AN ABSTRACT OF THE THESIS OF

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Title: VITAMIN E EFFECTS ON STERILITY OF WHITE LEGHORN MALES FOLLOWING PROLONGED VITAMIN E DEFICIENCY

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Dr. G.H. Arscott

One group of four adult White Leghorn male chickens was fed a diet containing 7.3% linoleic acid and 32.4 mg/kg of added vitamin E (group IV) for 50 weeks which was designated as the positive control. A second lot (group I) containing 14 males received the same level of linoleic acid but no added vitamin E until the 34th week when four males (group III) having the lowest fertility based on the results of the 29th week were transferred to the vitamin E supplemented diet. At 44 weeks, based on determinations made at the 38th week, the deficient group was again divided into two groups of five males each, one retained on the deficient diet (group I) which served as the negative control, the other (group II) was placed on the vitamin E supplemented diet.

On the basis of first setting data (2nd-9th day after insemination) fertility of group I, the negative control, decreased to 35.0% at 29 weeks. Fertility of group IV, supplemented throughout the trial with 32.4 mg/kg of vitamin E, was significantly higher than group I

beginning at 24 weeks and throughout the remainder of the experiment. At 50 weeks, fertility of the negative control group had decreased to 4.2%. Fertility of group III, (3.3%), supplemented with 32.4 mg/kg vitamin E at 34 weeks, and group II, (16.2%), supplemented with the above level of vitamin E at 44 weeks, had increased to 60.6% and 65.4%, respectively, at 50 weeks. At 47 weeks fertility in all supplemented groups was significantly greater than the negative control.

No meaningful differences were evident between any group for semen volume, semen concentration, hatchability of fertile eggs, feed consumption or body weights. Second setting data (10th through 17th day) for fertility and hatchability of fertile eggs gave results similar to the first setting, although lower fertility values were obtained due to the decreased fertility of sperm after nine days in the oviduct. Insemination of larger volumes of semen from vitamin E deficient birds did not increase fertility, nor did a reduced volume of semen from supplemented birds result in decreased fertility.

No conclusive results were obtained from the analyses of fatty acids in the sperm. It was noted, however, that sperm motility decreased in vitamin E deficient males.

From these findings one may conclude the following:

1. Male chickens fed diets high in linoleic acid maintained their fertilizing capacity when supplemented with 32.4 mg/kg of vitamin E.

2. Sterility in male chickens induced by a high linoleic acid and low vitamin E diet was reversible by supplementation with 32.4 mg/kg of vitamin E, even after prolonged deficiency periods of 34 and 44 weeks duration.

3. Results obtained from the second setting of eggs would indicate that one eight-day period of collection would be sufficient.

4. Increased volume of semen from deficient birds did not increase fertility, nor did reduced volume of semen from supplemented males reduce fertility.

5. Sperm motility was decreased in vitamin E deficient birds.

Vitamin E Effects on Sterility of White Leghorn Males Following Prolonged Vitamin E Deficiency

by

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INTRODUCTION

Since Adamstone and Card (1934) reported that vitamin E was essential for reproduction in male chickens, it has been shown 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 also been shown that this low fertility is reversible by supplementation with vitamin E or antioxidants such as ethoxyquin.

Work has also been done of the function of vitamin E in oxidative phosphorylation and the changes that are observed in membranes of vitamin E deficient animals. Fatty acid composition of different tissues from deficient and supplemented animals have been determined by several workers.

The purpose of this research was to determine whether (1) sterility in male chickens following prolonged vitamin E deficiency was still reversible, (2) there was any microscopic differences between sperm of vitamin E deficient or supplemented males, and (3) there was any differences in the fatty acid composition of sperm from deficient or supplemented males.

LITERATURE REVIEW

Vitamin E was discovered by Evans and Bishop (1922), when they concluded that the resorption during gestation occurring in their feeding experiments with rats was due to a deficiency of a fat-soluble factor in the food. Since this discovery, it has been found that most other animals would have died from myopathy, anemia or encephalomalacia before reproduction could have occurred. A review of the early history of vitamin E research has been given by Evans (1962).

Vitamin E requirements may be influenced by different dietary factors, the effect being dependent on the species studied and the tissue affected. Such factors are: synthetic antioxidants (encephalomalacia, embryonic degeneration, in vitro haemolysis), selenium (exudative diathesis, liver necrosis, myopathy), the amino acids, cystine and methionine (myopathy) and polyunsaturated fatty acids (encephalomalacia, in vitro haemolysis, myopathy) (Jager, 1975; Scott, 1970). Most factors have a beneficial effect, but polyunsaturated fatty acids (PUFA) may increase the need for vitamin E. The PUFA contained in edible fats and margarines relates almost exclusively to linoleic acid.

Some work to determine the relation between linoleic acid intake and vitamin E requirement was reported by Weber et al. (1964) on rats and by Søndergaard and Dam (1966) on chickens. The former found that linoleic acid had a profound effect on the vitamin E

requirement, whereas no such effect was noted by the latter. Jager (1975) worked with rats and ducklings in order to quantify the effect of linoleic acid intake on vitamin E requirement. The criteria used were in vitro haemolysis in rats (Jager, 1968) and myopathy in ducklings (Jager et al., 1969; Jager and Vles, 1970; Jager and Verbeck-Raad, 1970) as these symptoms developed within a short time and could express the degree of disease quantitatively. Jager (1975) found that 1.5 mg D-α-tocopherol acetate/kg of food was sufficient to prevent testis degeneration in male rats consuming a diet containing 35 cal % lard. In ducklings, 6 mg D-α-tocopherol acetate/kg food was sufficient to protect the gizzard from myopathy, and even less was required to protect the heart musculature.

Myopathy of the skeletal muscles is the most frequently occurring manifestation of vitamin E deficiency in mammals and birds. The study of myopathy in vitamin E deficient animals is complicated by the fact that the disease may be prevented in part or completely by the addition of cystine or methionine to the diet (Dam et al., 1952; Machlin and Shalkop, 1956; Scott and Calvert, 1962; Witting and Horwitt, 1964). Another complication is that only trace amounts of selenium in the diet are needed to almost completely prevent myopathy in vitamin E deficient chicks (Dam and Søndergaard, 1957; Scott, 1962), ducklings (Jager, 1972), and rats (Moore and Sharman, 1964). Selenium, however, was found ineffective in rabbits (Hove et al., 1958; Proctor et al., 1961). A survey of selenium treatment against myopathy in a wide range of different animals has been given by Wolf

et al. (1963), and the vitamin E requirement of chickens in relation to dietary treatment was determined by Scott (1962).

The function of vitamin E in the prevention of myopathy is still not clear. Zalkin et al. (1962) found a release of lysozymal enzymes in vitamin E deficient rabbit muscle and advanced the theory that for the lack of an antioxidant the primary effect would consist of free radical damage to lipoprotein membranes of the cell and its subcellular organelles. Morphologic studies on rats (Howes, et al., 1964), chickens (Cheville, 1966) and rabbits (van Vleet, et al., 1968) indicated that the primary injury involves mitochondria.

With respect to the mitochondria, there are indications that vitamin E is somehow involved in oxidative phosphorylation (Corwin, 1965; Naito, et al., 1966; Carabello, et al., 1971). Molenaar, et al. (1968, 1970) have observed in electron microscopy of intestinal epithelial cells of man and ducklings a decreased membrane contrast in the state of vitamin E deficiency, which was most distinct in the outer mitochondrial membrane. This contrast-loss in membranes was explained as a critical loss of double bonds and therefore probably represented a visualization of lipid peroxidation. Vos et al. (1972) found an analogous loss of contrast in liver tissue of vitamin E deficient ducklings, and demonstrated that on analyzing the fatty acids of these liver mitochondria the largest decrease in arachidonic acid was found in the outer membranes (Vos, 1972; Molenaar et al., 1972).

These observations strongly suggest that vitamin E may function in some way as an antioxidant *in vivo*. This view on vitamin E, supported by many researchers (Tappel, 1962, 1965, 1970; Horwitt, 1965; Witting, 1965), proposes that vitamin E inhibts the peroxidation of unsaturated fatty acid moieties of lipid *in vivo* by molecular oxygen through a nonenzymatic reaction; and that the oxidative attack is random on free and membrane-bound unsaturated fatty acids and is inhibited by biological antioxidants (Molenaar, *et al.*, 1972). Bunyan *et al.* (1967), however, were not able to detect an increase in lipid peroxides in different parenchematous tissues of vitamin E deficient rats. Diplock, *et al.* (1967) found the same tocopherol levels in the cerebral tissues of both normal chicks and those with incipient encephalomalacia. Green, *et al.* (1967) found that whenever lipid autoxidation occurred *in vitro* there was always a concomitant destruction of tocopherol, whether a second antioxidant was present or not. The results of *in vitro* studies also contrast sharply with those obtained from *in vivo* studies, as no difference in the rates of depletion of vitamin E of tissues could be observed when rats were fed a vitamin E deficient diet supplemented with either polyunsaturated linseed oil fatty acids or oleic acid. This suggests that vitamin E does not function solely as an *in vivo* antioxidant.

Several authors have tried to produce experimental evidence for the peroxidation of lipids *in vivo* by studying the proposed relation

between vitamin E and lipids in tissues. Carpenter (1966) found no differences in testicular lipids of vitamin E deficient and normal rats until the testes were very degenerate. He found that the percent composition remained the same when the total lipid content decreased, and also that the level of docosenoic acid (C 22:5ω6) (this notation for fatty acids specified the number of carbons in the chain, the number of double bonds, and the number of carbon atoms after the terminal double bond in the molecule), decreased with age instead of increasing as normally occurs. Carney and Walker (1971) found that the formation of C 22:5 ω6 from linoleate was blocked at the step between C 20:4 ω6 and C 22:5 ω6. Harman, et al. (1966) showed that an increase in arachidonic acid during vitamin E deficiency is only temporary and is followed by a phase of decreased arachidonic acid content of liver lipids in rats. Tinsley, et al. (1971) showed a decrease in the pro- of 18:2 ω6 and an increase in the proportion of 18:1 in vitamin E deficient chickens fed rations containing 10% safflower oil or coconut oil.

Arscott, et al. (1965) reported a decrease in sperm concentration and fertility in male White Leghorn chickens fed a low vitamin E and high linoleic acid diet. Either vitamin E or ethoxyquin, when added to the high linoleic acid diet, would maintain fertility and sperm concentration. Males would also remain fertile when fed a diet low in both vitamin E and linoleic acid. Arscott and Parker (1967) showed

that fertility and sperm concentration of male White Leghorn chickens once approaching zero could be restored to normal levels when vitamin E was added to a diet high in linoleic acid and low in vitamin E. Kuhns and Arscott (1969) found that lower levels of ethoxyquin or vitamin E would also restore fertility and sperm concentration in male White Leghorn chickens fed a high linoleic acid and low vitamin E diet. Mason (1949) found that sterility was reversible in the vitamin E deficient hamster, while male mice fed a vitamin E deficient diet for 400 days maintained their fertilizing capacity and showed no testicular degeneration (Bryan and Mason, 1940). Price (1968) reported that the reduction of fertility of male Japanese quail fed a low vitamin E diet was reversible upon the addition of vitamin E. No testis damage was noted. Kuhns (1969) reviewed the literature on the effects of vitamin E on female fowl reproduction and chick deficiency symptoms.

EXPERIMENTAL PROCEDURE

Eighteen dubbed White Leghorn Babcock-300 cockerels hatched on April 25, 1975 were placed in separate wire cages (30.5 x 45.7 cm) on October 14, 1975, involving 16 cages to a row. The males were housed in a windowless, forced-draft, thermostatically controlled positive pressure room, and were supplied with 14 hours of artificial lighting per day from 4 am to 6 pm. Food was provided ad libitum in individual trough feeders; water was provided for 15 minute periods eight times a day at about two hour intervals in plastic lined troughs. Preliminary records were taken for feed consumption, fertility, hatchability, body weights, semen volume and concentration. Males were housed in such a way as to minimize transfer of vitamin E from the supplemented birds to those on the deficient diet. This was accomplished by housing and maintaining males on the deficient diet at the water-intake end of the cage row. The trial commenced on May 13, 1976.

Based on the preliminary determinations, the males were divided into two groups so that each group had comparable fertility, hatchability and semen volume and concentration. The four males of Group IV were fed a diet supplemented with 32.4 mg/kg vitamin E[1], and were designated the positive control.

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[1] See footnote 10, Table 1.

The 12 males of Group I were fed a vitamin E deficient diet as were 2 surplus or replacement birds located in another row. Table 1 shows the diet composition, and indicates the modifications involved for male chickens and the sources of supply.

Group I males were subdivided twice, with the males having the lowest fertility values for a given measurement period being transferred into the new group, which were then fed the vitamin E supplemented diet. Accordingly, Group III, consisting of four males selected from week 29 fertility values, was placed on supplemental vitamin E at 34 weeks. Group II, involving five males selected from week 38 fertility values, was placed on the supplemented diet at 44 weeks. Group I, now consisting of five males, remained on the vitamin E deficient diet, and served as the negative control.

The tocopherol content of the safflower oil was destroyed by air-oxidation[2], and the oil stabilized by the addition of 0.1% Tenox 6, an antioxidant. The oxidized oil was stored in a freezer at -12°C until needed. Rations were mixed in 45.4 kg lots and also stored in the freezer. Small amounts of feed (ca. 12 kg lots) were kept at room temperature for use during the experiment.

Fertility was determined by ejaculating the males using the massage technique of Burrows and Quinn (1939), and artificially inseminating the ejaculate into approximately three White Leghorn

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[2] See footnote 3, Table 1.

Table 1. Composition of experimental rations[1]

Ingredients	Positive Control (%)	Negative Control (%)
Isolated soybean protein[2]	25.00	25.00
Air oxidized[3] safflower oil[4]	10.00	10.00
Glucose monohydrate[5] (cerelose)	49.15	49.25
Cellulose[6] (solka floc)	8.00	8.00
Salts[7] (G.B.I.)	6.00	6.00
Methionine hydroxy analogue[8]	.4	.4
Vitamin A, dry (30,000 IU/g)	.067	.067
Vitamin D_3, dry (26,455 ICU/g)	.014	.014
Vitamin K, B-complex vit. mixture[9]	.6	.6
Choline Cl (44%)	.57	.57
Vitamin E (44.1 IU/g)[10]	.1	--
Na_2SeO_3 in glucose monohydrate[11]	.1	.1
Actual analysis:[12]		
Vitamin E, mg/kg	36.7	4.3
Linoleic acid, %	7.3	7.3

[1] Adapted from the report of Machlin et al., (1969), and used previously by Kuhns and Arscott (1969), as diet S-25-B modified by reducing Ca and P levels to values specified for chicks by using salts N reported by Fox and Briggs (1960) including the optional level of Se.

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

[3] 4.54 kg samples were air oxidized in a 12 liter round bottom flask for 32 hrs. @ 72°C by means of an aspirator and stabilized with .1% Tenox 6, (Eastman Chemical Products, Inc., Kingsport, Tenn.) which contains 10% butylated hydroxytoluene, 10% butylated hydroxyanisole, 6% propyl gallate, 6% citric acid, 12% propylene glycol and 56% mixed glycerides.

[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, N.H.).

[7] Salts N - Fox and Briggs, (1960)(General Biochemicals, Chagrin Falls, Ohio) supplies as % of diet with N.F. grade salts: Ca, 1.24;

Table 1 (continued)

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; $FeC_6H_5O_7 \cdot 5H_2O$, .025; KIO_3, .001; $ZNCO_3$, .013; $CuSO_4$, .001.

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

[9] Vitamin mixture-Machlin and Gordon, (1958)(Nutritional Biochemicals Corp., Cleveland, Ohio) 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.

[10] Myvamix 44.1 IU/g (Distillation Products Industries, Rochester, N. Y.). Level based on report of Kuhns and Arscott (1969).

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

[12] Based on ingredient analysis.

females, housed under conditions similar to the males, at approximately four to eight week intervals. When low semen volumes were obtained, only one or two hens were inseminated. The males were pre-ejaculated two days prior to the collection and insemination period to eliminate any old sperm present.

The volume of ejaculate from each male was measured using a 1cc tuberculin syringe prior to insemination. Semen from each male was then artificially inseminated into the females, using a dose of 0.05 ml of undiluted semen. Semen from a given male was inseminated into a different group of females each time in order to minimize any dam effects. During the twelfth week, the hens normally used for insemination were replaced by pullets just coming into egg production.

Eggs were saved for two eight-day periods, beginning the second day after insemination, and incubated in Jamesway (model 252) incubators. Fertility was determined by candling the eggs no earlier than the seventh day of incubation. Eggs which appeared infertile were broken out and examined macroscopically for early embryonic development. Upon hatching, all unhatched eggs were also broken out and examined macroscopically for the presence of any development. Hatchability data were obtained on fertile eggs, and are expressed as the hatchability of fertile eggs. A total of 4,899 eggs were incubated during the experiment, 2,526 eggs in the first setting and 2,373

eggs from the second.

Semen concentration data were obtained soon after collection by utilizing a Phillips-Drucker microhematocrit reader (model L-550, Astoria, Oregon) and 75 mm capillary tubes with an outside diameter of 1.4 to 1.6 mm. The tubes were filled with semen, sealed at one end with potter's green clay, and then spun for five minutes in a microcapillary centrifuge (International Equipment Company). The amount of sperm accumulated at the base was quantitatively measured by the hematocrit reader in relation to the total volume, and expressed as the percent volume the sperm occupies in the semen. Periodically, semen samples were examined microscopically to determine the relative motility, and scored one to four, where four represents the greatest motility.

At the 34th week, an experiment was run to determine whether inseminating hens with a larger volume of semen would have any effect on male fertility. Within a two-day period, several males on the deficient diet and the four males on the positive control diet were used, first to inseminate the females with the normal volume of 0.05 ml semen, and then, with the maximum amount of semen possible, up to 0.15 ml, for the deficient birds, and 0.025 ml for the supplemented birds.

Body weights and feed consumption data were taken at about four week intervals.

To extract the fatty acids from the sperm, the semen was first centrifuged in 400 µl plastic tubes in an ultramicrocentrifuge (Beckman model 152 microfuge) for five minutes. Sperm and total volumes were recorded, sperm removed and extracted with chloroform-methanol (2:1 v/v). The bottom layer was removed, evaporated with nitrogen. Methyl esters were formed using ethyl ether and 5% HCl (gas) in methanol (1:1) by heating for 90 minutes at 80°C. Equal volumes of water and hexane were added, and then centrifuged for three minutes after mixing. Following removal of the top layer, the mixture was re-extracted with ether-hexane (1:1). The two top layers were combined, evaporated under nitrogen, and then resuspended in 0.5 ml hexane.

Five to 50 µl samples were run using a Hewlett-Packard gas chromatograph with a flame ionization detector (model 700-12) equipped with a 200 ft. capillary column (0.03 in I. D.). The open tubular column was wall-coated with ethylene glycol succinate. The relative percent of each fatty acid was determined from the peak "area", which was the product of retention time, peak height and attenuation. Peak identify was determined by comparison with chromatographs of known fatty acids. To determine the mass of each fatty acid present in the sample, a known volume of the methylated fatty acids was evaporated and then weighed. This mass, when multiplied by the relative percent values obtained from the chromatograms, gave the mass of individual

fatty acids present in the sample.

Standard errors were computed for all data except motility and fatty acid composition data. Significance at the 5% level was determined by students "t" test (Snedecor and Cochran, 1967) and are designated by lower case superscripts. Significance at the 1% level was indicated by capital letter superscripts when appropriate.

RESULTS

The results are summarized in Figures 1-9 and Tables 2-11.

The data for fertility are shown in Figure 1 and Table 2. During the depletion period fertility in Group I, the unsupplemented group, decreased to 35.0% at 29 weeks. From 24 weeks to the conclusion of the experiment, fertility of the males supplemented with 32.4 mg/kg of vitamin E (Group IV) was significantly higher than that of the unsupplemented group. Group I was subdivided twice, with the males having the lowest fertility values for a given measurement period being transferred into the new group, which was then fed the vitamin E supplemented diet. Group III, consisting of four males selected from week 29 fertility values, was placed on vitamin E supplemented ration at 34 weeks. Group III fertility increased to 79.2% at 38 weeks, after four weeks on the supplemented diet. The increase seen in Group I fertility at 38 weeks was probably due to the transferal of the males having the lowest fertility values (3.3%) to form the new group. Group II, involving five males selected from week 38 fertility values, was placed on the supplemented diet at 44 weeks. Group II fertility, 16.2%, was significantly lower than both Group III, 90.0%, and Group IV, 91.4%, at week 44, but by week 47, Group II fertility had increased to 83.1%, which was now significantly higher than Group I, and comparable to the fertilities of both Groups III and IV. Group I fertility,

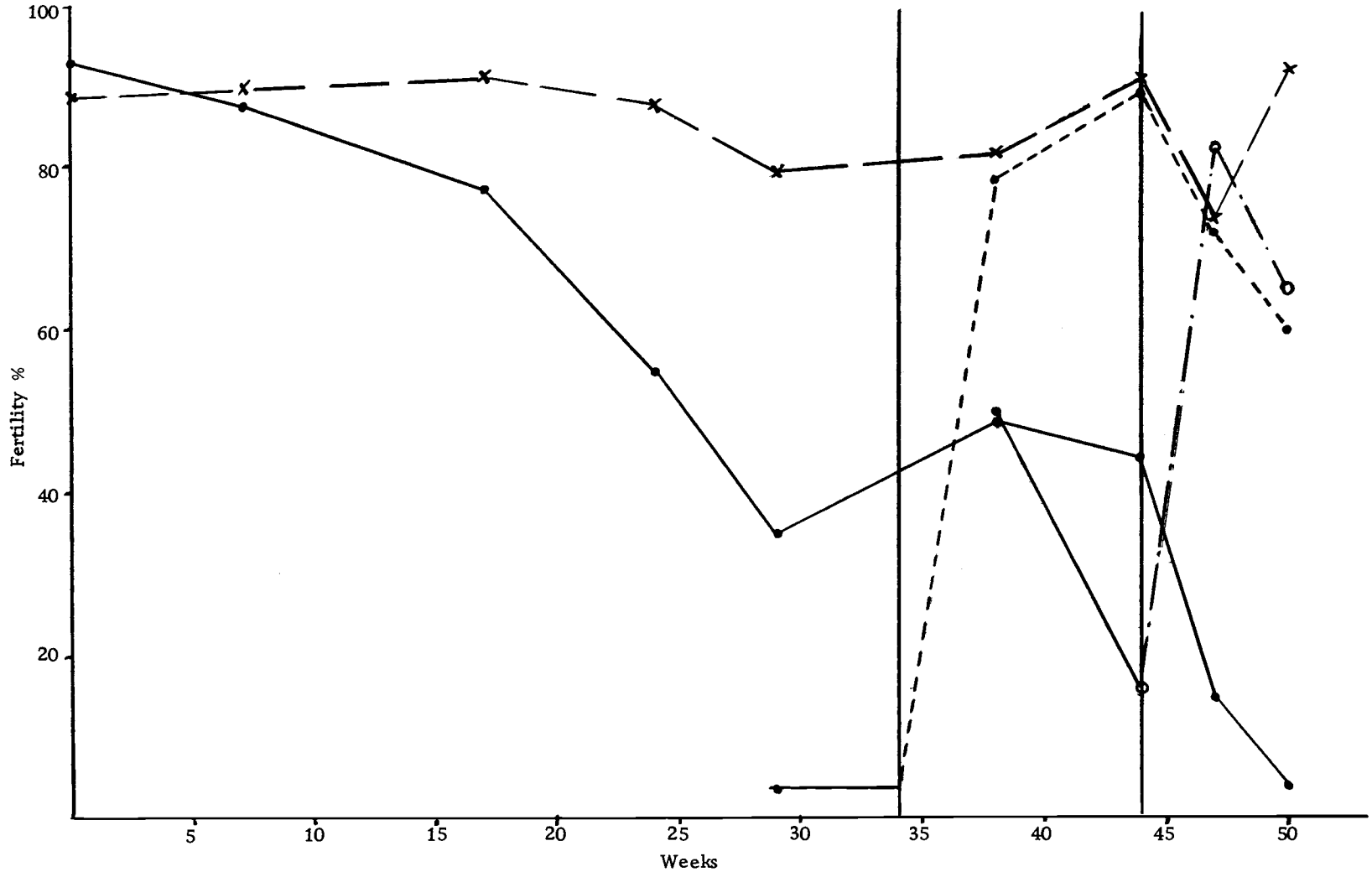

Figure 1. Effect of vitamin E on fertility of male chickens. Legend: Group I, •——•, negative control; Group II, ⊙—·—⊙, as Group IV after 44 weeks; Group III, •– – –•, as Group IV after 34 weeks; Group IV, ×—×, 32.4 mg/kg vitamin E. (first setting)

Table 2. Effect of vitamin E on fertility of male chickens

Group \ Weeks	0	8	17	24	29	38	44	47	50
	[1,2]			(%)					
I	92.8±1.7^{a}	87.8±3.3^{a}	77.5±9.2^{a}	55.3±11.5^{a}	35.0±11.7^{A}	49.1±11.6^{a}	44.4±16.5^{a}	15.1±9.2^{A}	4.2±4.2^{aA}
II						(51.0±42.3)[3]	(16.2±16.1^{aA})	83.1±7.2^{B}	65.4±23.0^{b}
III					(3.3±6.6)	79.2±9.1^{ab}	90.0±1.8^{b}	72.6±9.0^{B}	60.6±30.7^{ab}
IV	89.2±2.4^{a}	90.2±5.5^{a}	91.6±4.9^{a}	87.8±2.9^{b}	79.8±7.2^{B}	82.3±3.1^{b}	91.4±3.3^{bB}	74.5±12.5^{B}	93.0±3.9^{Bb}

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

determined by five males from week 44 on, steadily decreased from 44.4% at week 44, to 15.1% at week 47 and 4.2% at week 50. Group III fertility decreased from 90.0% at week 44, to 72.6% and 60.6% at weeks 47 and 50, respectively, but remained significantly higher than Group I fertility until week 50. Group II fertility decreased from 83.1% at week 47 to 65.4% at week 50, but remained significantly greater than Group I fertility of 4.2% at week 50.

Fertility data for the second setting are given in Figure 2 and Table 3. Fertility of Group I was significantly lower than Group IV at weeks 38 and 50, and lower than both Group II and IV at week 47. Group II fertility was significantly lower than Group IV at week 44.

Semen volume data, Figure 3 and Table 4, show that Group I volume was significantly lower than Group IV only at weeks 29 and 50. Group III volume was also significantly lower than Group IV at week 50. The data for semen concentration are shown in Figure 4 and Table 5. Concentration for Group I was significantly lower than Group IV at week 29, otherwise there were no significant differences.

Data for hatchability, shown in Figure 5 and Table 6, indicates that Group I hatchability is significantly lower than Group IV at week 29, and Group II hatchability is significantly lower than Groups III and IV at week 44. Hatchability for the second setting eggs are given in Figure 6 and Table 7, and shows that Group I hatchability was significantly lower than Group III at week 38, and lower than groups

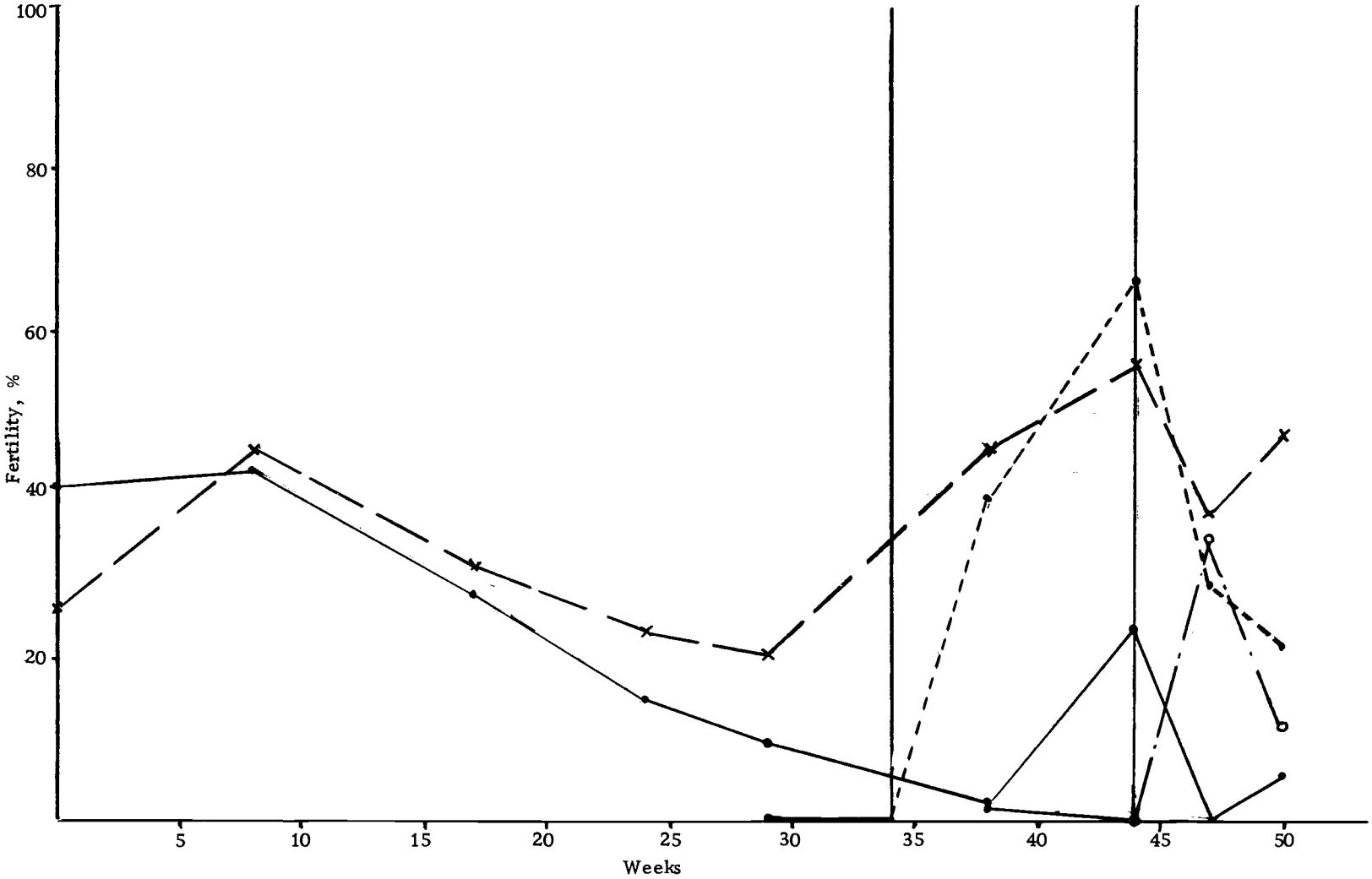

Figure 2. Effect of vitamin E on fertility of male chickens, (second setting) Legend: Group I, ●——● , negative control; Group II, ○—·—○, as Group IV after 4 weeks; Group III, ●- - -●, as Group IV after 34 weeks; Group IV, ×— —× , 32.4 mg/kg vitamin E.

Table 3. Effect of vitamin E on fertility of male chickens (second setting).

Group \ Weeks	0	8	17	24	29	38	44	47	50
					(%)				
I	41.3±19.7	43.0±16.8	27.7±6.3^{a}	14.8±4.3^{a}	9.6±5.2^{a}	2.2±1.4^{A}	23.4±19.4^{ab}	0.1±0^{A}	5.6±5.5^{a}
II						(1.8±4.1)[3]	(0.1±0^{a})	34.6±6.1^{B}	11.5±7.8^{ab}
III					(0.1±0)	39.8±6.7^{B}	66.5±33.5^{ab}	29.2±13.1^{AB}	21.7±11.6^{ab}
IV	26.0±22.7	45.4±15.6	30.9±11.7^{a}	22.8±2.7^{a}	20.3±6.7^{a}	46.1±12.9^{B}	56.3±21.3^{b}	37.6±10.1^{B}	47.5±13.2^{b}

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

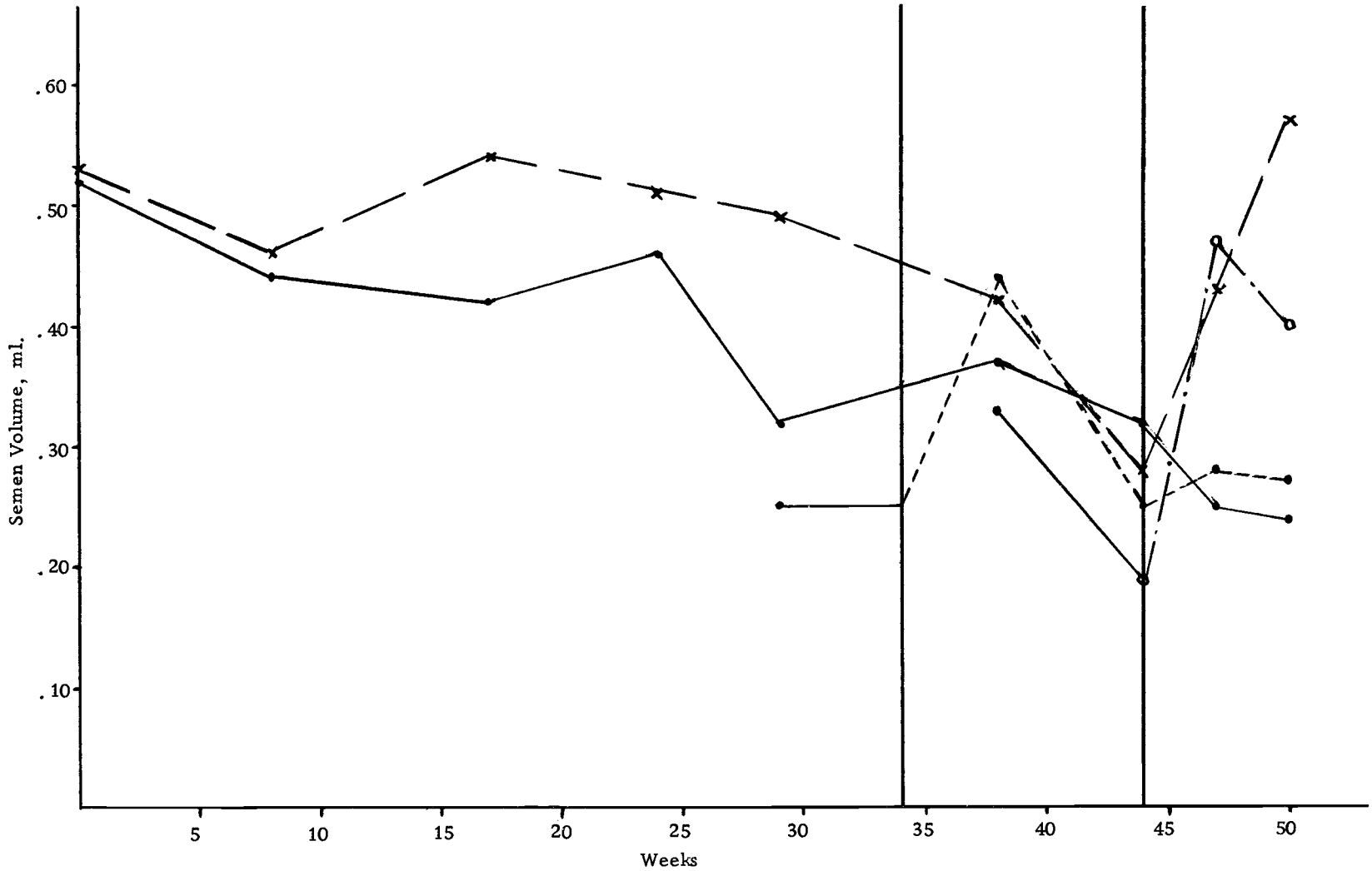

Figure 3. Effect of vitamin E on semen volume of male chickens. Legend: Group I, ●——●, negative control, Group II, o—·—o, as Group IV after 44 weeks; Group III, ●- - - -●, as Group IV after 34 weeks; Group IV, ×—— ——×, 32.4 mg/kg vitamin E.

Table 4. Effect of vitamin E on semen volume of male chickens

Group	Weeks 0	8	17	24	29	38	44	47	50
	1,2				(ml)				
I	.52±.06[a]	.44±0.7[a]	.42±0.4[a]	.46±.06[a]	.32±0.6[a]	.37±0.8[a]	.32±.05[a]	.25±.06[a]	.24±.04[a]
II						(.33±.30)[3]	(.19±.08[a])	.47±.12[a]	.40±.22[ab]
III					(.25±.14)	.44±.06[a]	.25±.10[a]	.28±.07[a]	.27±.02[a]
IV	.53±.05[a]	.46±.12[a]	.54±.07[a]	.51±.06[a]	.49±.02[b]	.42±.06[a]	.28±.09[a]	.43±.06[a]	.57±.10[b]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

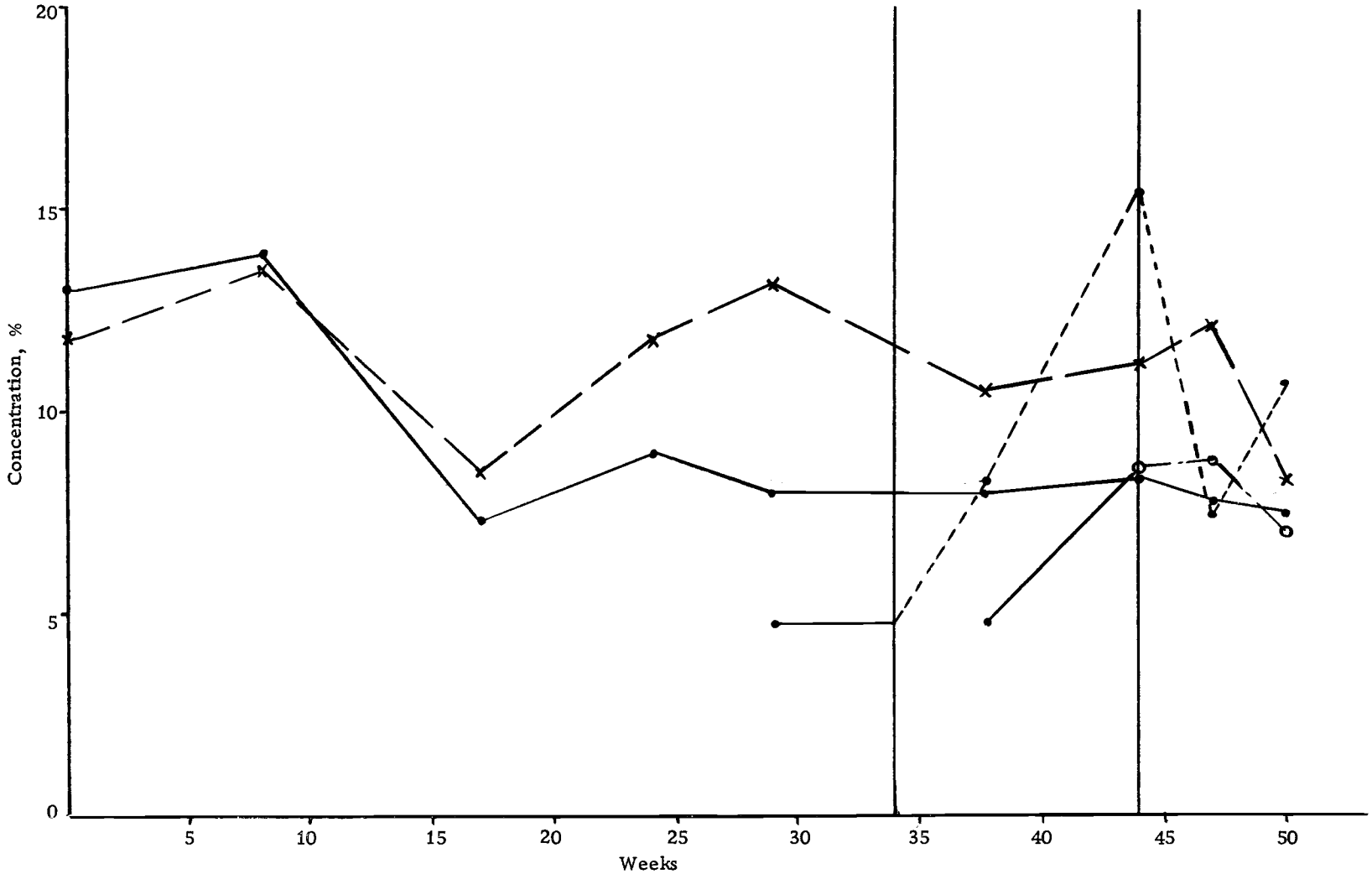

Figure 4. Effect of vitamin E on semen concentration of male chickens. Legend: Group I, ●——●, negative control; Group II, o— - —o, as Group IV after 44 weeks; Group III, ●- - - -●, as Group IV after 34 weeks; Group IV, ×— —×, 32.4 mg/kg vitamin E.

Table 5. Effect of vitamin E on semen concentration of male chickens

Group \ Weeks	0	8	17	24	29	38	44	47	50
					(%)				
	1,2								
I	13.0±1.0[a]	13.9±1.3[a]	7.3±1.1[a]	9.0±2.0[a]	8.0±1.8[a]	8.0±1.7[a]	8.4±2.4[a]	7.8±2.8[a]	7.5±2.3[a]
II						(4.8±5.4)[3]	(8.6±2.9[a])	8.8±2.2[a]	7.0±1.5[a]
III					(4.8±3.9)	8.3±3.2[a]	15.5±1.5[a]	7.5±1.8[a]	10.7±3.8[a]
IV	11.8±1.0[a]	13.5±2.1[a]	8.5±1.0[a]	11.8±1.4[a]	13.2±1.3[a]	10.5±2.4[a]	11.2±1.5[a]	12.2±1.3[a]	8.3±0.9[a]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

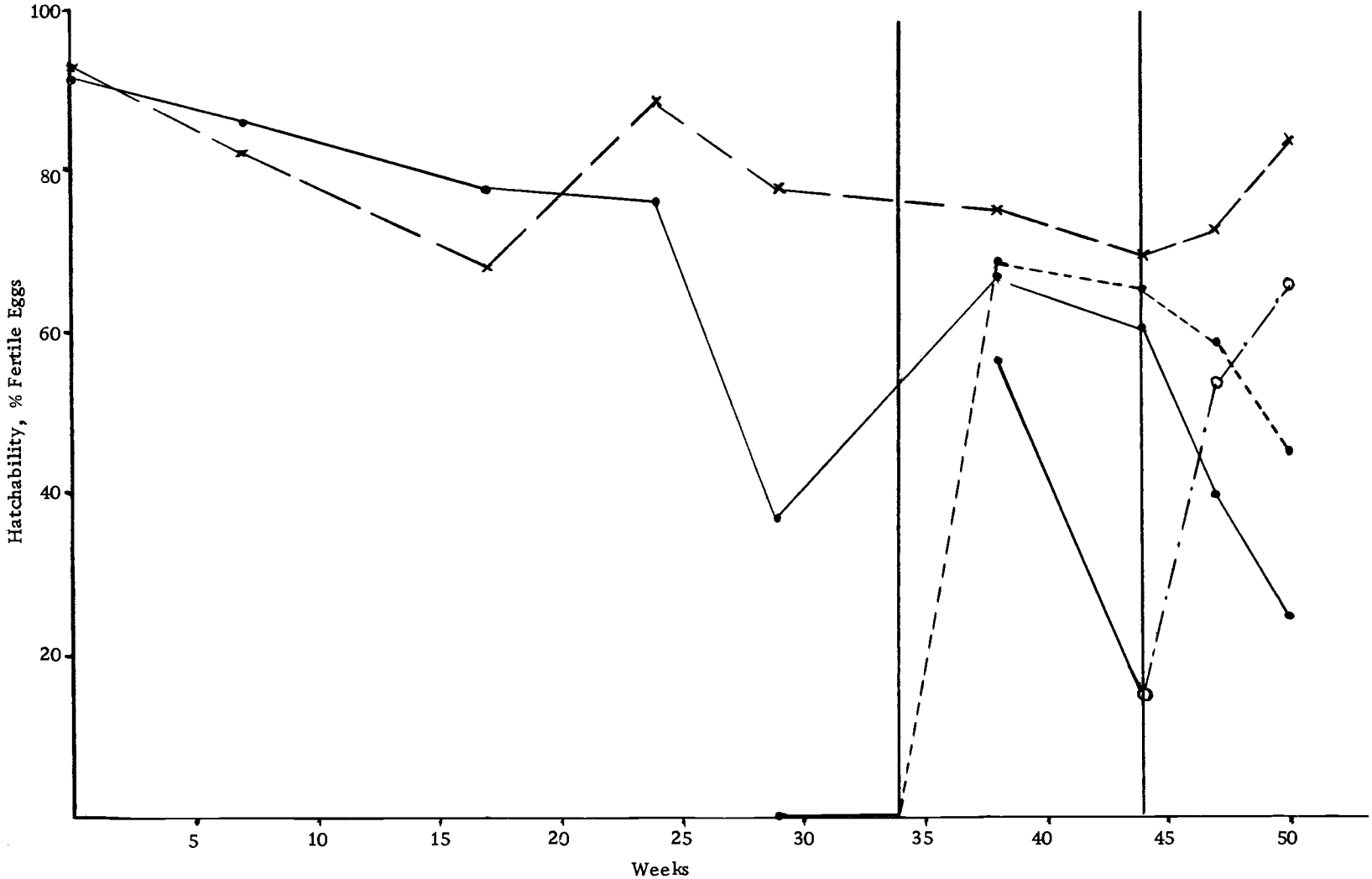

Figure 5. Hatchability of fertile eggs as related to males fed varying levels of vitamin E. Legend: Group I, ●——●, negative control; Group II, o— · —o, as Group IV after 44 weeks; Group II, ●- - - -●, as Group IV after 34 weeks; Group IV, ×—— ——×, 32.4 mg/kg vitamin E (first setting).

Table 6. Hatchability of fertile eggs as related to males fed varying levels of vitamin E (first setting)

Group \ Weeks	0	8	17	24	29	38	44	47	50
	[1,2]				(%)				
I	91.6±1.3[a]	86.2±3.8[a]	77.7±5.0[a]	76.0±10.7[a]	37.1±13.2[a]	67.0±13.9[a]	60.7±16.7[ab]	40.1±24.5[a]	25.1±25.0[a]
II						(56.7±52.2)[3]	(15.6±15.5[a])	54.0±14.1[a]	66.1±22.4[a]
III					(0.1±0)	68.5±12.0[a]	65.2±1.6[b]	58.9±21.8[a]	45.8±24.1[a]
IV	92.6±4.3[a]	82.0±5.1[a]	68.1±16.0[a]	88.6±5.2[a]	77.4±11.2[b]	75.2±5.6[a]	69.7±11.3[b]	72.8±5.7[a]	84.1±8.3[a]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

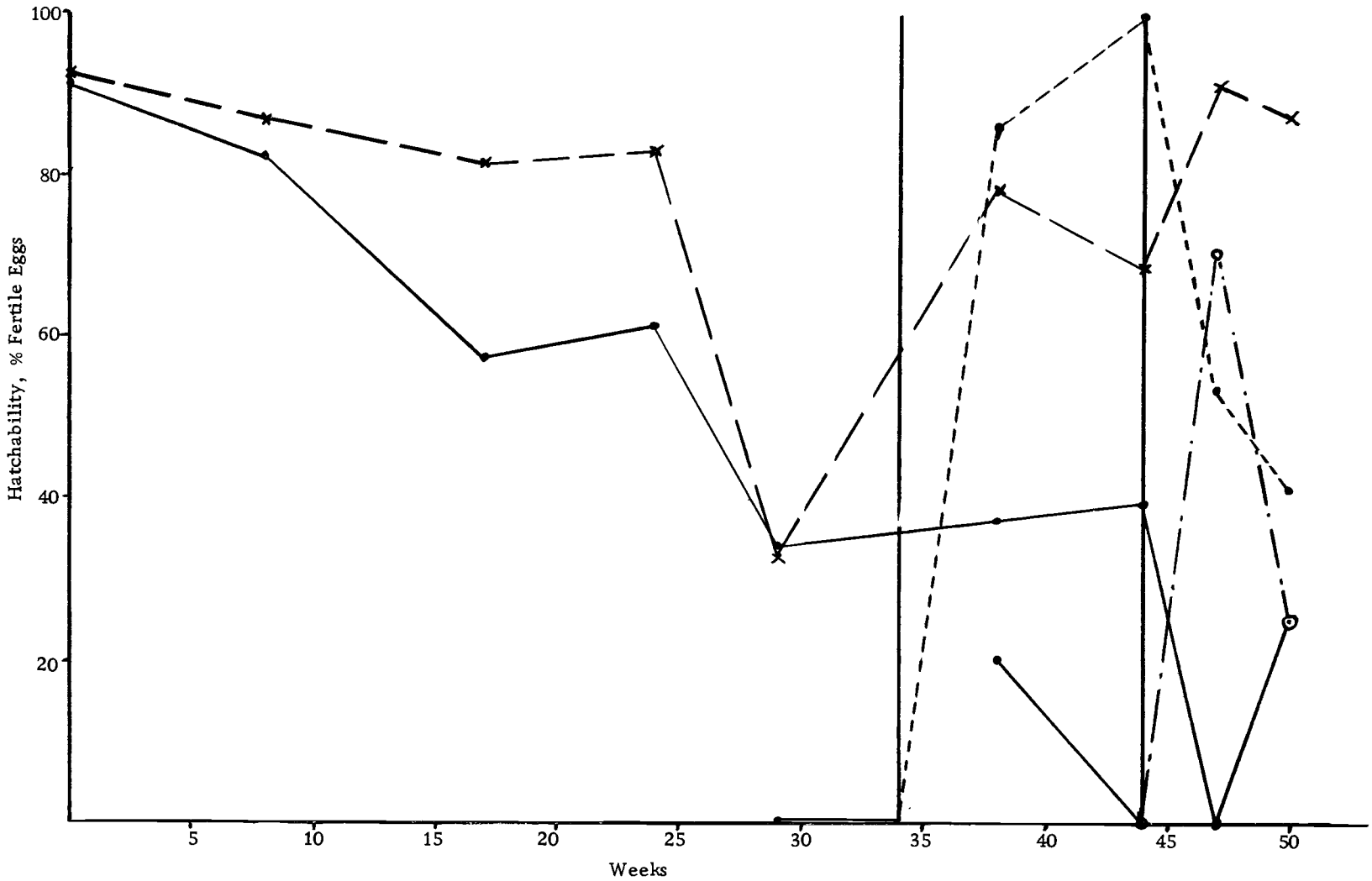

Figure 6. Hatchability of fertile eggs as related to males fed varying levels of vitamin E, (second setting). Legend: Group I, ●——●, negative control; Group II, o—·—o, as Group IV after 44 weeks; Group III, ●- - -●, as Group IV after 34 weeks; Group IV, ×——×, 32.4 mg/kg vitamin E.

Table 7. Hatchability of fertile eggs as related to males fed varying levels of vitamin E (second setting)

Group \ Weeks	0	8	17	24	29	38	44	47	50
					(%)				
I	91.7±1.3	82.9±22.7	57.8±10.6[a]	61.7±14.6[a]	34.6±14.5[a]	37.6±18.3[a]	40.1±24.5[ab]	0.1±0[aA]	25.1±25.0[a]
II						(20.0±47.7)[3]	(0.1±0[a])	70.4±12.4[B]	25.1±25.0[a]
III					(0.1±0)	86.1±7.4[b]	100.0±0[b]	54.2±20.8[b]	41.7±30.0[a]
IV	92.6±6.4	87.1±10.2	81.4±5.0[a]	83.4±9.6[a]	33.0±14.0[a]	78.5±7.8[ab]	68.8±23.6[b]	91.7±8.3[B]	87.4±8.4[a]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

II, III and IV at week 47. Group II hatchability was significantly lower than Group IV at week 44.

Feed consumption data, Figure 7 and Table 8, shows that Group I feed consumption was significantly higher than Group IV at week 0, and lower at weeks 20 and 27. Data for body weights, Figure 8 and Table 9, shows that Group II body weight was significantly lower than Groups III and IV at week 42 and Group I was significantly lower than Groups III and IV at weeks 46 and 50.

Table 10 shows that increasing the semen volume from vitamin E deficient males had no beneficial effect on fertility. Decreasing the insemination volume used from Group IV males also did not decrease fertility.

Table 11 shows the relative sperm motilities as determined through microscopic observation of semen samples on different dates. It appears that motility was decreased on the vitamin E deficient diet, and motility is rapidly regained when the males are given vitamin E. More abnormal sperm (hooked heads, broken or twisted tails) were observed in the semen from deficient males.

Figure 9 gives the results of the fatty acid analysis of sperm samples from February 7, 1977 (ca. 38 weeks) expressed in terms of sperm volume/total volume of semen versus total milligrams fatty acid/total volume of semen. The results are widespread and erratic, although the sperm from males on a diet containing vitamin E

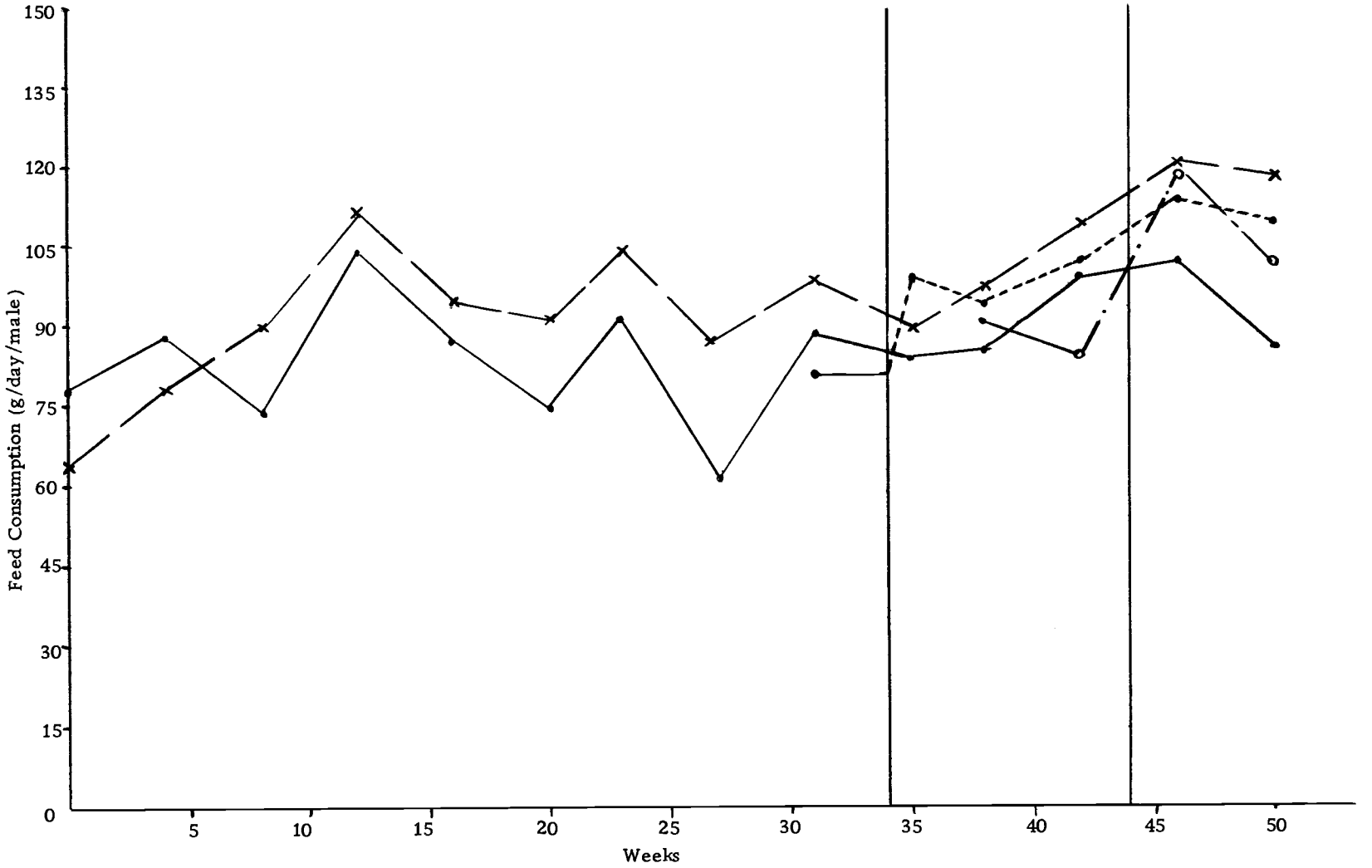

Figure 7. Effect of vitamin E on daily feed consumption of male chickens. Legend: Group I; ●——●, negative control; Group II, ○——⊖, as Group IV after 44 weeks; Group III, ●- - -●, as Group IV after 34 weeks; Group IV, ×—×, 32.4 mg/kg vitamin E.

Table 8. Effect of vitamin E on daily feed consumption of male chickens

Group \ Weeks	0	4	8	12	16	20	23	27
	[1,2]			(g)				
I, II, III	78.2±5.5[a]	87.6±4.6[a]	74.1±7.2[a]	103.9±11.5[a]	87.5±6.8[a]	74.8±5.7[a]	91.1±9.0[a]	61.9±14.7[A]
IV	64.0±1.1[b]	78.2±2.7[a]	89.8±6.8[a]	111.5±11.3[a]	94.5±11.0[a]	91.2±5.0[b]	104.0±5.6[a]	86.8±13.7[B]

Group \ Weeks	31	33	38	42	46	50
			(g)			
I	88.3±8.3[a]	84.2±18.5[a]	85.8±7.9[a]	105.2±20.2[a]	102.4±12.2[a]	86.4±15.1[a]
II			(90.6 ± 30.0)[3]	(84.6±9.0[a])	123.0±9.3[a]	102.2±13.4[a]
III	(81.5 ± 16.4)	98.8±12.7[a]	95.0±0.9[a]	102.5±11.6[a]	113.8±7.2[a]	109.5±12.6[a]
IV	99.8±6.6[a]	89.5±7.3[a]	97.8±4.2[a]	109.8±15.0[a]	121.0±10.9[a]	118.2±5.3[a]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

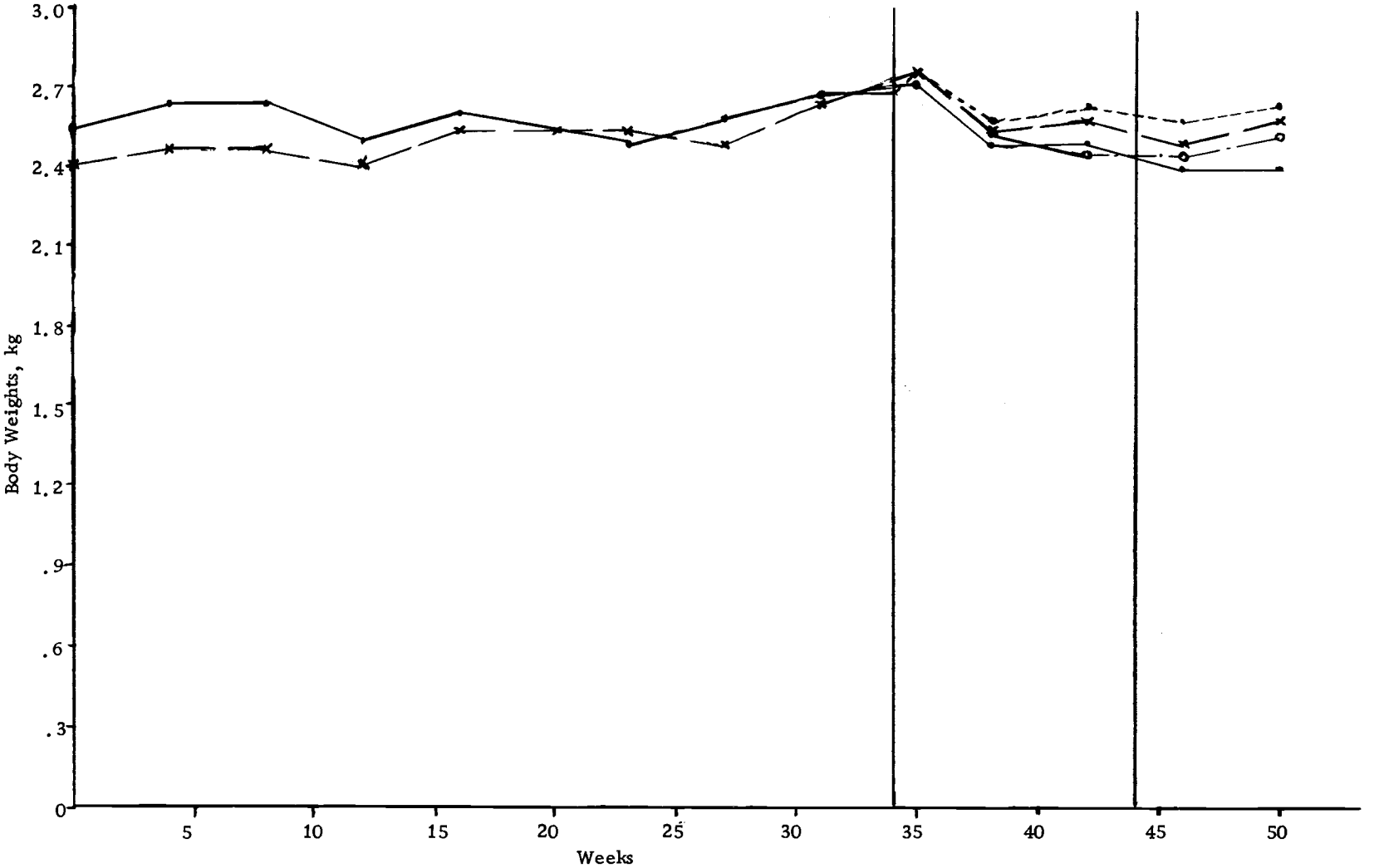

Figure 8. Effect of vitamin E on body weight of male chickens. Legend: Group I, •——•, negative control; Group II, o—·—o, as Group IV after 44 weeks; Group III, •- - - -•, as Group IV after 34 weeks; Group IV, ×— —×, 32.4 mg/kg

Table 9. Effect of vitamin E on body weight of male chickens

Group \ Weeks	0	4	8	12	16	20	23	27
	[1,2]			(kg)				
I, II, III	2.54±0.1[a]	2.63±0.1[a]	2.63±0.1[a]	2.49±0.2[a]	2.59±0.1[a]	2.54±0.2[a]	2.49±0.2[a]	2.59±0.1[a]
IV	2.40±0.2[a]	2.45±0.1[a]	2.45±0.2[a]	2.40±0.1[a]	2.54±0.1[a]	2.54±0.1[a]	2.54±0.1[a]	2.49±0.1[a]

Group \ Weeks	31	35	38	42	46	50
				(kg)		
I	2.68±0.1[a]	2.72±0.1[a]	2.49±0.1[a]	2.51±0.14[ab]	2.40±0.07[aA]	2.39±0.07[aA]
II			(2.54±0.2)[3]	(2.47±0.09[b])	2.43±0.14[ab]	2.51±0.14[aB]
III	(2.68±0.4)	2.77±0.1[a]	2.59±0.1[a]	2.62±0.10[a]	2.58±0.11[b]	2.63±0.09[B]
IV	2.63±0.1[a]	2.77±0.1[a]	2.54±0.1[a]	2.59±0.06[a]	2.54±0.04[bB]	2.58±0.05[B]

[1] Figures noted by same letter(s) are not significantly different. Lower case letters ($P < .05$); upper case letters ($P < .01$).

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

[3] Figures in parentheses were computed before addition of vitamin E.

Table 10. Effect of increased semen volume on fertility of vitamin E deficient White Leghorn males.

	% Fertility[1]	
	Normal Volume[2]	Adjusted Volume[3]
Deficient Males	0.0	0.0
Group IV Males	86.8 ± 9.2	96.3 ± 4.4

[1] 34th week

[2] normal volume = 0.05 ml

[3] adjusted volume
deficient males = up to 0.30 ml depending on amount of collection
group IV males = 0.025 ml

Table 11. Sperm Motility

Number	Date: 3-21 (ca 44 wks) Motility	Treatment	Date: 4-6 (ca 47 wks) Motility	Treatment
		Group I		
2	1	negative	1	negative
3	<2	control	2	control
4			1	
		Group II		
5	2		2	
6	3	5 days on	4	vitamin E
7		vitamin E	4	
8			4	
		Group III		
9			3	
10	4	one month	3	vitamin E
11		on vitamin E	3	
12	3		4	
		Group IV		
13			4	
14	4	32.4 mg/kg	4	positive
15	4	vitamin E	4	control
16			3	
		Group I		
17			1	negative control

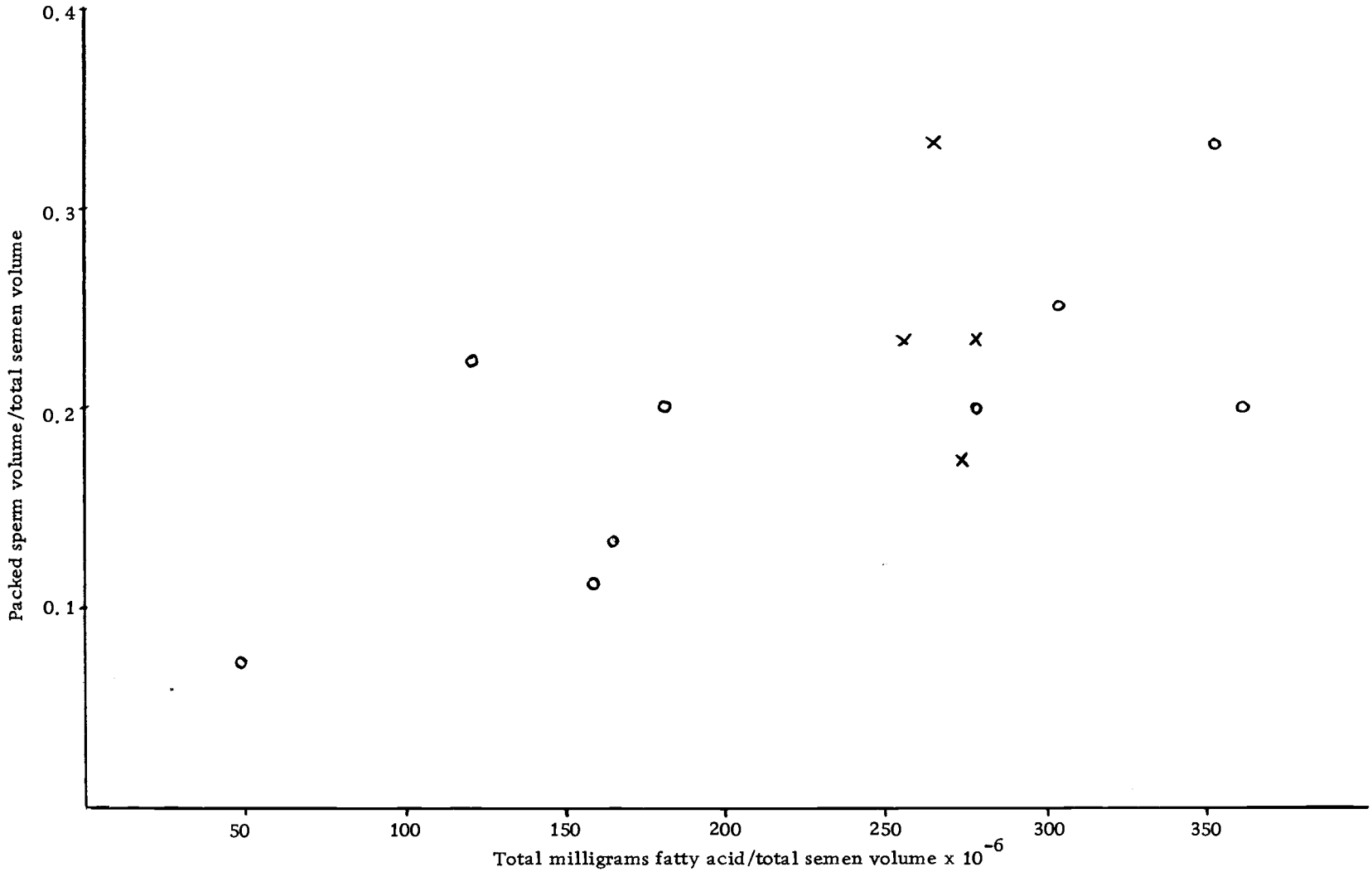

Figure 9. Effect of vitamin E on sperm volume and total fatty acid in sperm. Legend: x, supplemented; o, unsupplemented.

did fall into a narrow band in terms of total milligrams fatty acid/ total semen volume (255-276). Other samples were analyzed on the gas chromatograph, but the values were not included because no trends were present, even in the positive control birds.

There was no mortality during the course of the experiment, which was terminated when the birds were about 25 months old.

DISCUSSION

From the results obtained in this trial, it appears evident that fertility of vitamin E deficient male chickens may be restored upon the addition of vitamin E to their diet. This is true even when the birds have been deficient in vitamin E for extended periods. It is also apparent that the improvement is rapid. Group III, placed on a vitamin E diet at 34 weeks, increased from about 3.3% fertility at week 29 to 79.2% fertility at week 38. Group II with 16.2% fertility at week 44 increased to 83.1% fertility after three weeks on vitamin E ration.

The resulting increase in fertility of Group I from week 29 to week 38 was due to the removal of four males having the lowest fertility values at week 29 to form Group III at week 34. The small decrease in Group I fertility between weeks 38 and 44 was again due to the selection of the least fertile males to form Group II. The decrease of fertility noticed in Groups II and II at weeks 50 and 47, respectively, was most likely due to the increasing age of the males, approximately 25 months old.

These results are similar to those observed by Arscott, et al. (1965) and Arscott and Parker (1967), but the length of time required to get below 20% fertility varied greatly. In both their reports, fertility was below 10% at 27 weeks on the deficient diet, while in this trial, the deficient group did not drop below 20% fertility until week

47. A possible explanation for the delayed depletion in this trial might be the incomplete destruction of vitamin E in the safflower oil. In the previous trials, large amounts of air were drawn through the heated oil by means of water aspiration. In this trial, smaller amounts of air were drawn through the oil due to a decrease in water pipe diameter. The last two batches of safflower oil were oxidized by means of an electric air pump. Individual differences were also apparent among male chickens on the same diet. Several birds on the deficient diet seemed quite resistant to the vitamin E deficiency as reflected in the higher fertility values than other birds on the same diet. Fertility for the second settings essentially follows the same trends, the lower values being expected due to the eight-day period in which the sperm are most active.

Semen volume and semen concentration data follow the same pattern, both showing a significant difference at week 29, which was in December after a period of freezing weather. Both the weather conditions and the season may have affected the deficient birds more than the supplemented birds since they were already being stressed (Scott et al., 1976). The decrease in semen volume seen at week 50 was probably due to a different person doing the ejaculation, and the generally lower volume may also be a reflection on the age of the roosters. Semen volume was not expected to differ significantly between the deficient and supplemented males since a vitamin E deficiency has been reported

to have no effect on seminal fluid (Arscott and Parker, 1967). The lack of differences in semen concentration of the groups would indicate that males on deficient diets do not stop producing sperm, but the sperm are not viable.

Significant differences in hatchability appear at weeks 29 and 44. The difference at week 29 again may be due to the season and prior cold spell. The low hatchability of Group II at week 44 may indicate that vitamin E deficient males do produce some viable but defective sperm which cause the embryo to die prior to hatching. These lower hatchabilities may also be explained on the basis of the hens and the rations they were being fed since hatchability usually is more closely associated with the hen. However, it should be noted that the hens were fed diets believed capable of supporting normal hatchability. Second setting hatchability follows the same trends as the first setting from week 38 on. The greater variability may be due to the small sample sizes, since only fertile eggs are being considered, and not hatchability of all eggs.

Arscott et al. (1965) indicated that increased volume of semen from vitamin E deficient males inseminated into hens did not increase fertility. These results support this finding. Also, in an attempt to equalize sperm concentrations, lower volume of semen from supplemented birds were inseminated. The increased volume of semen from deficient birds did not increase the fertility, nor did the reduced

volume of semen from the supplemented birds reduce their fertility. It is apparent therefore that the sperm, although present, are defective in some way, as reflected in the decreased motility and fertility.

Sperm motility apparently was reduced when males were placed on low vitamin E-high linoleic acid diets. An increase of motility of the sperm was observable within five days of the males being placed on vitamin E.

Fatty acid analyses were run on sperm samples to see if fatty acid composition differed significantly between the deficient and supplemented males since most membranes include a lipid layer. No obvious differences were obtained. One would expect a difference in composition of the mitochondrial membranes as reported by Vos, 1972 and Molenaar, et al., 1972, and reflected by the decrease of motility of the sperm. The graphical representation of the third set of samples is presented, which shows the wide variability in total fatty acid composition that was obtained. The fatty acid content of the supplemented birds does fall in a fairly narrow band, but the deficient birds do not. One would expect the fatty acid content of deficient birds' sperm to be more closely related. Not only were no trends apparent in total fatty acid content but there were no obvious trends in fatty acid composition between the two groups. The method of determining fatty acid composition was satisfactory. For further

work, it would be advisable to determine the mass of lipid present in each sample so that one might have a better basis of comparison than just relative percent as obtained from the chromatograph. Larger sample sizes would also be greatly desirable so that the normal range of values for birds on vitamin E may be more clearly delineated. A better method of determining actual semen volume and sperm volume than used here would be greatly desirable.

SUMMARY AND CONCLUSIONS

One lot of four White Leghorn male chickens was fed a diet containing 7.3% linoleic acid and 32.4 mg/kg of added vitamin E for 50 weeks. A second lot containing 14 males received the same level of linoleic acid but no added vitamin E until the 34th week when the first subdivision was made. Four males were transferred to the added vitamin E diet at this time. At 44 weeks, the deficient group was divided into two groups of five males each, one remaining on the deficient diet and the other being supplemented with 32.4 mg/kg of vitamin E.

From week 24 on, the fertility of the unsupplemented group was significantly lower than the group which had been supplemented throughout the trial with vitamin E. The deficient birds, when placed on a vitamin E containing ration at 34 or 44 weeks, rapidly regained their fertility, which reached levels comparable to the continuously supplemented group.

No meaningful differences were evident between any group for semen volume, semen concentration, hatchability, feed consumption or body weights. The trends apparent in the second settings were similar to the first setting results, although fertility was much lower due to the approximately eight day life span of sperm in the oviduct. Insemination of larger volumes of semen from vitamin E deficient

birds did not increase their fertility, nor did a reduced volume of semen from supplemented birds reduce their fertility.

Sperm motility was decreased in vitamin E deficient males. Fatty acid analyses of sperm did not give conclusive results, although one might expect an obvious difference in the fatty acid composition of sperm from deficient and supplemented birds due to lipid peroxidation or the high level of linoleic acid. With refined techniques and larger sample sizes so as to minimize individual variability, one would expect a difference between the two groups in fatty acid composition although not in total lipid content.

From these findings one may conclude the following:

1. Male chickens fed diets high in linoleic acid maintained their fertilizing capacity when supplemented with 32.4 mg/kg of vitamin E.

2. Sterility induced by linoleic acid and a deficiency of vitamin E in male chickens was reversible by supplementation with 32.4 mg/kg of vitamin E, even after prolonged deficiency periods.

3. The results obtained from the second setting of eggs would indicate that one eight-day period of collection would be sufficient.

4. Increased volumes of semen from deficient birds did not increase their fertility, nor did smaller volumes of semen reduce the fertility of supplemented birds. Thus, inseminating with a standard volume of semen is not skewing the fertility results.

5. Sperm motility is decreased in vitamin E deficient birds.

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