation with vitamin E. Antioxidants have also been reported to prevent sterility in male chickens fed a diet low in vitamin E but high in linoleic acid.

Since fertility in the male chicken is a sensitive measure of a deficiency of vitamin E and since the male can be depleted of this vitamin yet still survive, it provides one with a tool to study the nutrient and antioxidant properties of vitamin E. Therefore, it is the purpose of this research to determine whether (1) levels of vitamin E and ethoxyquin, lower than those reported earlier, can maintain fertility in male chickens fed diets high in linoleic acid,

(2) vitamin E has biological activity other than that of an antioxidant, and (3) an easier method can be used for measuring the amount of sperm per unit volume of semen in place of the standard semen density procedure.

REVIEW OF LITERATURE

Vitamin E, Antioxidants and Linoleic Acid in Reproduction of the Male Fowl

Adamstone and Card (1934a) reported a decrease in fertilizing capacity in Rhode Island Red male chickens fed a vitamin E free diet for two years; however, no change was apparent at the end of one year. The impaired fertility was based on pen matings and testicular damage although it should be noted that no positive control group was available. Testis degeneration was evident in some males while others appeared normal. The nuclear material of the sperm head developed abnormally in some sperm. The testis degeneration noted by Adamstone and Card was later confirmed by Adamstone (1942).

Adamstone and Card (1934b) also postulated that a fatty material on the sperm head carried a reserve of vitamin E and other nutrients. It was proposed that this substance maintained sperm viability from ejaculation until fertilization and, according to the authors, this could be an explanation why chickens are able to fertilize for long periods on a vitamin E deficient diet. While the permanency of sterility was not mentioned by Adamstone and Card (1934a), it is of interest to note the results of Evans and Burr (1927) and Mason (1933) in which male rats fed a vitamin E deficient diet developed irreversible sterility and testicular degeneration.

More recently, Arscott et al. (1965) reported a decrease in sperm concentration and fertility in White Leghorn male chickens fed a diet high in linoleic acid but low in vitamin E and the antioxidant, ethoxyquin. The addition of either vitamin E or ethoxyquin to the high linoleic acid diet maintained fertility and sperm concentration as did a low linoleic acid diet when vitamin E and ethoxyquin were absent. Increasing the volume of semen inseminated and therefore the number of sperm, did not improve the fertility of males fed the sterility inducing diet. On all diets semen volume, hatchability of fertile eggs, feed consumption and body and testes weights were not significantly affected. Arscott and Parker (1967) later confirmed the above report and showed that fertility and sperm concentration of male White Leghorn chickens could be restored to normal levels when vitamin E was added to a diet high in linoleic acid but low in vitamin E and ethoxyquin. A search of the literature reveals similar findings for the vitamin E deficient hamster (Mason, 1949). Male mice fed a vitamin E deficient diet for 400 days maintained their fertilizing capacity and showed no testicular degeneration (Bryan and Mason, 1940) while the reversibility of testicular damage demonstrated in the vitamin E deficient guinea pig is not known (Pappenheimer and Schogoleff, 1944). More recently, Price (1968) reported that a vitamin E deficient diet resulted in a significant reduction in fertility of male Japanese quail although no testis

degeneration was noted. Supplementing the diet with vitamin E restored fertility to normal.

Other Nutritional Factors Affecting Fertility in the Male Fowl

Parker and McSpadden (1943b) reported testes degeneration and a reduction in fertility, semen volume and the number of spermatozoa per ejaculate in Rhode Island Red male chickens fed 42 to 72% of their normal feed intake. Boone et al. (1967) reported similar results in White Plymouth Rock males completely restricted of feed and water. Parker and Arscott (1964) noted a decrease in testis size, fertilizing capacity and semen volume in White Leghorn males fed a low calorie diet. Arscott and Parker (1963) reported no adverse effect on reproduction performance of White Leghorn males fed a 6.9% protein diet. Gleichauf (1967) reported that animal protein was essential for normal semen volumes in cockerels of the same breed.

Paredes and Garcia (1959) reported an adverse effect on semen quality in Leghorn males fed a vitamin A deficient ration. No significant differences were noted in semen volumes. On the other hand, Ferrand and Bohren (1948) reported normal semen quality and fertility in New Hampshire and Barred or White Plymouth Rock males fed a carotenol deficient diet. Perek and Snapir (1963) have shown that supplementary vitamin C significantly increased semen

volume in White Rock males while Krzanowska (1956) reported that vitamin B_{12} slightly increased fertility but had no effect on sperm production in male chickens. Cooper et al. (1963) have shown that feeding low levels of manganese had no adverse effect on fertility or semen quality in Rhode Island Red males.

Although this research deals specifically with male chickens a review of the literature related to the female fowl as well as the chick is believed desirable.

Vitamin E, Antioxidants and Linoleic Acid in Reproduction of the Female Fowl

The relationship of vitamin E to reproduction of the female fowl was first noted by Card et al. (1930). A diet treated with ferric chloride to destroy vitamin E resulted in a marked drop in hatchability when fed to female Rhode Island Red chickens. Daily supplementation with 1/2 cc of wheat germ oil, which is high in vitamin E, restored hatchability to normal. Jensen et al. (1953) reported a decrease in hatchability of eggs from Empire White turkey hens fed a vitamin E deficient diet while Ferguson et al. (1956) reported similar results with Broad Breasted Bronze turkey hens fed essentially the same diet. Adding vitamin E to the diet in both reports restored hatchability, while supplemental niacin had no effect in Jensen's report. Jensen also noted that poults hatched from the vitamin E

deficient group were weak compared to the vitamin E supplemented group. No differences were reported in egg production, feed consumption or body weights. Jensen and McGinnis (1957a) went one step further and injected vitamin E into eggs laid by Broad Breasted Bronze hens fed a vitamin E deficient diet with a resultant increase in hatchability over those injected with distilled water. Injection with methylene blue resulted in early death of all embryos while ascorbic acid was without effect.

Singsen et al. (1954) found that a vitamin E deficiency had no effect on egg production or adult mortality in Barred Plymouth Rock layers. They noted that the exclusion of a vitamin mixture from the diet resulted in a marked drop in fertility which was restored by vitamin E supplementation. Vitamin E addition gave no response when added to a diet containing ascorbic acid, vitamin K and B complex vitamins. Nutritional encephalomalacia was observed in dead embryos and day old chicks hatched from hens receiving the deficient diet. Chicks hatched from hens fed the vitamin E supplemented diet were heavier than those from the unsupplemented group.

Atkinson et al. (1955), on the other hand, noted no change in fertility or egg production of Beltsville Small White Turkey hens fed an all vegetable diet plus 20 mg/lb of vitamin E although Jensen et al. (1956), working with Broad Breasted Bronze hens, reported an increase in fertility by adding a similar amount of α-tocopherol acetate

to a diet with or without 2% fish liver oil. Both groups of investigators observed a decrease in hatchability and a high incidence of embryonic mortality between 24 and 28 days of incubation in embryos from dams fed the vitamin E deficient diet. Jensen and his coworkers also reported an increase in hatchability when vitamin E or the antioxidant, N, N-diphenyl-para-phenylene diamine (DPPD) was added to the diet with or without 2% fish liver oil even though the tocopherol content of the egg yolk remained low. Jensen's group also reported that embryonic mortality was slightly reduced by adding vitamin E to the 2% fish liver oil diet. Atkinson's group along with Ferguson et al. (1954) and Jensen and McGinnis (1957b) observed a cloudiness of the cornea or blindness in many of the embryos or poults hatched from hens deficient in vitamin E.

Jensen and McGinnis (1957b) also reported no increase in fertility of Broad Breasted Bronze hens when several levels of vitamin E were added to a vitamin E deficient diet containing 1% fish liver oil. Hatchability increased with increased levels of vitamin E. These workers felt that at least 12 IU of added vitamin E per pound of ration were needed for maximum hatchability in the turkey. They also reported that butylated hydroxy toluene (BHT), another antioxidant, increased hatchability to a level equivalent to 3-6 IU of vitamin E/lb of diet. Poor growth was noted in poults hatched from vitamin E deficient hens. Creger et al. (1960), working with Beltsville Small White hens, noted

an increase in fertility when 5% dried brewers yeast was supplemented to a diet deficient in vitamin E, although hatchability was not increased. Hatchability and fertility were increased by supplementing 10 IU/lb of vitamin E while .1 ppm of selenium was without effect.

Jensen and McGinnis (1960) noted an increase in hatchability and viability of White Leghorn chicks hatched from hens fed torula yeast by adding vitamin E to the diet. The addition of DPPD increased hatchability slightly but did not affect the viability of chicks while the addition of 3% fat had no influence on hatchability, viability or egg production. Substituting dried brewers yeast in place of torula yeast reduced chick mortality but had little effect on hatchability. Death loss of embryos as well as day old chicks in the group receiving torula yeast deficient in vitamin E was high. The addition of vitamin E to the torula yeast and dried brewers yeast diets increased hatchability whereas BHT supplementation had no effect. Adding selenium to the torula yeast diet failed to increase hatchability or lower mortality of chicks.

Machlin et al. (1962) observed a decrease in egg production when female White Leghorn chickens were fed a diet high in linoleic acid (7.2%) but low in vitamin E and ethoxyquin. When birds were fed the same basal diet with only .5% linoleic acid, egg production was equivalent to birds receiving ethoxyquin. The addition of 20 or

100 IU of vitamin E/lb of feed, after eight weeks on the basal diet, restored egg production to levels obtained by the control group supplemented with both vitamin E and ethoxyquin. The addition of .075% ethoxyquin failed to increase egg production to control levels but prevented a further decline. Fertility of 30% was reported in the high linoleic acid - low vitamin E diet whereas 60% fertility resulted when vitamin E or ethoxyquin was added. Hens receiving a diet with 7.2% linoleic acid had lower fertility than those fed less than 1%. Hatchability decreased from 77 to 0% in hens fed the high linoleic acid diet. One hundred IU/lb of vitamin E and .3% ethoxyquin prevented this drop while .075% ethoxyquin and 20 IU of vitamin E/lb of feed had little effect. Since it took 100 IU/lb of vitamin E and .3% ethoxyquin to prevent this drop, these workers felt that vitamin E was 14 times more effective on a weight basis than ethoxyquin for hatchability. With a low linoleic acid concentration, no added vitamin E or ethoxyquin was required to maintain egg production, fertility or hatchability of fertile eggs.

Jensen (1968), based on the report of Coggeshall and Bieri (1960), felt that the low level of fertility, hatchability and egg production reported by Machlin and his coworkers was due to the adverse effect produced by the lipids, resulting possibly in a deficiency of vitamin A. Bieri et al. (1960) fed two groups of New Hampshire

female chicks to maturity with and without added vitamin E. Egg production began during the 19th week in both groups. Fertility was normal in both groups even though tissue tocopherol was absent in the deficient group by five weeks of age. Although these results do not support those of Machlin et al. (1962), it should be noted that low levels of linoleic acid were used. Olson et al. (1962) also reported that fertility was not affected in hens fed a 10% safflower oil diet deficient in vitamin E and antioxidants although hatchability was adversely affected and chicks hatched were in poor condition.

Price (1968) reported no adverse effects on fertility, egg production, or egg weight in female Japanese quail fed a vitamin E deficient diet. In an unpublished paper cited by Jensen (1968), male and female Japanese quail were fed a basal diet low in selenium and vitamin E. A decrease in hatchability of fertile eggs resulted when the quail were fed the basal diet with or without .3% ethoxyquin compared to those supplemented with either 1 ppm of selenium, 30 IU/kg of α-tocopherol acetate or a combination of the two. Adding selenium to the diet supported hatchability at levels corresponding to a vitamin E supplemented group. Young quail hatched from hens receiving the selenium deficient diet were unthrifty and weak. Egg production and fertility were similar in all groups.

Functions and Deficiency Symptoms of Vitamin E in the Chick

A deficiency of vitamin E may result in nutritional encephalomalacia, exudative diathesis and muscular dystrophy in the chick and poult as well as cloudy cornea or enlarged hock disorder in turkeys.

An excellent review has been published by Scott (1962), in which he notes that vitamin E is not associated with a specific metabolic function, although it is believed that this vitamin functions in several metabolic systems. These proposed functions are:

1. As a biological antioxidant.
2. In synthesis of ubiquinone.
3. In metabolism of nucleic acids.
4. In synthesis of ascorbic acid.
5. In the cytochrome C reductase system.
6. In phosphorylation reactions.

Scott's review, as well as a review by Calvert (1964) indicated that the most important function of vitamin E was that of an antioxidant. They noted that vitamin E functioned as an antioxidant in the prevention of nutritional encephalomalacia (crazy chick disease). Vitamin E is closely associated with the metabolism of linoleic acid. Several workers cited by Scott felt that vitamin E guarded against encephalomalacia by preventing linoleic acid from being broken down

to 12-oxo-cis-9-octadecenoic acid. Both reviewers cited evidence that the antioxidants DPPD, BHT, ethoxyquin and methylene blue protected chicks against encephalomalacia when fed a vitamin E deficient diet. DPPD was also effective in preventing encephalomalacia in chicks fed a completely rancid vitamin E and antioxidant free diet. The exclusion of DPPD from the rancid diet left 75% of the chicks dead from encephalomalacia. Scott indicated that it is not known whether antioxidants take the place of vitamin E in its metabolic role or prevent the destruction of minute amounts of vitamin E present in vitamin E deficient diets.

These reviewers noted that under certain conditions a vitamin E deficiency caused exudative diathesis. Dried brewers yeast contained a factor which reduced the amount of vitamin E necessary to prevent exudative diathesis in chicks. This factor was identified as selenium. On a vitamin E deficient diet sodium selenite prevented exudative diathesis. Scott further states that torula yeast fed to chicks receiving a diet deficient in selenium and vitamin E but containing 8 mcg of sodium selenite/100 g of diet gave a superior growth response compared to chicks supplemented with vitamin E and equivalent to a vitamin E and selenium supplemented diet. The sodium selenite supplemented ration produced no sign of exudative diathesis in chicks and restored blood albumen levels equivalent to the vitamin E supplemented group. Omitting sodium selenite from the vitamin

E - selenium deficient diet resulted in poor growth and high mortality. Mortality reached a peak when chicks were between five and eight weeks of age. Selenium prevented exudative diathesis in chicks fed a vitamin E - selenium deficient ration when isolated soya bean or crystalline amino acids were used as the source of protein.

Both Scott and Calvert cited several references noting the inability of antioxidants in preventing muscular dystrophy although they reported that small amounts of vitamin E or cystine were effective. Excess linoleic acid had no effect on the disease. Methionine took the place of cystine in preventing the disorder when low levels of vitamin E and cystine were fed but further evidence showed that cystine was specific for the prevention of muscular dystrophy. Scott noted that when diets low in vitamin E, methionine and cystine were fed, the addition of . 2% methionine resulted in no improvement of muscular dystrophy, but when . 15% cystine was added, muscular dystrophy was corrected. The . 2% methionine and . 15% cystine contained the same amount of sulfur. Methionine provided a growth stimulating effect while cystine did not. Further evidence showed that D-α-tocopherol acetate gave a growth depressing effect when added to a diet deficient in vitamin E, methionine and cystine, although muscular dystrophy was prevented. Methionine addition overcame the growth depression.

Scott noted that a deficiency of arginine prevented muscular

dystrophy in chicks even when vitamin E and cystine were deficient. The addition of 1% arginine monohydrochloride produced muscular dystrophy in 90% of the chicks by five weeks of age. Increasing the level of arginine to 2, 3 and 4% of the diet resulted in no increase in symptoms than was produced at the 1% level. The addition of vitamin E, methionine or cystine to the diet containing arginine prevented the disease.

In both reports it was noted that selenium prevented exudative diathesis in chicks and poults although it had no effect in preventing encephalomalacia. The low level of selenium required to prevent exudative diathesis on a vitamin E, methionine and cystine deficient diet had little effect in preventing muscular dystrophy. The addition of 2.5 mg of D-α-tocopherol or 1.0 mg of selenium/kg of diet did not completely prevent muscular dystrophy although both supplemented in a vitamin E deficient diet were effective.

Since only minute quantities of antioxidants such as DPPD and ethoxyquin prevent encephalomalacia in chicks and fail to prevent exudative diathesis and muscular dystrophy, Scott feels that vitamin E acts as a nonspecific antioxidant since antioxidants provide the same protection. It takes very large quantities of antioxidants to prevent muscular dystrophy and exudative diathesis while only small amounts of vitamin E are needed. Thus, it appears that vitamin E functions in metabolic processes in preventing muscular dystrophy

and exudative diathesis but functions as an antioxidant in preventing encephalomalacia.

In a review, Singsen (1955) discussed an enlarged hock disorder in turkeys which was prevented by supplementing the diet with 5 IU/lb of vitamin E and 1% phosphorus. Hunt and McGinnis (1959) reviewed the occurrence of leg weakness in turkeys due to vitamin E and interrelating nutrients. They acknowledged that mature turkeys had a greater amount of perosis and other leg weaknesses if an enlarged hock was present at two to four weeks of age. At an early age this was prevented by adding 30 mg of niacin and 5 mg of tocopherol acetate/lb of feed to the diet. The addition of 20 mg of niacin/lb of feed with the same level of vitamin E prevented the disorder in developing poults showing enlarged hocks at two to four weeks of age. Niacin or vitamin E supplementation alone was ineffective.

Their trial showed that vitamin E and niacin supplementation did not prevent leg weakness in mature turkeys or poults. They also showed that leg weakness at maturity had no relationship to enlarged hocks at two to four weeks of age. The authors accounted for the controversy by noting that the previous workers produced the disease with stress conditions which made necessary additional vitamin E and niacin while they induced the disease by feeding an isolated soybean diet. They also noted that leg weakness was highly

transferrable from the sire to offspring which may have played some role in the differing results.

The relationship of vitamin E, niacin and enlarged hock disorder to a leg weakness in turkeys recently reported by Jensen (1967) and Richardson and Wilgus (1967) is unknown. Biotin supplementation was effective in correcting or preventing this form of leg weakness.

EXPERIMENTAL PROCEDURE

Forty-two dubbed White Leghorn cockerels hatched on April 14, 1967 were placed in separate wire cages on September 27, 1967. The males were housed in a windowless forced draft room throughout the experiment. To aid in equalizing groups, preliminary records were taken for fertility; hatchability; body weights; sperm live-dead percent and semen volume, concentration and density. The males were distributed into their respective experimental groups in such a way as to avoid positional effects. Fourteen hours of artificial lighting per day were supplied throughout the trial. Feed and water were provided ad libitum. The trial commenced on December 5, 1967.

Based on the preliminary records, the males were divided into three groups of six (lot I), six (lot II), and 30 (lot III) males each so that all groups were quite similar for fertility, hatchability and semen volume. The rations fed are shown in Table 1, and were developed from the report of Machlin et al. (1962) using females and were modified for males by Arscott et al. (1965). The diets consisted of isolated soybean protein, glucose monohydrate plus added vitamins, minerals and methionine. The hen diet (S-25-B) was modified by reducing the levels of calcium and phosphorus to slightly above those required by the chick by utilizing the salt mixture[1] reported by Fox and

[1] See footnote 7, Table 1.

Table 1. Composition of experimental rations. [1]

Ingredients	Lots I and IV %	Lots II and VI %	Lot III %	Lot V %	Lot VII %
Isolated soybean protein[2]	25.00	25.00	25.00	25.00	25.00
Air oxidized[3] safflower oil[4]	10.00	10.00	10.00	10.00	10.00
Glucose monohydrate[5]	48.6	48.575	48.675	48.375	48.175
Salts N[7]	6.00	6.00	6.00	6.00	6.00
Cellulose[6]	8.00	8.00	8.00	8.00	8.00
Methionine hydroxy analogue[8]	.4	.4	.4	.4	.4
Vitamin A, dry (10,000 IU/g)	.2	.2	.2	.2	.2
Vitamin D_3, dry (15,000 ICU/g)	.025	.025	.025	.025	.025
Vitamin K, B-complex vit. mixture[9]	.6	.6	.6	.6	.6
Ethoxyquin[10]	.075	---	---	.3	---
Vitamin E (20,000 IU/454g)[13]	---	.1	---	---	.5
Choline Cl (25%)	1.00	1.00	1.00	1.00	1.00
Na_2SeO_3 in glucose monohydrate[11]	.1	.1	.1	.1	.1
Actual analysis:[12]					
Vitamin E, mg/kg	4.3	36.7	4.3	4.3	166.3
Linoleic acid, %	7.3	7.3	7.3	7.3	7.3

[1] Adapted from the report of Machlin et al. (1962).

[2] Assay protein C-1, (Skidmore Enterprises, Cincinnati, Ohio).

[3] Tenox 6, which contains 10% butylated hydroxytoluene, 10% butylated hydroxyanisole, 6% propyl gallate, 6% citric acid, 12% propylene glycol and 56% mixed glycerides (Eastman Chemical Products, Inc., Kingsport, Tenn.).

[4] Alkaline refined safflower oil, (Pacific Vegetable Oil Corp., San Francisco, Calif.).

[5] Cerelose, (Corn Products Co., New York).

[6] Solka Floc BW-100, (Brown Co., Berlin, New Hampshire).

[7] Salts N - Fox and Briggs (1960), supplies as % of diet: Ca, 1.24; P, .8; K, .37; Na, .384; Cl, .58; Mg, .06; Fe, .00334; Mn, .00813; I, .0006; Zn, .00728; Cu, .0004 or $CaHPO_4$, 2.84; $CaCO_3$, 1.; Na_2HPO_4, .7; NaCl, .4; KCL, .7; $MgSO_4$, .3; $FeC_6H_5O_7 \cdot 5H_2O$, .02; $MnSO_4 \cdot H_2O$, .025; KIO_3, .001; $ZNCO_3$, .013; $CuSO_4$, .001, (General Biochemicals, Chagrin Falls, Ohio).

[8] Ca-DL-2-hydroxy, 4-methylthiobutyrate, (Monsanto Chemical Co., St. Louis, Mo.).

[9] Vitamin mixture-Machlin and Gordon (1958), supplies in mg/kg of diet: vit. K (menadione), 1.2; vit. B_{12}, .036; thiamine.HCl, 28.8; riboflavin, 19.2; Ca-D-pantothenate, 24.; niacin, 120.; pyridoxine.HCl, 9.6; folacin, 4.8; biotin, .36, (Nutritional Biochemicals Corp., Cleveland, Ohio).

[10] Santoquin, 1,2-dihydro-6-ethoxy-2, 2, 4-trimethylquinoline, (provided by Monsanto Chemical Co., St. Louis, Mo.).

[11] Optional trace mineral - Fox and Briggs (1960) with 21.9 mg of Na_2SeO_3 mixed per 100 g of glucose monohydrate.

[12] Based on ingredient analysis (Arscott et al., 1965).

[13] Myvamix, 20,000 IU/454 g, (Distillation Products Industries, Rochester, N. Y.).

Briggs (1960), and including the optional level of selenium. Substitutions or additions to the diet were made at the expense of glucose monohydrate.

All groups were fed diets high in linoleic acid (7. 3%) throughout the trial by means of 10% air oxidized safflower oil. Diets fed to lots I and II contained supplementary ethoxyquin[2] (. 075%) and vitamin E[3] (32. 4 mg/kg), respectively, throughout the trial. The levels of vitamin E and ethoxyquin used in this trial were taken from the report of Machlin et al. (1962). Males in lot III, the sterility inducing diet, received no supplementary vitamin E or ethoxyquin until the 38th week, when they were subdivided into five lots containing approximately six males each. The lot was subdivided into its respective groups and equalized for fertility based on the results of the 34th week. One of the groups was retained on the negative control diet (lot III). Lots IV and V were supplemented with . 075% and . 3% ethoxyquin, respectively, while lots VI and VII received 32. 4 and 162. 0 mg/kg of added vitamin E.

The tocopherol content of the safflower oil was destroyed by passing air into a 4. 54 kg sample from an aspirator pump for 32 hours at approximately 72° C. The oil was contained in a 12 liter

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[2]See footnote 10, Table 1.

[3]See footnote 13, Table 1.

round bottom flask surrounded by an electric heating mantle. To prevent rancidity, .1% Tenox 6[4], an antioxidant, was added to each 4.54 kg sample of oil. The oil was stored in a deep freeze at -12°C until needed. The vitamin E and linoleic acid content of the untreated and air oxidized oil have been reported by Arscott et al. (1965) as 490 to 41.1 mcg/g and 75 to 72.6 %, respectively.

The rations were mixed in 45.4 kg lots and stored in the above mentioned freezer. Small amounts of feed were kept at room temperatures for use during each week of the experiment.

Fertility was determined by ejaculating the males using the massage technique (Figure 1) of Burrows and Quinn (1939), and artificially inseminating the ejaculate into three White Leghorn females, housed in wire cages, at no less than three week[5] intervals. When low semen volumes were encountered only one or two hens were inseminated. In all cases, the males were pre-ejaculated two days prior to the collection and insemination period to eliminate the possibility of differences in fertility due to the length of time between testing.

The ejaculate from each male was measured for volume in a

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[4]See footnote 3, Table 1.

[5]It was established during the preliminary period that 11.2% fertility resulted in eggs saved during the 14th day after insemination while fertility was 0% for eggs saved during the 21st day.

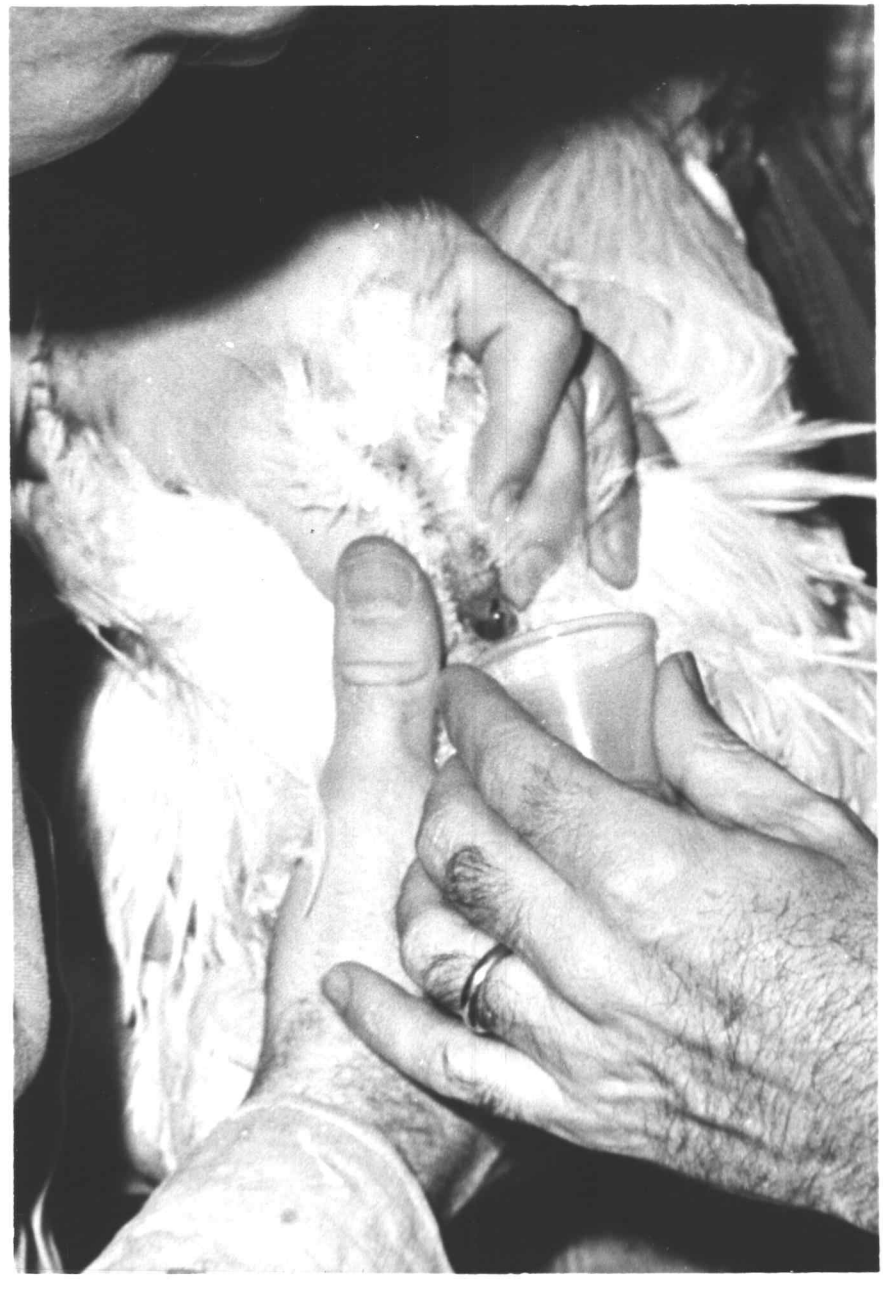

Figure 1. Obtaining semen using the massage technique.

1 cc tuberculin syringe prior to insemination (Figure 2). Semen from each male was then artificially inseminated into the females using a dose of .05 ml of undiluted semen (Figure 3). The process was done on a rotational basis in such a way that semen from a given male was inseminated into a different group of females each time. During the 38th week, hens normally used for insemination were replaced by pullets just coming into egg production.

Eggs were saved for incubation from the second through the ninth day after insemination. Fertility was determined by candling the eggs no earlier than the sixth day of incubation. Eggs which appeared infertile were broken out and examined macroscopically for early embryonic development. At hatching time all unhatched eggs were broken out and examined to check the presence of dead germs or early embryonic mortality.

Hatchability data were obtained on eggs which were fertile and are expressed as hatchability of fertile eggs. A total of 5,411 eggs were incubated during the experiment.

Body weights were obtained at the start of the trial and thereafter at four week intervals while the feed consumed per 24 hour period per male was taken once a week with four week averages computed for presentation in graphic form.

Semen concentration and transmittancy data were obtained the day after each ejaculation and insemination period. Semen not used

Figure 2. Measuring the volume of semen using a 1 cc syringe.

Figure 3. Inseminating . 05 cc of semen into a female.

for insemination was stored overnight in a refrigerator at 5° C. The next day semen was diluted 1:100 by placing .05 cc of semen into 5 cc of .85% saline solution followed by mixing on a Sensaur lab mixer for ten seconds. Transmittancy readings were obtained by placing the solution into tubular absorption cells and inserting the cells into a Cenco-Sheard-Sanford-Photelometer equipped with a 640 mμ filter (Kosin and Wheeler, 1956). These readings were converted to optical density values for expression on a linear basis (Hawk et al., 1947). Concentration readings were obtained with a Phillips - Drucker micro hematocrit reader (model L-550 Astoria, Oregon) using 75 mm capillary tubes with an outside diameter of 1.4 - 1.6 mm. The tubes were filled with semen, sealed at one end with putty and placed in a micro capillary centrifuge (International Equipment Company) for six minutes. The amount of sperm accumulated at the base was quantitatively measured by the hematocrit reader in relation to the amount of serum accumulated at the top. The value obtained is the percent volume the sperm occupies in the semen. During the course of this experiment Cherms (1968) reported spermatocrit values for turkey semen using a procedure similar to the one used in this investigation for semen concentration. In his report he showed no significant differences between various lines of turkeys.

Live-dead sperm counts were obtained using a procedure from

the report of Cooper and Rowell (1958) and modified by Harper (1967) using a staining period of three to four minutes rather than one minute as suggested by the original authors. Three drops each of 1% eosin "B" and 5% nigrosin stain were added to a slide containing a small drop of fresh undiluted semen, stirred and allowed to stain for three to four minutes. A second slide was used to spread a thin layer of the solution on a new slide. The slides were air dried and examined microscopically so that two fields of 100 sperm were counted on each slide for dead sperm. Spermatozoa stained to any degree were considered dead.

Standard errors were computed for all data and significance at the 5% level was determined by students "t" test (Snedecor and Cochran, 1967).

RESULTS

The results are summarized in Figures 4 - 10 and Tables 2-9.

The data for fertility are shown in Figure 4 and Table 2. During the depletion period fertility in lot III, the unsupplemented group, decreased[6] to 21.9% at 34 weeks although it increased to 29.2% at 38 weeks. Beginning at 25 weeks and throughout the depletion period, fertility of lot III was significantly lower than for males supplemented throughout the trial with .075% ethoxyquin (lot I) while males supplemented with 32.4 mg/kg of vitamin E (lot II) was significantly higher beginning at 30 weeks. During the depletion period no significant differences were observed between males in lots I and II except during the 25th week. Based on the results of the 34th week, lot III was subdivided at 38 weeks, after ejaculating for fertility determination, into five lots, equalized for fertility, including the lot retained as the negative control. At least two sterile males were included in each of the subdivided lots. At 41 weeks, the first measurement after the subdivision of lot III, fertility of the lot retained as the negative control increased to 48.3% but dropped to 32.5 and 4.8% at 44 and 47 weeks, respectively. During the same periods, fertility increased to

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[6] Since fertility did not substantially decrease by 20 weeks, barriers or shields were installed to eliminate any possibility of males consuming adjacent diets.

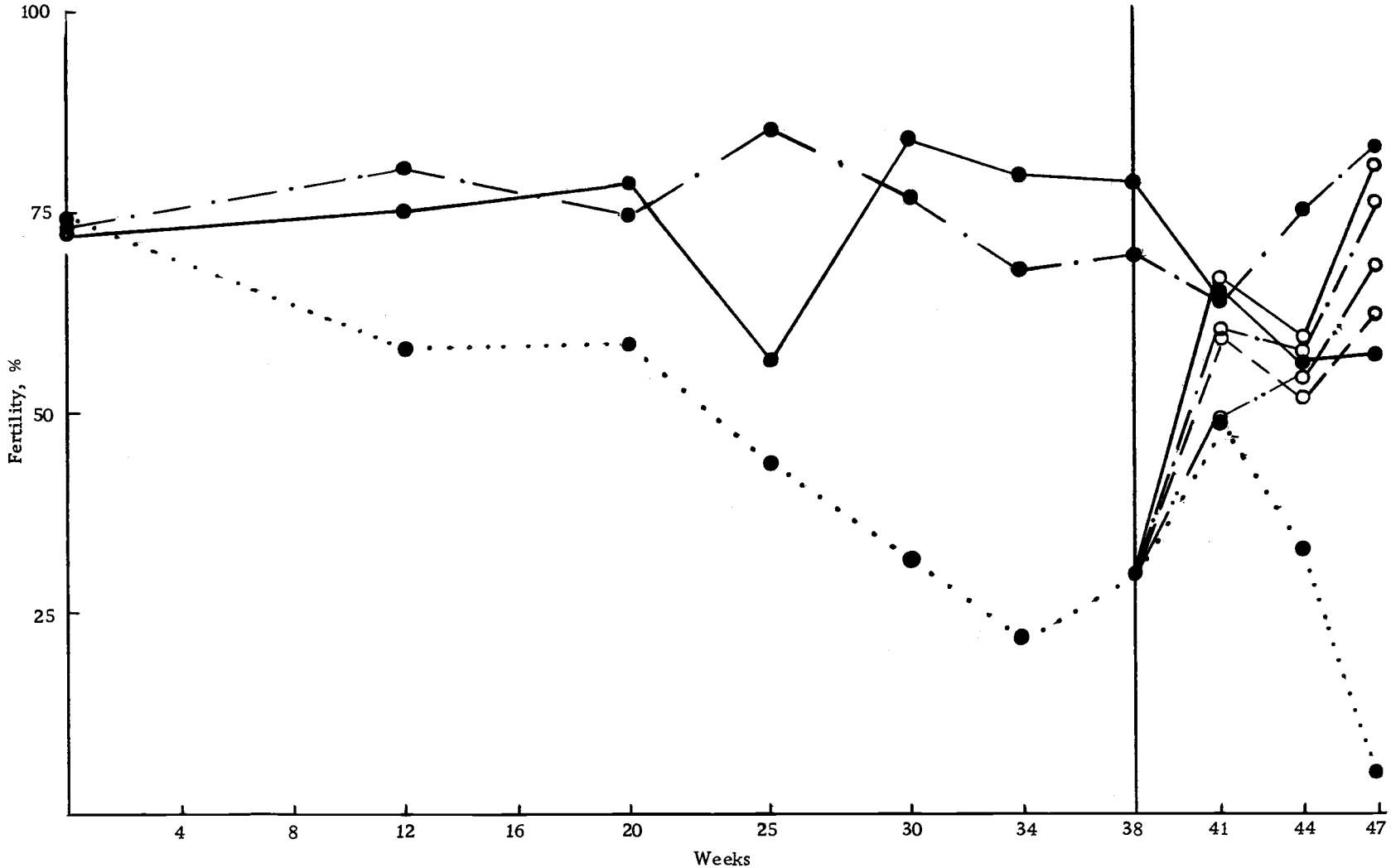

Figure 4. Effect of vitamin E and ethoxyquin on fertility of male chickens. Legend: lot I, ●——— . ———●, .075% ethoxyquin; lot II, ●———●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O——— . ———O, .075% ethoxyquin; lot V, O——— .. ———O, .3% ethoxyquin; lot VI, O———O, 32.4 mg/kg vitamin E; lot VII, O—— —— ——O, 162 mg/kg vitamin E.

Table 2. Effect of vitamin E and ethoxyquin on fertility of male chickens (%).

Lots \ Weeks	0	12	20	25	30	34	38	41	44	47
I	73.1±7.5[a]	80.6±11.0[a]	74.7± 7.0[a]	85.0±9.3[a]	77.1±19.5[ac]	67.8±4.8[ac]	69.4±14.6[ac]	63.5± 6.6[a]	75.2± 6.3[b]	83.2±7.5[a]
II	72.6±5.8[a]	75.0±13.4[a]	78.9±16.4[a]	56.3±8.9[bc]	84.0±10.2[c]	79.7±8.5[c]	78.5± 5.8[c]	64.0±10.5[a]	56.2±17.2[ab]	56.9±10.7[a]
III	73.4±2.5[a]	58.1± 6.0[a]	58.3± 5.4[a]	43.7±6.1[b]	31.2± 6.5[b]	21.9±6.0[b]	29.2± 5.7[b]	48.3±11.1[a]	32.5±16.1[a]	4.8± 4.8[b]
IV								60.1±12.4[a]	56.7±17.6[ab]	76.0±15.8[a]
V								48.6±15.7[a]	54.2±14.0[ab]	68.7±10.0[a]
VI								66.5±10.0[a]	59.0±12.4[ab]	80.6± 5.2[a]
VII								60.0±11.3[a]	51.9±12.2[ab]	62.0±12.9[a]

[1,2]

[1]Figures noted by same letter(s) are not significantly different.

[2]Comparisons for significance made within weeks and not between weeks.

levels approaching the positive control groups in lots supplemented with . 075 or . 3% ethoxyquin (lots IV and V) and 32. 4 or 162. 0 mg/kg of vitamin E (lots VI and VII). No significant differences existed at 41 weeks while at 44 weeks all lots were substantially higher than the negative control although only lot I was significantly greater. All lots were significantly greater than the negative control at 47 weeks. All sterile males (15)[7] fed vitamin E or ethoxyquin beginning at 38 weeks exhibited some degree of fertility with the exception of one male in lot IV. The average fertility of these males at 47 weeks was 71. 5% compared to 11. 9% for the sterile males maintained on the negative control ration (lot III).

The data for semen density are shown in Figure 5 and Table 3. Semen density from males in lot III decreased throughout the depletion period. Lot I had a significantly higher density than the negative control beginning at 20 weeks and throughout the remainder of the experiment, except during the 30th week while lot II was significantly greater during the 34th and 38th weeks of the depletion period and at 47 weeks. No significant differences were observed between birds supplemented throughout the trial with . 075% ethoxyquin (lot I) or 32. 4 mg/kg of vitamin E (lot II). At 41 weeks, semen density from males supplemented at 38 weeks with . 075% ethoxyquin (lot IV) and 162. 0 mg/kg of vitamin E (lot VII) increased substantially but not significantly over lot III while males supplemented with . 3%

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[7] Those males with 0% fertility at 34 weeks.

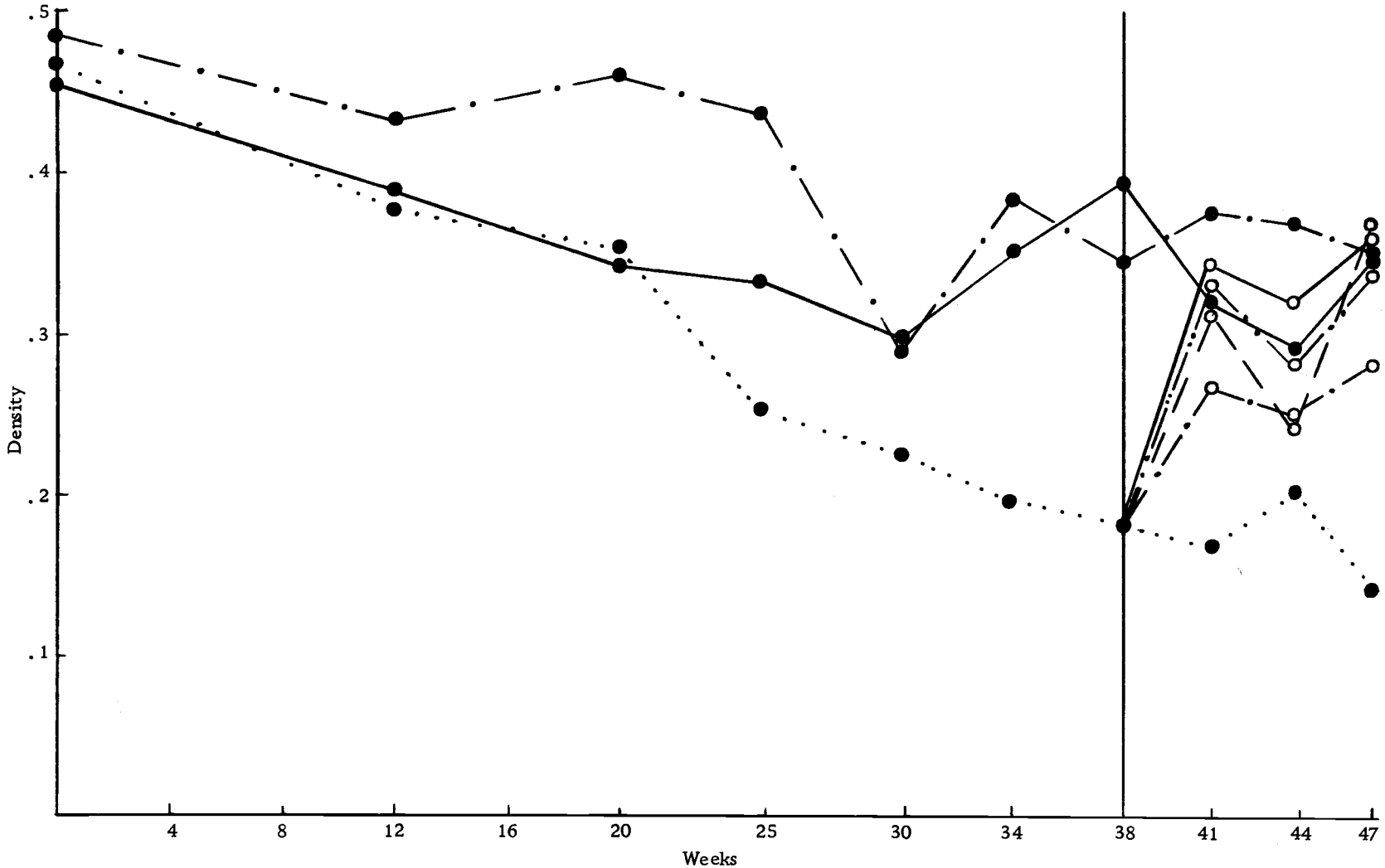

Figure 5. Effect of vitamin E and ethoxyquin on semen density of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●————●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— .. ——O, .3% ethoxyquin; lot VI, O————O, 32.4 mg/kg vitamin E; lot VII, O— — —O, 162 mg/kg vitamin E.

Table 3. Effect of vitamin E and ethoxyquin on semen density of male chickens.

Lots \ Weeks	0	12	20	25	30	34	38	41	44	47
I	.485±.03[a][1,2]	.433±.01[a]	.462±.01[a]	.435±.04[a]	.290±.06[a]	.385±.02[a]	.346±.06[a]	.375±.04[a]	.371±.02[a]	.346±.06[a]
II	.455±.03[a]	.390±.27[a]	.344±.07[ab]	.334±.04[ab]	.296±.04[a]	.350±.07[ac]	.395±.07[ac]	.323±.07[ab]	.293±.06[ab]	.348±.06[a]
III	.468±.02[a]	.378±.11[a]	.355±.02[b]	.254±.03[b]	.225±.02[a]	.197±.02[b]	.182±.03[b]	.167±.05[b]	.201±.03[b]	.142±.04[b]
IV								.267±.06[ab]	.248±.05[b]	.281±.06[ab]
V								.326±.02[a]	.282±.05[ab]	.342±.04[a]
VI								.345±.06[a]	.322±.08[ab]	.349±.03[a]
VII								.315±.05[ab]	.246±.04[b]	.371±.03[a]

[1] Figures noted by same letter(s) are not significantly different.

[2] Comparisons for significance made within weeks and not between weeks.

ethoxyquin (lot V) and 32.4 mg/kg of vitamin E (lot VI) were significantly greater. At 44 weeks semen density from males in lot I was significantly higher than for males in lots III, IV and VII while at 47 weeks density in all lots was significantly higher than the negative control, with the exception of lot IV.

The results for semen concentration are shown in Figure 6 and Table 4. The pattern of significant differences for concentration (Table 4) was quite similar to that for semen density (Table 3) during the depletion period except lot I was significantly greater than lot II at 12 weeks and lot III at 30 weeks. In all lots semen concentration was substantially greater than the negative control (lot III) at 41 and 44 weeks but the only significant difference at these intervals appeared between the negative control and the lot supplemented throughout the trial with .075% ethoxyquin (lot I). With the exception of lot IV, semen concentration in all lots was significantly higher than the negative control at 47 weeks. Although significant differences were not present at 47 weeks between lot IV and the negative control for either semen density or concentration, it should be noted that a substantial margin was present between these lots.

The data for hatchability, semen volume, sperm live-dead percent, feed consumption and body weights are shown in Figures 7-10 and Tables 5-9. No meaningful differences were noted for hatchability of fertile eggs (Figure 7, Table 5), semen volume (Figure 8,

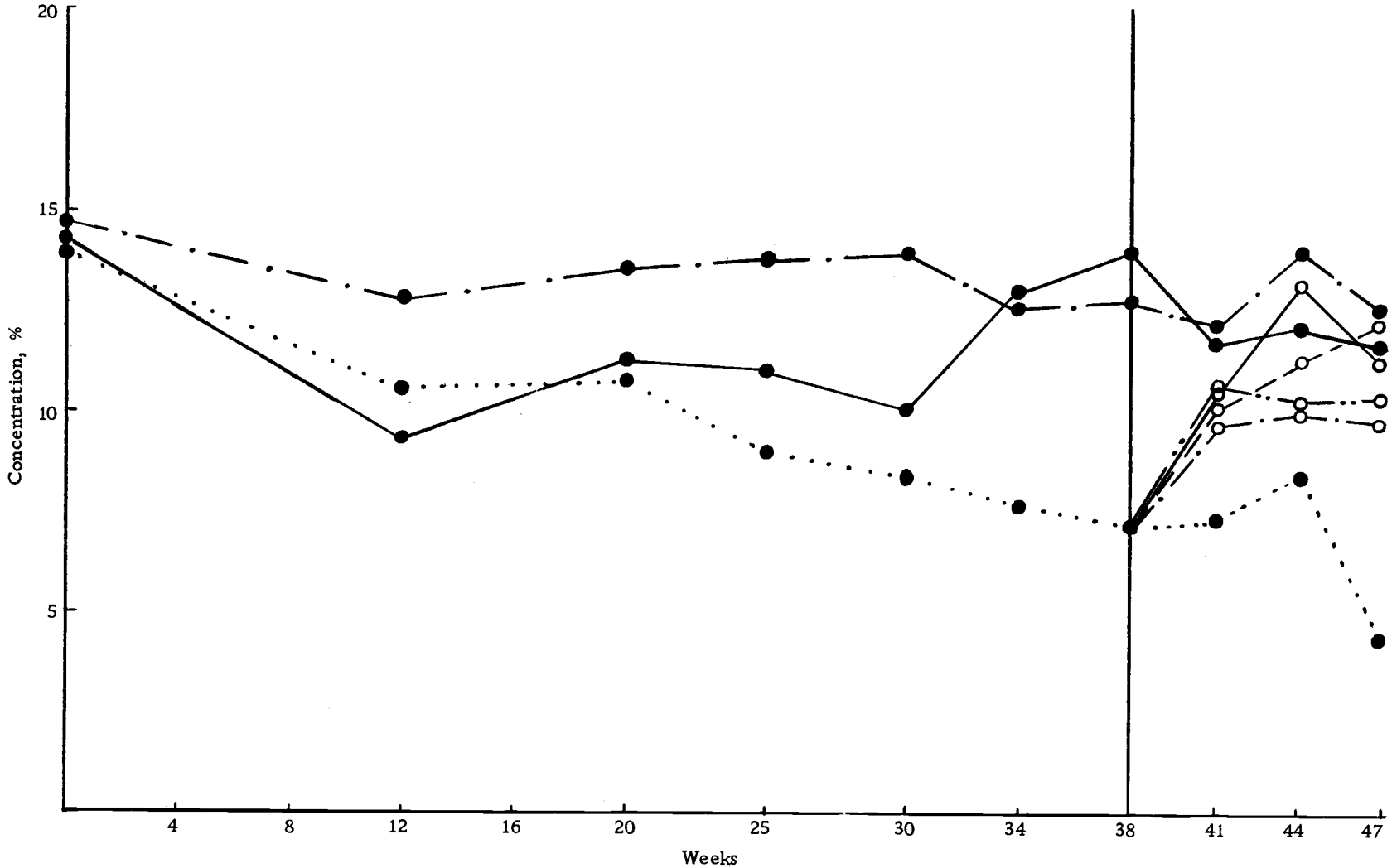

Figure 6. Effect of vitamin E and ethoxyquin on semen concentration of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●———●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— . . ——O, .3% ethoxyquin; lot VI, O———O, 32.4 mg/kg vitamin E; lot VII, O— — —O, 162 mg/kg vitamin E.

Table 4. Effect of vitamin E and ethoxyquin on semen concentration of male chickens (%).

Lots \ Weeks	0	12	20	24	28	34	38	41	44	47
I	14.5±1.1[a]	12.8±1.0[a]	13.6± .93[a]	13.8± .49[a]	14.0±1.6[a]	12.6±1.2[a]	12.8±2.0[a]	12.2±1.2[a]	14.0±1.5[a]	12.6±2.3[a]
II	14.3±2.4[a]	9.3±1.0[b]	11.3±2.4[ab]	11.0±2.1[ab]	10.0±1.7[ab]	13.0±2.5[ac]	14.0±1.8[ac]	11.8±2.4[ab]	12.2±2.3[ab]	11.7±1.4[a]
III	14.1± .56[a]	10.6± .77[ab]	10.8± .83[b]	9.0± .84[b]	8.4±1.0[b]	7.7± .97[b]	7.2±1.1[b]	7.4±2.0[b]	8.4±1.9[b]	4.4±1.7[b]
IV								9.7±2.2[ab]	10.0±2.2[ab]	9.7±2.3[ab]
V								10.7±1.3[ab]	10.2±1.5[ab]	10.3±1.0[a]
VI								10.7±1.0[ab]	13.2±2.4[ab]	11.3±1.2[a]
VII								10.2±2.0[ab]	11.2±1.4[ab]	12.2±1.6[a]

[1,2] [1] Figures noted by same letter(s) are not significantly different.

[2] Comparisons for significance made within weeks and not between weeks.

Table 6), sperm live-dead percent (Table 7), feed consumption (Figure 9, Table 8) or body weights (Figure 10, Table 9) throughout the trial.

The average body weight of males in lot VII increased substantially at 38 weeks when the group was subdivided from lot III. This rise was due to the inadvertent placement of heavier males in this group.

One male died during the experiment. The postmortem examination showed the male had a pendulous crop. The death took place during the 11th week while the male was in the process of being ejaculated when it seemed to suffocate.

One male was discarded from the experiment when it appeared to be sterile after the preliminary period from causes other than that due to the diet treatments. The male was in lot I.

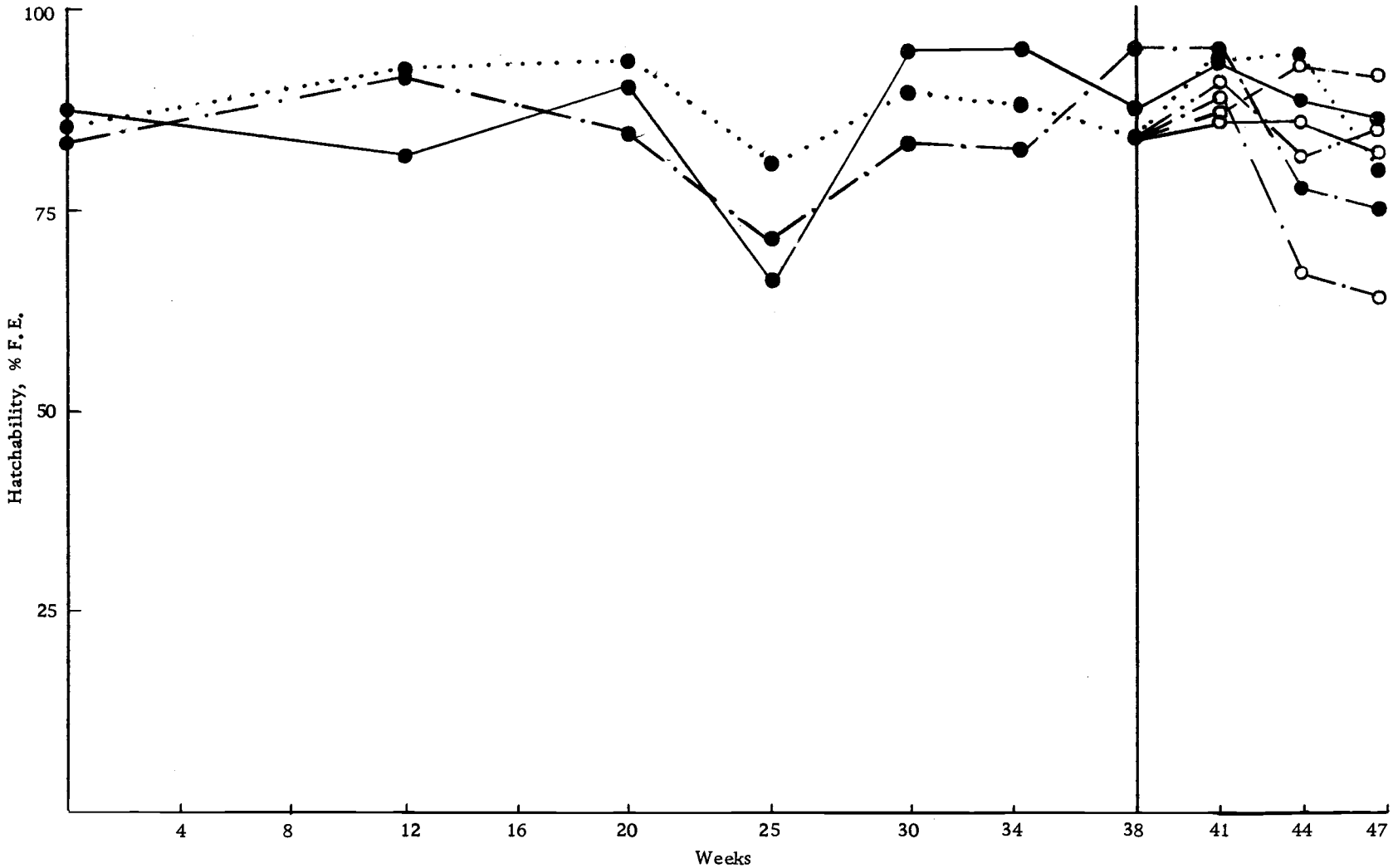

Figure 7. Effect of vitamin E and ethoxyquin on hatchability of fertile eggs of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●———●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— .. ——O, .3% ethoxyquin; lot VI, O———O, 32.4 mg/kg vitamin E; lot VII, O— — — —O, 162 mg/kg vitamin E.

Table 5. Effect of vitamin E and ethoxyquin on hatchability of fertile eggs of male chickens(%).

Lots \ Weeks	0	12	20	24	28	34	38	41	44	47
I	83.7±5.5[a][1,2]	92.2± 4.8[a]	85.0±4.8[a]	71.8± 6.5[a]	84.0±1.2[a]	82.8±7.0[a]	95.3±3.6[a]	95.3±4.7[a]	78.2±6.0[a]	75.3±4.2[ae]
II	87.8±3.8[a]	82.2±10.2[a]	90.4±4.4[a]	66.7±13.9[a]	95.5±2.0[b]	95.4±3.0[a]	88.2±4.0[a]	93.3±3.6[a]	89.0±4.1[ab]	86.9±5.1[bce]
III	85.6±1.8[a]	92.4± 1.6[a]	94.0±2.1[a]	81.3± 4.6[a]	90.0±3.5[ab]	88.7±7.8[a]	84.7±4.4[a]	93.5±4.0[a]	94.5±4.3[b]	80.0±0.0[be]
IV								89.5±3.2[a]	67.6±17.2[ab]	64.2±8.9[ad]
V								91.3±6.5[a]	81.8±8.1[ab]	85.3±6.5[abc]
VI								85.8±4.4[a]	85.2±9.5[ab]	82.3±7.4[ace]
VII								86.3±5.8[a]	93.8±3.3[b]	91.9±4.6[c]

[1]Figures noted by same letter(s) are not significantly different.

[2]Comparisons for significance made within weeks and not between weeks.

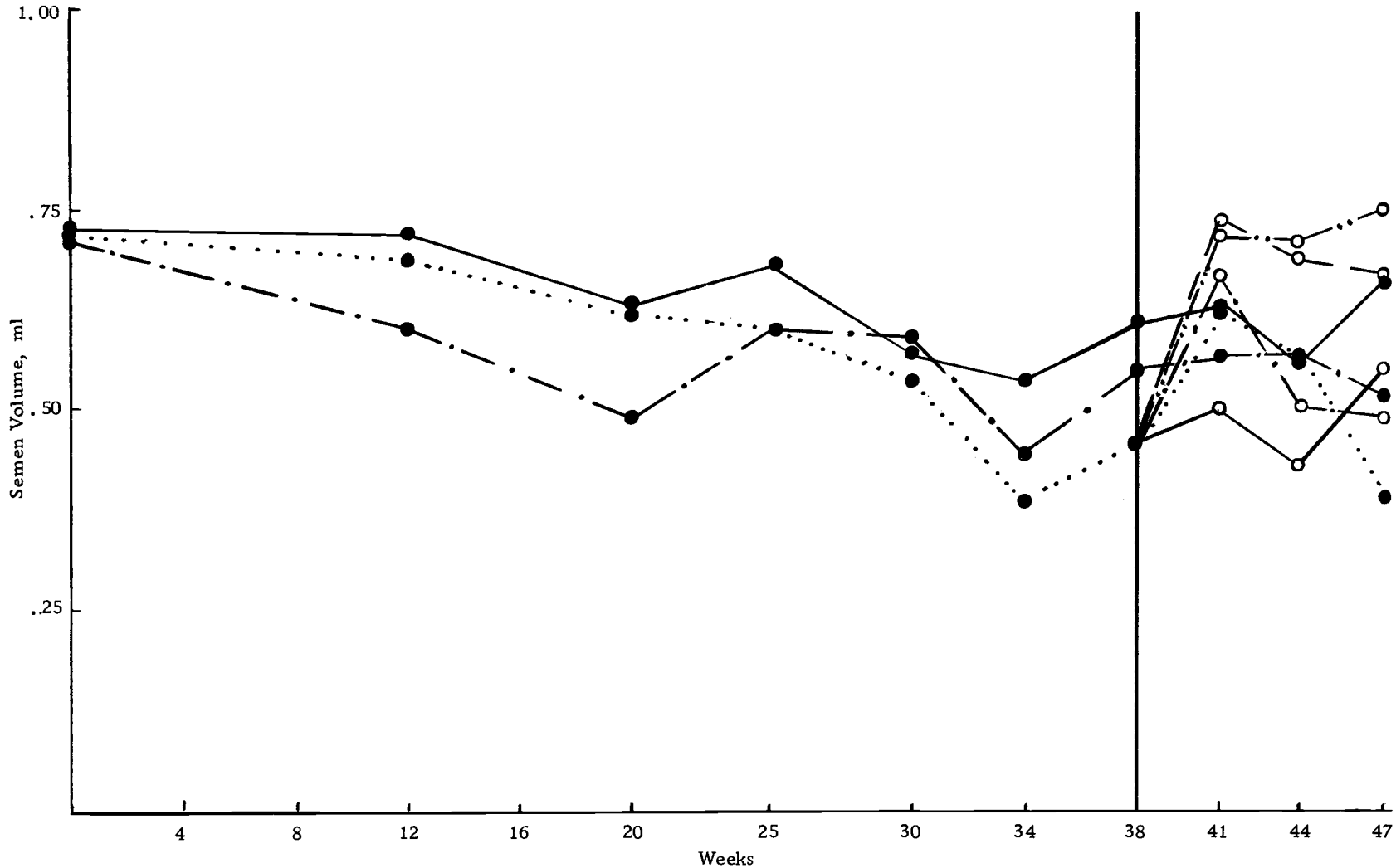

Figure 8. Effect of vitamin E and ethoxyquin on semen volume of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●———●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— .. ——O, .3% ethoxyquin; lot VI, O———O, 32.4 mg/kg vitamin E; lot VII, O— — —O, 162 mg/kg vitamin E.

Table 6. Effect of vitamin E and ethoxyquin on semen volume of male chickens (ml).

Lots \ Weeks	0	12	20	24	28	34	38	41	44	47
I	.71±.09[a][1,2]	.60±.14[a]	.49±.07[a]	.60±.11[a]	.59±.10[a]	.45±.10[ab]	.55±.07[ab]	.57±.09[a]	.57±.07[ab]	.52±.09[ab]
II	.73±.09[a]	.72±.08[a]	.63±.07[a]	.68±.04[a]	.57±.07[a]	.54±.04[a]	.61±.06[a]	.63±.07[a]	.56±.05[ab]	.66±.04[ac]
III	.72±.04[a]	.69±.06[a]	.62±.05[a]	.60±.05[a]	.54±.04[a]	.39±.04[b]	.46±.03[b]	.62±.12[a]	.57±.10[ab]	.39±.11[b]
IV								.67±.13[a]	.50±.08[ab]	.49±.08[bc]
V								.72±.07[a]	.71±.12[a]	.75±.08[a]
VI								.50±.13[a]	.43±.04[b]	.55±.16[ab]
VII								.74±.14[a]	.69±.13[a]	.67±.13[ab]

[1]Figures noted by same letter(s) are not significantly different.

[2]Comparisons for significance made within weeks and not between weeks.

Table 7. Effect of vitamin E and ethoxyquin on sperm live-dead percent of male chickens (percent dead).

lots \ weeks	0	16	36	50
I	24.4±6.4[ab][1, 2]	25.7± 6.2[a]	15.2±7.6[ab]	9.4±2.0[a]
II	11.5±2.6[a]	27.7±10.6[a]	7.7±2.4[a]	13.2±5.0[abc]
III	18.6±2.0[b]	29.3± 3.3[a]	22.9±4.3[b]	30.5±9.7[b]
IV				15.0±.50[c]
V				15.1±3.3[abc]
VI				17.4±3.9[abc]
VII				15.8±4.2[abc]

[1]Figures noted by same letter(s) are not significantly different.

[2]Comparisons for significance made within weeks and not between weeks.

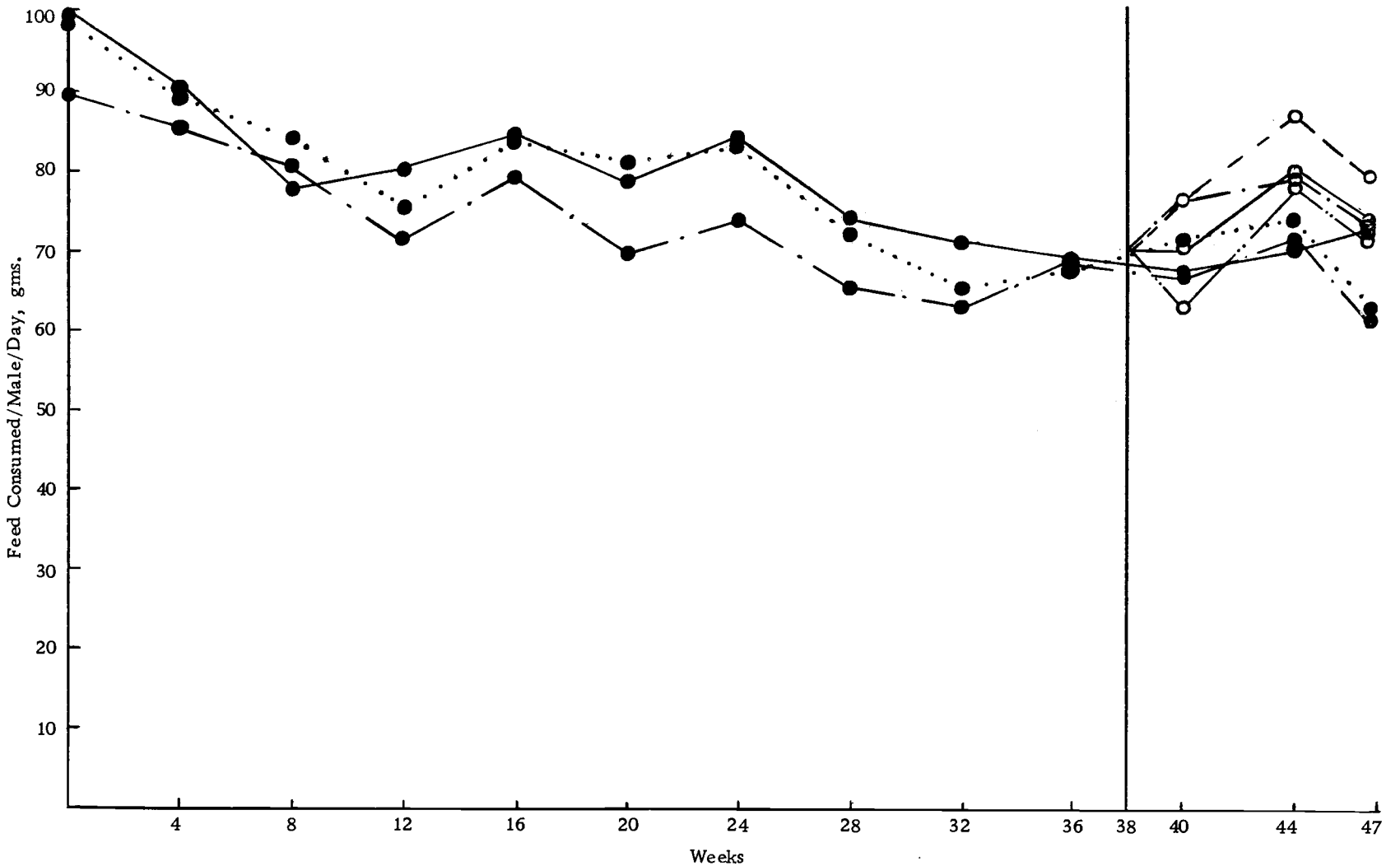

Figure 9. Effect of vitamin E and ethoxyquin on feed consumption of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●————●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— .. ——O, .3% ethoxyquin; lot VI, O————O, 32.4 mg/kg vitamin E; lot VII, O— — —O, 162 mg/kg vitamin E.

Table 8. Effect of vitamin E and ethoxyquin on feed consumption/day of male chickens (gms).

Lots \ Weeks	0	4	8	12	16	20
	[1,2]					
I	89.4±4.0[a]	85.5±6.9[a]	80.2±3.8[a]	71.6±4.5[a]	79.3±4.6[a]	69.9±4.3[a]
II	100.2±3.2[a]	90.6±4.3	78.9±5.1[a]	80.0±3.6[a]	83.3±3.9[a]	79.0±3.2[ab]
III	98.7±2.6[a]	89.9±2.2[a]	83.0±2.1[a]	75.2±2.7[a]	83.8±1.8[a]	81.0±1.7[b]

Lot \ Weeks	24	28	32	36	40	44	47
I	74.2±5.2[a]	65.8±6.5[a]	63.2±4.3[a]	68.6±3.9[a]	66.8±3.3[ab]	71.3±3.8[a]	61.4±4.8[a]
II	84.8±2.7[a]	74.8±2.8[a]	71.3±3.1[a]	68.1±2.6[a]	67.2±4.0[ab]	70.5±4.4[a]	72.4±1.9[bc]
III	83.3±2.2[a]	72.0±1.9[a]	65.7±1.7[a]	67.9±1.6[a]	71.5±4.5[ab]	74.2±6.7[ab]	61.7±5.5[ac]
IV					76.5±3.5[a]	79.3±4.5[ab]	72.5±7.7[ab]
V					63.4±1.8[b]	78.6±2.8[a]	72.3±4.4[ab]
VI					70.8±5.5[ab]	79.8±4.5[ab]	72.6±5.0[ab]
VII					76.5±5.4[a]	87.2±2.0[b]	79.8±3.1[b]

[1] Figures noted by same letter(s) are not significantly different.

[2] Comparisons for significance made within weeks and not between weeks.

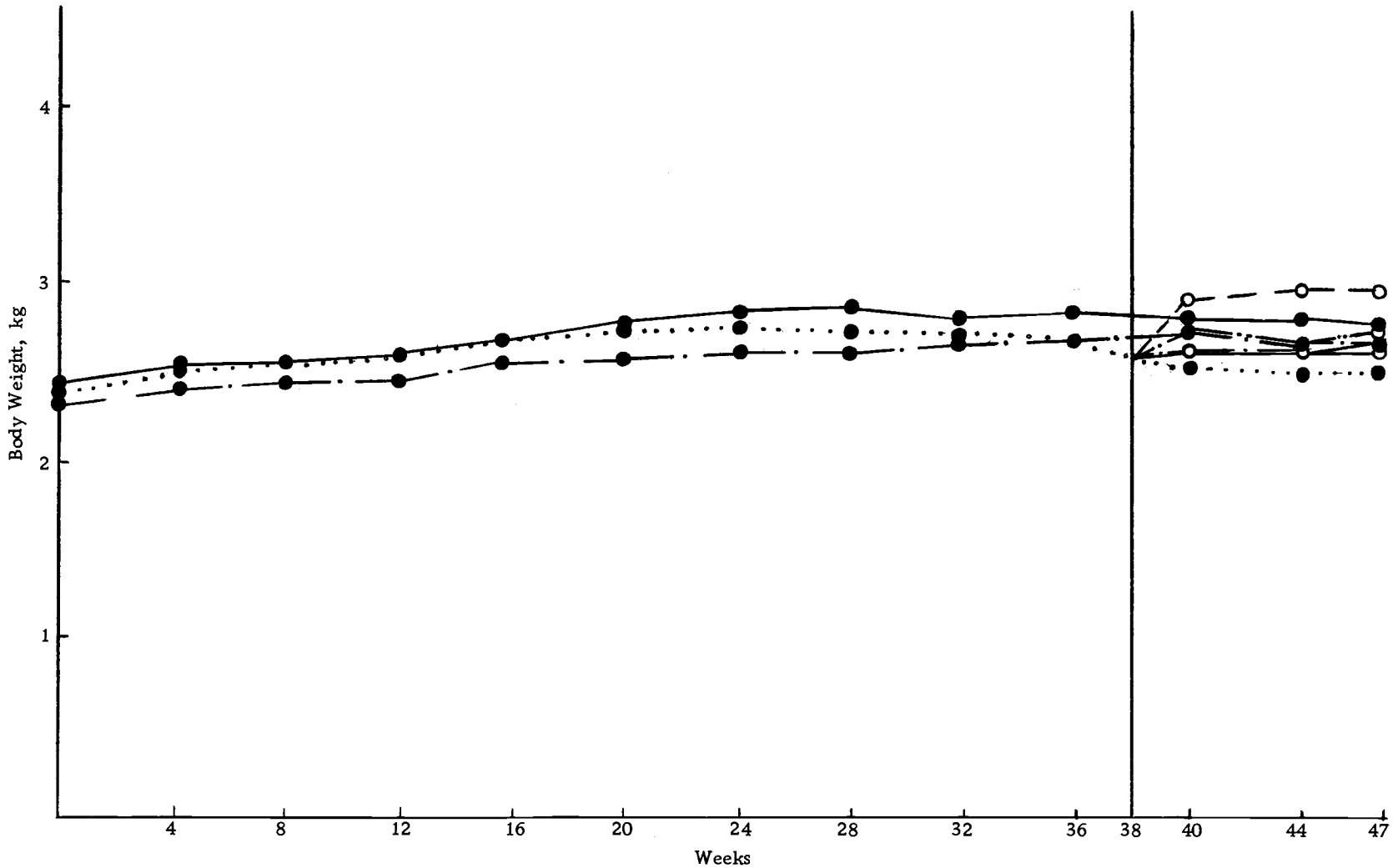

Figure 10. Effect of vitamin E and ethoxyquin on body weights of male chickens. Legend: lot I, ●—— . ——●, .075% ethoxyquin; lot II, ●————●, 32.4 mg/kg vitamin E; lot III, ●.....●, negative control; lot IV, O—— . ——O, .075% ethoxyquin; lot V, O—— .. ——O, .3% ethoxyquin; lot VI, O————O, 32.4 mg/kg vitamin E; lot VII, O—— — —O, 162 mg/kg vitamin E.

Table 9. Effect of vitamin E and ethoxyquin on body weights of male chickens (kg).

Lots \ Weeks	0	4	8	12	16	20
I	2.3±.15[a][1,2]	2.4±.18[a]	2.5±.16[a]	2.5±.16[a]	2.5±.15[a]	2.6±.17[a]
II	2.5±.07[a]	2.5±.09[a]	2.5±.10[a]	2.6±.11[a]	2.7±.11[a]	2.8±.14[a]
III	2.4±.05[a]	2.5±.05[a]	2.5±.05[a]	2.6±.05[a]	2.7±.07[a]	2.7±.05[a]

Lots \ Weeks	24	28	32	36	40	44	47
I	2.6±.16[a]	2.6±.16[a]	2.7±.17[a]	2.7±.16[a]	2.7±.16[ab]	2.7±.15[ab]	2.7±.14[ab]
II	2.9±.16[a]	2.9±.17[a]	2.8±.17[a]	2.9±.16[a]	2.8±.17[ab]	2.8±.16[ab]	2.8±.13[ab]
III	2.8±.05[a]	2.7±.05[a]	2.7±.05[a]	2.7±.05[a]	2.5±.07[b]	2.5±.10[b]	2.5±1.0[b]
IV					2.6±.09[b]	2.6±.09[b]	2.6±.09[b]
V					2.7±.15[ab]	2.7±.11[ab]	2.7±.11[ab]
VI					2.6±.15[ab]	2.6±.14[ab]	2.7±.14[ab]
VII					2.9±.09[a]	3.0±.10[a]	3.0±.10[a]

[1] Figures noted by same letter(s) are not significantly different.

[2] Comparisons for significance made within weeks and not between weeks.

DISCUSSION

It appears evident from the results obtained in this trial that vitamin E and ethoxyquin function in a similar manner in restoring normal fertilizing capacity of the vitamin E deficient male chicken. This is believed the case since the low fertility and semen density or concentration induced by a vitamin E-antioxidant low diet (lot III) were restored to levels essentially comparable to the positive control groups (lots I and II) by using varying levels of either vitamin E or the antioxidant, ethoxyquin.

Although no significant differences for fertility were present at 41 weeks and the only significant difference observed at 44 weeks was between the negative control and the lot supplemented continuously with .075% ethoxyquin (lot I), it is evident from Figure 4 that all lots supplemented at 38 weeks with either level of vitamin E or ethoxyquin (lots IV-VII) increased in fertility equally well to levels comparable to the positive control groups (lots I and II) during this period. Furthermore, fertility in these lots was significantly greater than the negative control at 47 weeks with no differences evident between lots I, II and IV - VII. This is supported further by noting no apparent differences at 47 weeks in fertility of the sterile males supplemented with either level of ethoxyquin beginning at 38 weeks as compared to males supplemented with either level of

vitamin E with values of 66. 1 and 76. 2%, respectively. Fertility of sterile males retained on the negative control ration was 11. 9%.

The unanticipated rise in fertility of the negative control at 41 weeks may be explained in part by referring to the data at the 38th week. The subdivision of lot III, the negative control, was based on a fertility value of 21. 9% obtained during the 34th week. At this time the prospective groups to be supplemented with either level of vitamin E or ethoxyquin plus the group to be retained as the negative control were equalized so each group had an average fertility of about 22%. The undivided lot III remained on the deficient diet until the 38th week at which time the average fertility obtained was 29. 2%. However, examination of the data on the basis of the subdivided lots (lots III - VII) reveals that a substantial increase took place in the lot to be retained as the negative control (lot III, subdivided). These values at 38 weeks were 37. 8, 29. 0, 28. 5, 31. 9, and 20. 3 for lots III - VII, respectively. Since fertility in the negative control only increased to 48. 3% from the 37. 8% noted above after the 38th week and, in fact, actually decreased to 4. 8% at 47 weeks, it is possible that this effect may have been due to simple biological variability. As an alternative explanation, the increase in fertility of lot III at 38 and 41 weeks may be attributed to contamination of feed with vitamin E or ethoxyquin either by improper mixing or feeding or by using a sample of air oxidized safflower oil in which the vitamin E

had not been completely destroyed. If vitamin E contamination had occurred the relationship between the vitamin and the antioxidant may not still be clearly established since it is known that antioxidants may spare the requirement for this nutrient by preventing oxidation of small amounts of vitamin E (Scott, 1962).

The reversibility of low fertility with vitamin E when induced by a vitamin E deficient diet is in agreement with the results of Mason (1949), working with the hamster, but is at variance with the results of Evans and Burr (1927) and Mason (1933) in which male rats fed a vitamin E deficient diet developed irreversible sterility. It also appears that both levels of vitamin E and ethoxyquin were as effective in reversing sterility as the 162 mg/kg of vitamin E used by Arscott and Parker (1967).

It was also shown that 32. 4 mg/kg of vitamin E (lot II) and . 075% ethoxyquin (lot 1) were effective in maintaining fertility for a relatively long period of time. It is of interest to note that these levels are less than the 162.0 mg/kg of vitamin E and . 3% ethoxyquin used by Arscott et al. (1965) to maintin fertility in male chickens fed the same diet.

Although the results obtained in this trial are similar to those observed by Arscott et al. (1965) and Arscott and Parker (1967), it should be noted that fertility was below 10% in both their reports at 27 weeks on the deficient diet while in this trial it took 47 weeks to

reach approximately the same level. A possible explanation could be the fact that these workers began their experiments in March while the present trial commenced in December. It is possible, therefore, in addition to the reasons noted earlier, that seasonal variation may have also been a factor in delaying the depletion since Parker and McSpadden (1943a, 1943b) have reported an increase in certain semen characteristics and fertility during the spring months. It should also be noted that a tolerance difference to a vitamin E deficiency appears to exist between male chickens fed the same diet and housed under the same conditions. From lot III, the sterility inducing diet, a number of males which had a high fertility during the preliminary period dropped markedly at 12 and 20 weeks on the deficient diet while the fertility of others appeared essentially normal throughout the depletion period.

It is of interest to note that the patterns for semen density and semen concentration were similar throughout the trial (Figures 5 and 6). Since less time and less chance of error are involved using the method outlined in the procedure for semen concentration, this method may possibly be used to replace the procedure in future experiments for semen density in determining the amount of sperm per unit volume of semen. Significant differences for semen density and concentration between lots I, II and III were nearly identical up to the time that lot III was subdivided into five groups. After the

negative control was subdivided some variability existed between the significance of semen density and semen concentration. It needs to be recalled that the negative control contained 29 males prior to being subdivided while each group contained approximately only six males after the subdivision which may explain the variation since samples containing a large number of individuals certainly yield smaller standard errors.

Examination of Figures 4-6 reveals a positive correlation between semen density, semen concentration and fertility. In most instances when semen density increased or decreased, the values for semen concentration and fertility were correspondingly altered.

SUMMARY AND CONCLUSIONS

Two lots of six White Leghorn male chickens each were fed a diet containing 7. 3% linoleic acid and either . 075% ethoxyquin or 32. 4 mg/kg of added vitamin E for 47 weeks. A third lot containing 30 males received the same level of linoleic acid but had no added ethoxyquin or vitamin E until the 38th week when fertility decreased to 29. 2%. At this time the lot was subdivided into five lots with approximately six males each. The lots were supplemented with either . 075% ethoxyquin, . 3% ethoxyquin, 32. 4 mg/kg of vitamin E or 162. 0 mg/kg of vitamin E while one group was retained on the unsupplemented ration.

During the 38th week, before the unsupplemented group was subdivided into its respective lots, fertility, semen concentration and semen density were significantly greater in the lots supplemented throughout the trial with . 075% ethoxyquin and 32. 4 mg/kg of vitamin E when compared to the unsupplemented group.

Supplementing the negative control with either . 075 or . 3% ethoxyquin and 32. 4 or 162. 0 mg/kg of vitamin E at 38 weeks restored fertility, semen concentration and semen density to levels comparable to the continuously supplemented groups. Fertility in the lot retained as the negative control decreased to 4. 8% at 47 weeks. At the end of the experiment a significant difference for

fertility existed between the negative control and all lots supplemented with vitamin E or ethoxyquin.

No meaningful differences were evident between any group for hatchability, sperm live-dead percent , semen volume, feed consumption or body weights.

From these findings one may conclude the following:

1. Male chickens fed diets high in linoleic acid maintained their fertilizing capacity when supplemented with . 075% ethoxyquin or 32. 4 mg/kg of vitamin E when compared to the . 3% and 162. 0 mg/kg, respectively, reported by Arscott et al. (1965).

2. Sterility induced by linoleic acid and a deficiency of vitamin E and ethoxyquin in male chickens was equally reversible by supplementation with . 075% ethoxyquin and 32. 4 mg/kg of vitamin E as well as . 3% ethoxyquin and 162. 0 mg/kg of vitamin E. Thus, vitamin E appears to act similarly to an antioxidant in reversing sterility of male chickens fed diets high in linoleic acid.

3. The procedure for semen concentration outlined in this thesis gave results similar to the procedure for semen density (Kosin and Wheeler, 1956) in determining the percent of sperm per unit volume of semen.

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