AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF DDE AND DDT ON THE PERFORMANCE OF COTURNIX QUAIL

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Two experiments were conducted to investigate the effects of p, p'-DDE and p, p'-DDT on the performance of Coturnix quail (Coturnix coturnix japonica) fed an adequate and deficient calcium diet.

In Experiment I four hundred day-old Coturnix quail of mixed sex were fed 0 and 50 ppm of p, p'-DDE on a soybean meal-glucose monohydrate diet to 2 weeks of age. Thereafter the dosage was increased to 100 ppm on a corn-soybean meal diet. At the beginning of 25 percent egg production (10 wks.) the two populations were subdivided so that the pesticide treatments were reversed for one-half each population. The resulting four populations were further subdivided to receive diets containing 0.5 or 3.0 percent calcium and treatments were duplicated with 12 females per pen. The males were separated from the females, housed 20 birds per pen in duplicate lots and fed the lower calcium level.

In the second experiment two hundred day-old quail of mixed sex were distributed randomly into four groups of 50 each and fed the same breeder-type diet which contained either 0, 100, or 300 ppm of p, p'-DDE or 100 ppm of p, p'-DDT. Sexually mature males were housed with females at a ratio of 1:1. At 25 percent egg production each group was subdivided so that half received diets with either 3.0 or 0.5 percent calcium and the resulting eight treatments were duplicated with 12 birds per pen.

The quail through eight 28-day production periods in Experiment I seemed unaffected by the DDE intake during the developing period. Egg shell thickness, as indicated by specific gravity, and number of cracked eggs were not influenced by pesticide treatment in either experiment. Eggs from quail fed the lower calcium level showed markedly thinner shells and more cracks, however, there were no pesticide X calcium interactions. Egg production, feed consumption, egg weight and female body weight were not significantly affected by the pesticide treatments. Female mortality was higher with 3 percent calcium (associated with prolapse), and with the higher pesticide treatments.

Males seemed more sensitive to DDE toxicity than females and experienced greater mortality which was accompanied by a gradual loss of body weight along with decreased fertilizing capacity. Hatchability of fertile eggs and livability of chicks were not significantly

affected by the pesticide treatments, however hatchability was reduced on the lower calcium diet.

Effects of DDE and DDT on the Performance of Coturnix Quail

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF p, p'-DDE AND p, p'-DDT ON THE PERFORMANCE OF COTURNIX QUAIL

I. INTRODUCTION

Pest control is largely carried out by use of synthetic chemicals. These pesticides are designed to kill or inhibit plant or animal competitors that interfere with our health, comfort, or production of food and fiber. In the use of these pesticides attention initially was focused upon questions of acute toxicity. In recent years questions have arisen as to whether or not the effects of long term exposure to low levels of pesticides can cause alterations in nontarget organisms. This has come about largely from field observations of reproductive failures of several avian species plus the increased technology which has enabled chemists to accurately detect extremely small amounts of various stable pesticides in the tissues of these same birds.

DDT (dichloro-diphenyl trichlorethene) has had unprecedented development as a synthetic insecticide because of its unusual combination of properties such as wide spectrum of insecticidal action, simple structure permitting ease of manufacture, prolonged stability to the action of light and air which results in long term residual activity and relatively low mammalian toxicity. It is because of some of these very qualities that DDT, the "miracle" chemical of the

agriculturalists, has become the pollutant of the environmentalists.

Much research is currently being done on the effect of sub-lethal dosages of DDT and its metabolite DDE on various species of birds in controlled laboratory situations in order to assess the reproductive failures noted in the field. It was the purpose of this study to investigate the effects of moderate to high levels of p, p'-DDT and p, p'-DDE on the performance of Coturnix quail (Coturnix coturnix japonica), over an extended period of time. A primary objective was to attempt to duplicate the thin eggshell phenomenon using two calcium levels in the diet and secondarily to further assess the value of Coturnix quail as a suitable pilot animal in pesticide research.

II. REVIEW OF LITERATURE

Background

The compound 2, 2-bis-(p-chlorophenyl)-1, 1, 1-trichlorethane, known as DDT from the initial letters of the generic name Dichloro Diphenyl Trichloroethane, was first synthesized by Zelder in 1874. Its insecticidal properties were discovered by Müller in Switzerland in 1939 (Metcalf, 1955). With the advent of World War II, DDT was used extensively. One of its first uses was the dusting of many thousands of soldiers, refugees, and prisoners to combat lice. This was possible since DDT, in powdered form, is not readily absorbed through the skin. The success of this insecticide in halting a typhus epidemic in Italy in 1943 and 1944 was an unprecedented achievement which heralded the postwar era of unparalleled benefits in the use of pesticides for human health (Mrak, 1960). Thus, it was accepted that since so many people came into extremely intimate contact with DDT and suffered no immediate ill effects the chemical must certainly be relatively harmless. The United States alone has produced more than two billion pounds of DDT since 1949 and DDE, a DDT metabolite, is now an ubiquitous feature of the earth's environment (Bitman, 1969). It is estimated that there are a billion pounds of the substance in the world's ecosystem, and traces of it

have been found in animals everywhere, from polar bears in the Arctic to seals in the Antarctic (Peakall, 1970).

Stable Properties of DDT

Technical DDT is a white to cream colored amorphous powder, while the pure compound crystallizes into biaxial tabular crystals. It is produced by reacting chloral with chlorobenzene in the presence of sulfuric acid, oleum, or chlorosulfonic acid (Metcalf, 1955). The average commercial product contains 70 percent of the p, p'-isomer, the remainder consisting mainly of o, p'-DDT along with a little DDD (dichloro-diphenyl-dichloro-ethane), PDB (paradichlorobenzene), and excess chlorobenzene (Brown, 1951).

DDT, as an insecticide, has the advantage of prolonged stability. Its environmental half-life is 15-20 years, and a major portion remains in the upper soil layers (Bitman, 1969). It has an excessively low vapor pressure, 1.5×10^{-7} mm Hg at 20 degrees centigrade, and presumably this property is the cause of its remarkable persistence on surfaces, giving insect kills for 18 months on non-porous surfaces (Brown, 1951). In the presence of alkaline conditions, DDT is dehydrochlorinated to form 2, 2-bis-(p-chlorophenyl)-1, 1-dichloriethylene or DDE (O Brien, 1967).

Pure, p, p'-DDT may also be dehydrochlorinated at temperatures above melting point (108 °C) to form the non-insecticidal ethylene,

a reaction catalysed by ferric and aluminum chlorides and by ultra-violet light. It otherwise is stable and inert; unattacked by acid and alkaline permanganate, or by aqueous acids and alkalies. DDT is also extremely apolar, having a large oil-water partition coefficient and an excessively small water solubility. It is moderately soluble in hydroxylic and polar solvents such as alcohol, and in petroleum oils, and it is readily soluble in most aromatic and chlorinated solvents (Gunther, 1945).

DDT is very stable at normal temperatures. It decomposes at 195° C, but the decomposition may be inhibited by magnesium oxide, picolinic acid, or salicylalaminoguanidine (Gunther, 1945). DDT is not normally decomposed by sunlight. It is almost completely stable to ultra-violet light when in solid form, but in oil solution it is slightly decomposed (Brown, 1951).

Toxicity of DDT to Animals

The toxicity of DDT to animals is thought to be due to its excitatory effect on axons; it blocks the potassium flux associated with the falling phase of the action potential. It has been suggested that there is also an effect on the sodium flux associated with the rising phase of the action potential (Poulson and White, 1969). Symptoms following single exposure range from increased sensitivity and hyperexcitability with low dosages to characteristic severe tremors,

tonic contractions, and convulsions with high dosages. Immediate toxicity of purified material is gauged in laboratory animals by single administrations at different dosage levels through oral, dermal, and respiratory routes. The end result is acute toxicity. Because responses to toxicity vary greatly, individual toxicity values are judged in relation to a large test population. Toxic symptoms and sublethal effects are also noted. Death is the chief criterion. The effectiveness of the chemical at a given dose is gauged by the number of deaths in a test population. The most common standard for comparison is the LD_{50} (lethal dose for 50 percent), the amount of chemical resulting in the death of half of the test population (Rudd, 1964).

Mammals

DDT is only of moderate toxicity to mammals, the oral LD_{50} values which have been recorded are: rat 250 mg/kg of body weight, cats and dogs 150-300 mg/kg, guinea pigs and rabbits 300-500 mg/kg, cows and horses 300 mg/kg, and goats and sheep 1000 mg/kg (Metcalf, 1955). In humans a fatal case of DDT poisoning has been reported by Hill and Robinson (1945). The lethal dose was computed to be approximately 150 mg/kg of body weight of commercially pure DDT. Intravenously the compound is about ten times as toxic. For dermal application the LD_{50} for rabbits was 300 mg/kg, for

guinea pigs 1,000 mg/kg, for rats 3,000 mg/kg (Cameron and Burgress, 1945).

When it became apparent during World War II that DDT was effective for controlling the vectors of malaria, typhus, plague, and certain other diseases of military importance, the toxicity of the compound was studied by teams of investigators in several countries. Since the results of animal tests were encouraging, the studies were extended to volunteers. Conclusions from these studies recommended that the only precautions that the practical operator, who handles DDT in its various forms, need take are those dictated by common sense and experience (West and Campbell, 1952). The general opinion of DDT and public use in early post-war years can be summarized by Buxton who stated, "My conclusion, given without reserve and in simple words, is that DDT used as an insecticide is quite safe" (West and Campbell, 1952).

Birds

In general, birds are more resistant to acute DDT toxicity than are mammals. The median lethal concentrations (LC_{50}) and their 95 percent confidence limits for p,p'-DDD, p,p'-DDT, technical DDT, and p,p'-DDE in the diets of 7-day-old pheasants (Phasianus calchicus) were 522 (469-583) ppm, 550 (462-655) ppm, 935 (835-1,047) ppm, and 1,086 (928-1,271) ppm, respectively

(Gill, Verts and Christensen, 1970). The LC_{50} for o, p'-DDT was not established, but was estimated to be in excess of 5, 000 ppm. The LC_{50} for mallards (Anus platyrhynonous) was 850 to 1, 200 ppm; for pheasants, 300 to 700 ppm; for bobwhite quail (Colinus virginianus), 600 to 1, 000 ppm; and for Coturnix quail, 400 to 600 ppm of DDT in diets for 2-week-old birds when fed treated feed for 5 days followed by untreated feed for 3 days (Heath et al., 1969). The LC_{50} for mallards was 3, 300 to 3, 600 ppm; for pheasants, 750 to 950 ppm; for bobwhite quail, 750 to 950 ppm; and for Coturnix quail, 1, 200 to 1, 400 ppm of DDE in diets of 2-week-old birds when fed treated feed for 5 days followed by untreated feed for 3 days (Heath et al., 1969). When birds were given oral dosages of DDT in capsule form, the LD_{50} for young mallards was greater than 2, 240 mg/kg of body weight; for young pheasants, 1, 296 mg/kg; for young Coturnix quail, 841 mg/kg; for pigeons (Columba livia) greater than 4, 000 mg/kg; and for lesser sandhill cranes (Grus canadensis) greater than 1, 200 mg/kg (Tucker and Haegele, 1971).

Sublethal Effects of DDT and its Metabolites in Birds

Few mammals or birds are killed outright by DDT. However, from the standpoint of chronic poisoning, DDT presents considerably more of a hazard (Metcalf, 1955). The decided affinity of DDT for fatty materials has resulted in its pronounced storage in

animal fats and its excretion in milk and eggs. Field sampling has shown that DDT and its derivatives are present in the fat of many species of wild animals, including those in areas which are not known to have received pesticidal exposure (Mrak, 1960).

Field Observations

Declines in populations of some species of wild birds have been linked to increased levels of DDT and other pesticides in the environment. The decline is believed to be due to greater shell breakage caused by thinner shells. The incidence of broken eggs in nests of peregrine falcons (Falco peregrinus), sparrow hawks (Accipiter nisus), and golden eagles (Aquila chrysaetos) in Britain has increased considerably since 1950. For the species examined, frequency of egg-breakage, scale of decrease in eggshell weight, subsequent status of breeding population, and exposure to persistent organic pesticides has been correlated (Ratcliffe, 1967). The first statistical correlation study in the United States comparing DDT residues in eggs of birds with decreasing eggshell weight and declining bird populations showed the declines of three raptorial species, osprey (Pandion haliaëtus), bald eagle (Haliaeetus leucocephalus), and the peregrine falcon (Falco peregrinus). All had been accompanied by decreases in eggshell thickness that began in 1947 (Hickey and Anderson, 1968). The weights of raptor eggshells

in museums and private collections were measured to determine if there had been a change in the weights of these eggshells from the pre-DDT period (1886 to 1939) to the post-DDT period. In Brevard County, Florida bald eagle eggshells from the pre-DDT era weighed 12.5±0.127 g., 56 eggs measured; eggshells from 1947 to 1962 weighed 9.96±0.280 g., 12 eggs measured. Hence, there appears to be an 18 percent decrease in the weight of the eggshells. It has been reported that the population of bald eagles have declined in this area (Hickey and Anderson, 1968). A similar reduction in shell thickness in the eggs of American herring gulls (Larus argentatus) from five states has been shown to have been inversely correlated with the amount of DDE in the egg contents (Hickey and Anderson, 1968). Canada reported a significant drop of 11 percent in the thickness of prairie falcon (Falco mexicanus) eggs, compared with eggs sampled from the pre-organochlorine insecticide era. Although other chemicals were present in addition to DDT, a high correlation was found between eggshell thickness and DDE residue in the eggs. Associated with the decline in eggshell thickness was a 34 percent decline in the occupancy of territories known to have falcons during the previous 10 years (Fyfe et al., 1969). A high correlation has also been found between the amount of DDE in eggs and eggshell thickness of pelican (Pelecanus erythrorhynchos) and double-created cormorant (Phalacrocorax auritus) eggs (Anderson

and Hickey, 1969). In contrast peregrines of the Mackenzie system, although heavily contaminated with DDT and/or its metabolites, were apparently reproducing normally (Enderson and Berger, 1968). This phenomenon was also true with peregrine populations in Alaska and Canada, although the authors felt that these falcons were near the threshold level of organo-chlorine residues that initiate dysgenic reproductive behavior and eventual population decline (Cade et al., 1968).

Controlled Experiments

DDT, DDE and Eggshell Thickness

DDT and DDE have been shown to cause thin eggshells under laboratory conditions in several species of birds, however, many investigators have reported no effect. Studies with gallinaceous birds on high DDT diets including the chick (Gallus domesticus), pheasants (Phasianus calchicus), and Japanese quail (Coturnix coturnix), (Weihe, 1967; DeWitt, 1956; Hunt, et al., 1968; Cross et al., 1962; Jones and Summers, 1968), reported no obvious shell thinning. In these cases dietary calcium was apparently adequate or in excess.

Japanese quail on a calcium stress during egg laying (0.56 percent of calcium), demonstrated significantly thinner shells when

fed either 100 ppm of o, p' or p, p'-DDT than the control group on the same calcium stress (Bitman et al., 1969). The decrease in eggshell thickness was related to clutch size and was most noticeable towards the end of the clutch cycle.

Eggs laid by Japanese quail on an adequate calcium diet (3.5 percent of Ca) and treated with three levels of p, p'-DDT; 2.5 ppm, 10 ppm, and 25 ppm had 6.0, 6.4, and 7.3 percent thinner eggshells, respectively, than those laid by untreated birds. In contrast, eggshell thickness in this latter study was not related to the clutch cycle but to total egg production (Stickel and Rhodes, 1970).

Technical DDT was administered at 10 and 30 ppm to 2-year-old bobwhite quail (Colinus virginianus) and 1-year-old mallard ducks (Anus platyrhynonos). The birds were also treated with various calcium levels (3.0, 1.73 and 1.0 percent of calcium). Results showed very slight shell thinning for both species due to pesticide (5 percent) and a greater shell thinning due to calcium stress alone (13 percent). Mallard ducks were more sensitive to calcium stress and pesticide treatment than were the bobwhite quail (Tucker and Haegele, 1970).

DDE was found to be more detrimental to eggshell thickness in mallard ducks than DDT. DDE in concentrations of 10 and 40 ppm severely impaired reproductive success. DDE affected eggshell formation, so that shells were 13 percent thinner than normal. DDD

did not cause any significant changes in shells. DDT induced significant thinning of shells only when at a concentration of 25 ppm (Heath, et al., 1969).

Captive American sparrow hawks (Falco sparverius), given a diet containing a mixture of DDT and dieldrin produced eggshells from the parental group that averaged 8 to 10 percent thinner than those of controls of the parental group; and eggshells of first-generation dosed birds were thinner by 15 to 17 percent on the average than those of first-generation controls. The differences were significant in both the first and parental generations of hawks. In another experiment 10 ppm of p, p'-DDE alone was administered to American sparrow hawks for two years. No difference in eggshell thickness was recorded between treated and untreated birds the first year, but average shell thickness of eggs laid by DDE-treated hawks was 10 percent less the second year (Porter and Wiemeyer, 1969) and (Wiemeyer and Porter, 1970).

Dieldrin, when injected into ring doves (Streptopelia risoria), shortly before they laid their first eggs, did not produce any significant thinning of the eggshells. DDE, however, brought about a marked decrease in the thickness of the eggshells of ring doves when injected shortly before lay (Peakall, 1970).

In experiments with the Bengalese finch (Lonchura striata), a passeriform in which the pairs incubate their eggs and rear the

chicks themselves, DDT treatment resulted in shells of eggs that were thicker, rather than thinner, than those produced by untreated birds (Jefferies, 1967).

Onset and Rate of Egg Production and Egg Weight

A delay in onset of egg production due to moderate levels of DDT has been reported for Bengalese finch and Japanese quail (Jefferies, 1967), (Bitman et al., 1969).

Although a few investigators have reported statistically significant downward egg production trends for quail fed as low as 10 ppm of DDT (Stickel and Rhodes, 1969), in general, no significant drop in egg production has been reported for quail treated with DDT up to 300 ppm (DeWitt, 1955, Cross et al., 1962, Smith et al., 1970, and Bitman et al., 1969). However, in another experiment egg production did decrease when quail were treated with 400 ppm of DDT and production ceased at 700 ppm of DDT (Smith et al., 1970, and Cross et al., 1962). Pheasants have shown no effect on egg production when fed diets with as high as 500 ppm of technical grade DDT (Genelly and Rudd, 1956, and Azevedo et al., 1965); nor have mallard ducks with 10 and 40 ppm of DDE or 2.5, 10 and 25 ppm of DDT (Heath et al., 1969).

Little has been reported on the effect of DDT on egg weight.

Cross et al. (1962), found no effect on egg weight of Japanese quail that were administered levels of DDT as high as 300 ppm. Eggs layed by Japanese quail fed 10 ppm p,p'-DDT and low calcium (0.56 percent) were reported to be significantly smaller than those of the control with the same calcium stress (Bitman et al., 1969).

Fertility, Hatchability and Livability

In general, no adverse effects have been noted for quail or pheasant fertility or hatchability when DDT was administered up to 200 ppm (DeWitt, 1955, Genelly et al., 1956, Azevedo et al., 1965, Jones and Summers, 1968, and Smith et al., 1970); however quail fertility dropped sharply when treated with 400 ppm of DDT for over 30 days (Smith et al., 1970) and hatchability of quail was significantly affected at 200 ppm of DDT (DeWitt, 1955) and was reduced to zero at 500 ppm of DDT (Cross et al., 1962). Mallard ducks seemed more sensitive to DDE than DDT as 10 and 40 ppm of DDE reduced hatchability, by producing embryo mortality during the final (fourth) week of incubation while DDT at 2.5 or 10 ppm had no effect (Heath et al., 1969).

Two hundred ppm of DDT administered to parent stock produced a 79 percent chick mortality within the first 3 days after hatching in young quail fed a normal diet without pesticide. Young

birds from DDT treated parents exhibited ataxia and spasm (DeWitt, 1955, Jones et al., 1968, and Smith et al., 1970). Pheasant liveability was significantly reduced in day-old chicks from parents treated with 100 and 400 ppm of DDT (Genelly et al., 1956); however, in another experiment there was no significant difference with 100 ppm of DDT but liveability of chicks was significantly reduced when parents were fed 500 ppm (Azevedo et al., 1965). Livability of ducklings from parents treated with 10 and 40 ppm of DDE was normal; yet ducklings from parents that had been treated with 25 ppm of DDT showed a significant decrease in liveability (Heath et al., 1969).

Body Weight

Body weights of quail fed 200 ppm of technical grade DDT were not significantly different than those of controls during a breeding season of 154 days (DeWitt, 1955), nor were growth rates of Bengalese Finch significantly different than controls when dosed with moderate levels of DDT (Jefferies, 1967). However, 500 ppm of DDT fed to adult Coturnix quail for 10 days resulted in an increase in body weight (Ernst, 1967) and average body weights of adult Coturnix quail, in breeding condition, that survived DDT treated diets of 700 ppm in a two week period were significantly greater than those of controls. Average weights of both controls and

the treated survivors at time of sacrifice were significantly higher than the average weights of birds that died during DDT treatment. When birds from DDT treated groups and the controls were sacrificed, 100 percent of those birds surviving the treatments of DDT and 94.8 percent of the controls contained some body fat, including subcutaneous, abdominal, mesentery, and coronary fat. However, of those that died during pesticide treatment, only 0.4 percent contained some body fat, as determined visually (Gish and Chura, 1970).

Gonadal Development

DDT, when administered during a period of growth to cockerels, at a rate increasing from 15 to 300 mg/kg of body weight per day, resulted in an inhibition of testicular growth and secondary sexual characteristics. The testes of treated birds were only one-fifth the weight of control birds. Difference in weight was accounted for by decrease in tubular development in the treated birds; intertubular development showing a relative and perhaps an absolute increase (Burlington and Linderman, 1950). DDT, when fed to sexually mature Bengalese finch, caused no change in weight or size of the testes as compared to the controls (Jefferies, 1967). The average gonadal weight of adult Coturnix quail treated with high levels (700 ppm and higher) of DDT for 20 days was inversely proportional to the amount

of DDT in the food. The gonads of all controls were heavier than the gonads of those birds surviving DDT treatment or those that died from the treatment (Gish and Chura, 1970).

III. EXPERIMENTAL PROCEDURE

Foundation Stock

Coturnix quail (Coturnix coturnix japonica) used in all trials were from the O. S. U. strain maintained by the Department of Poultry Science, Oregon State University. This strain was obtained in 1960 from the Oregon Game Commission, Hermiston, Oregon, which originally secured the birds as mature stock from the Oklahoma State Game Commission.

Incubation of Eggs

To obtain chicks of a uniform age eggs were collected daily for a period of 10 days. After each day's collection the eggs were placed in flats specifically designed for quail and set in a wooden container which was tilted 45 degrees by hand twice a day in order to prevent the membranes of the eggs sticking to the shell. The eggs were stored in a room which averaged 70° F.

On the tenth day all eggs were set in a Jamesway incubator, Model 252[1]. The setting trays had been modified for quail eggs by Arscott. Modification of trays consisted of placing the quail eggs

[1] James mfg. Co., Fort Atkenson, Wisconsin.

between two sheets of hardware cloth with openings of 1.9 cm^2 mesh. The top mesh was overlaid with a finer screen of 0.63 cm^2 mesh which was secured in the setting tray by metal clips. Incubation conditions were similar to that of hen eggs except for length of time and an effort to operate units at a slightly higher humidity. Coturnix quail eggs hatch between 16 and 18 days of incubation (Howes, 1964). From 0 to 14 days incubating temperatures averaged 100° F dry bulb and 85° F wet bulb. On the fourteenth day the eggs were transferred into chicken pedigree hatching baskets and incubation was continued until the eighteenth day. Approximately two days before hatching the humidity was increased to 88-90° F wet bulb.

Housing and Management

Day-old birds were raised in a chick battery brooder manufactured by Oakes.[2] The battery had four levels each of which was partitioned into four compartments having the dimensions of 89 x 41 x 36 cm (length x width x height) which could house 50 birds per pen for the first 2 weeks. Temperature was controlled by overhead electric hovers which permitted the maintenance of 90-95° F at floor level for the first 2 weeks. The temperature was gradually reduced after the second week until it was maintained at room temperature (65-70° F) by the fourth week.

[2]Oakes mfg. Co., Tipton, Indiana.

Birds treated with pesticide were placed below control groups to minimize contamination by way of feed and waterers. Feed was provided ad libitum in gravity-flow self feeders which were located outside each cage. Water was provided continuously by the use of Hart watercups.

At 6 weeks of age the quail were wing-banded and transferred into a finishing battery[3]. The battery had 4 levels each of which was partitioned into 8 compartments having the dimensions 69 × 46 × 36 cm (length × width × height). The floors of each compartment were slightly inclined to facilitate egg collection and to prevent eggs from being broken by trampling or egg-eating. Heat was supplied to the finishing battery room by an electrical space heater which maintained a temperature of 65-70° F. Feed and water were provided ad libitum with self feeders placed on the outside of each cage and a continuous gravity flow water system. At all times during growth and production the quail were maintained under 24 hours of incandescent light.

Rations for Coturnix Quail

The rations used were developed by Arscott (1969) and were patterned after similar rations fed to turkeys (Table 1). In general, there were three distinct diets. The starter mash (No. 1410) fed

[3]Wes-Bilt mfg. Co., Inc., Hayward, California

Table 1. Composition of experimental rations for coturnix quail.

	Period I 0-2 wks (R-333)	II 3-5 wks[1] (1408m)	III 5 wks[1]-25% EP (1403m)	IV 25% EP - completion (1409m)	IV 25% EP - completion (1403m)
	%				
Glucose monohydrate[2]	20.9	--	--	--	--
Corn, yel. grd.	--	59.68	67.14	67.14	67.14
Fat, corn oil[3]	4.	2.	2.	2.	2.
Soybean meal (44% prot.)	69.	32.2	18.73	18.73	18.73
Alfalfa meal (20% prot.)	--	2.	3.	3.	3.
Cellulose[4]	--	--	--	6.65	--
DL-methionine (98%)	(.5)[5]	.12	.15	.15	.15
Glycine	(.2)	--	--	--	--
Limestone flour	--	1.6	6.15	--	6.15
Dicalcium phosphate	--	1.85	2.	1.5	2.
Salt, iodized	--	.3	.5	.5	.5
Salts, N[6]	6.	--	--	--	--
Salts, N, suppl't (SE+ Mo)[7]	.1	--	--	--	--
Vit.-tr. min. mix. (PM-1-65)[8]	--	.25	--	--	--
Vit.-tr. min. mix (PM-2-65)[9]	--	--	.33	.33	.33
Vit. K-B-complex mix[10]	(.6)	--	--	--	--
Vit. E (20,000 IU/lb)[11]	(.0938)	--	--	--	--
Choline Cl (25%)	(1.2)	--	--	--	--
Vit. A (30,000 IU/g)	(.0333)	--	--	--	--
Vit. D_3 (1500 ICU/g)	(.133)	--	--	--	--
BHT[12]	(.0125)	--	--	--	--
Totals	100.0	100.0	100.0	100.0	100.0

(Continued)

Table 1. Continued

	Period I 0-2 wks (R-333)	II 3-5 wks (1408m)	III 5 wks[1]-25% EP (1403m)	IV 25% Ep - completion (1409m)	IV 25% Ep - completion (1403m)
			%		
Calculated analyses:					
Prot., %	31.6	21.	15.2	15.2	15.2
Ca, %	1.24	1.29	3.0	.5	3.0
P, %	.8	.8	.7	.63	.7

1 Or 1st egg.

2 Cerelose, (Corn Products Co., New York).

3. Mazola (Corn products Co., New York)

4 Solka Floc BW-100 (Brown Co., Berlin, New Hampshire).

5 Fig. in ( ) not part of total %.

6 Min. mix. (Fox and Briggs), supplies as % of mix: Ca, 20.67; P, 13.33; K, 6.17; Na, 6.4; Cl, 9.67; Mg, 1.; Fe, .0556; Mn, .1355; I, .01; Zn, .1213; Cu, .0066 (General Biochemicals, Chagrin Falls, Ohio).

7 Optional tr. min. mix. (Fox & Briggs), supplies in mg/kg of mix.: Se, 100; Mo, 2000.

8. Supplies in amts/kg of mix.; Cu, .8 g; Fe, 8 g; I, .48 g; Mn, 24 g; Zn, 11 g; Co, 88 mcg; Vit. A, 1,320,000 IU; Vit. D_3, 440,000 ICU; Vit. E, 440 IU; Vit. K, .22 g; riboflavin, 1.32 g; d-pantothenic acid, 2.2 g; niacin, 8.8 g; Choline cl, 88 g; Vit. B_{12}, 2.2 mg; BHT, 50 mg; Zn bacitracin, 1.76 mg.

9 Supplies in amts/kg of mix.: Cu, .8 g; Fe, 8 g; I, .48 g; Mn, 24 g; Zn, 11 g; Co, 88 mcg; Vit. A, 1,320,000 IU; Vit. D_3, 440,000 ICU; Vit. E, 440 IU; Vit. K, .22 g; riboflavin, .88 g; d-pantothenic acid, 1.32 g; niacin, 8.8 g; choline cl, 44 g; Vit. B_{12}, 1.8 mg; BHT, 50 mg.

10 Vit. mix. (Gordon) supplies in mg/kg of mix.: Vit. K (meandione), 200; Vit. B_{12}, 4; thiamine HCl, 1600; riboflavin, 1600; Ca-d-pantothenate, 4000; niacin, 20,000; pyridoxine HCl, 1600, folacin, 600; biotin, 60; glucose monohydrate, qs., (Nutritional Biochemicals Corp., Cleveland, Ohio).

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

12 Butylated hydroxyanisole.

from 0 to 2 weeks of age consisted of a 28% protein soybean meal-glucose monohydrate diet. The grower or developer mash (No. 1408 m) consisted of a 20% protein corn-soybean meal diet and was fed from 3 to 5 weeks or to date of laying the first egg. At onset of lay (6 weeks) the quail were fed a laying mash (No. 1403 m) which consisted of 15% protein and a 3% calcium level in the diet. Another ration (1409 m) was developed for mature males, when housed separately from females, which was essentially the same as ration 1403 m except for a lower calcium level (0.5%) and was found to be adequate for male quail. Ration 1409 m was also used for females in production when introducing a calcium stress.

In order to uniformly distribute the pesticide throughout the feed the pesticide crystals were dissolved in corn oil using a Fisher Thermix unit. The solution was mixed into the ration in 25 pound lots by way of a Holbart mixer, care being taken to avoid contamination. Periodic pesticide analysis of the feed, eggs and birds were carried out by the agricultural chemistry department at Oregon State University under the direction of Claeys (Appendix Table 1). Statistical analysis of quail experiments was performed by analysis of variance (Snedecor and Cochran, 1967).

Individual Experiments

Experiment 1. Effects of p, p'-DDE on the Performance of Coturnix Quail

Approximately 400 day-old Coturnix quail of mixed sex were divided into two groups and fed 0 or 50 ppm of p, p'-DDE on a soybean meal-glucose monohydrate diet (Table 1) to two weeks of age, and 0 or 100 ppm of p, p'-DDE on a corn-soybean meal diet thereafter.

At 6 weeks of age all birds were wing-banded. The males were separated from the females and housed 20 birds per pen while the females were housed 12 birds per pen in the finisher battery.

At 10 weeks of age the two populations were subdivided so that one-half of those receiving no pesticide continued without pesticide while the other half received 100 ppm of p, p'-DDE. One-half of the birds then receiving 100 ppm of p, p'-DDE reverted to 0 ppm and the other half continued on the treatment throughout egg production (Figure 1).

At the beginning of 25% egg production (10 weeks) each of the four female populations was further subdivided so that half received diets containing 0.5% of calcium (ration 1409 m) while the others continued on the normal 3% of calcium for egg production (ration 1403 m). The males were maintained on the lower calcium level of 0.5% throughout the production period.

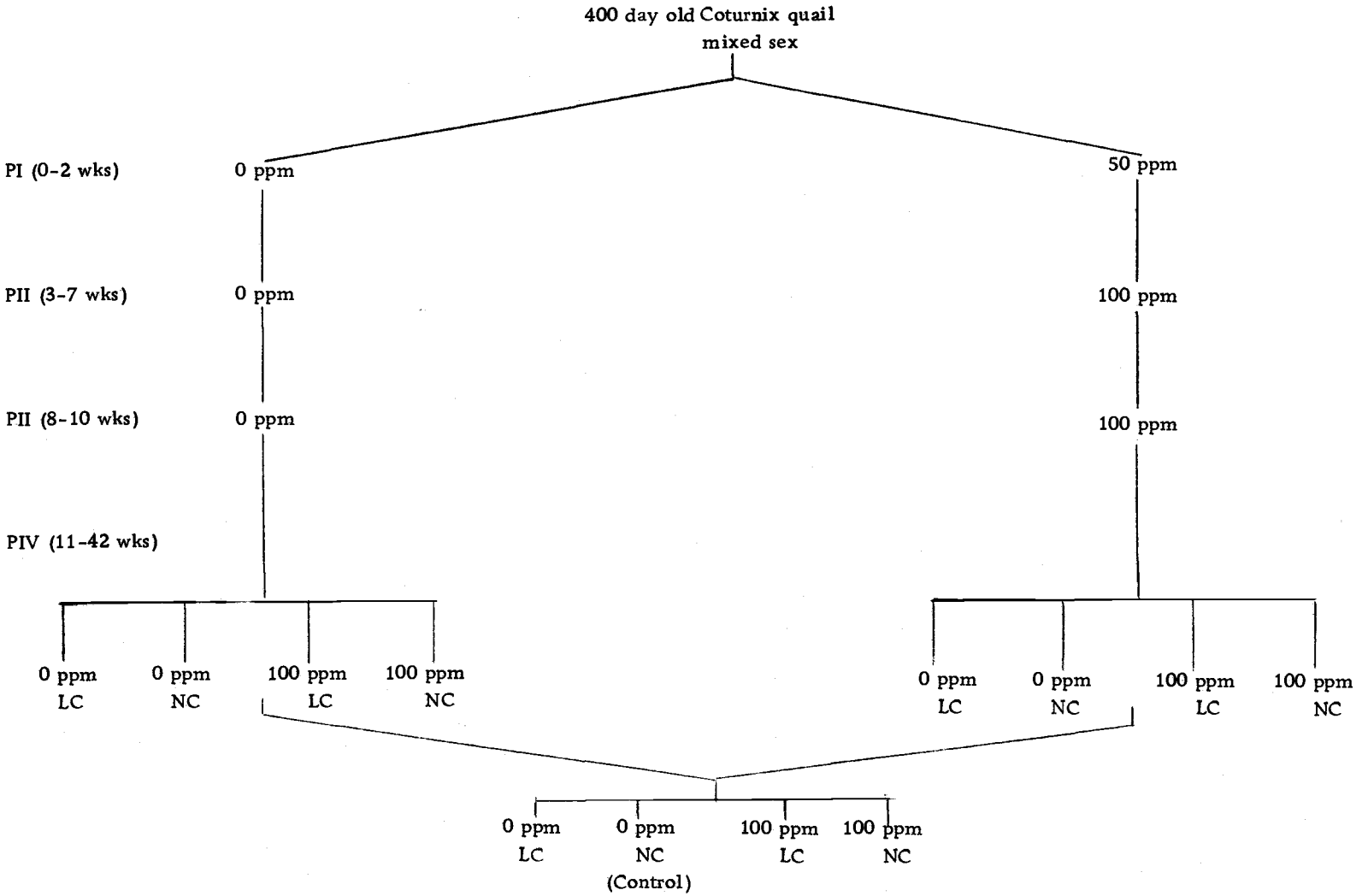

Figure 1. Experimental design for experiment 1.
Legend: ppm-p, p'-DDE; LC - 0.5% calcium, ration 1409 m;
NC - 3.0% calcium, ration 1403 m.

All treatments were duplicated so that variability within treatments could be estimated. Body weights and feed consumption data were taken on a two week basis from the start of the experiment until onset of lay (6 weeks) and again at the commencement of 25% of egg production. After 25% of egg production body weights and feed consumption data were taken once each 28-day period. Body weights were computed on a group basis and their average was taken as the weight per bird. Feed consumption was also calculated on a per bird basis.

Eggs were collected daily and egg production was calculated on a 28-day basis. Eggshell thickness was determined through specific gravity measurements. This method of determining eggshell thickness has not been commonly utilized in pesticide research. The specific gravity of an entire egg and its relationship to shell thickness has been recognized for many years by the poultry industry (Arscott and Bernier, 1961). Little correlation exists as to total egg weight and specific gravity. Romanoff and Romanoff (1949) observed that the internal contents of eggs have a specific gravity of one that remains unchanged while the shell has a specific gravity of two. The entire egg's specific gravity is therefore largely influenced by the proportional amount of thickness of the shell. Thus, factors influencing the percentage of shell will influence the specific gravity of eggs. Specific gravity was taken at the beginning of 25 percent egg production

and during the last 3 days of each production period. Egg weights were taken consecutively with specific gravity during production periods 4-6.

Hatchability and fertility tests were accomplished by placing males from corresponding treatments into female pens for various lengths of time. The males were then removed and eggs were collected and stored at room temperature (55° F) for eight days. On the eighth day all eggs were incubated. Hatchability (%) was determined as the number of chicks obtained from the number of fertile eggs incubated. Fertility (%) was calculated as the number of chicks that hatched plus the number of unhatched eggs containing an embryo at any stage of the development based on total eggs incubated.

After 30 weeks of age (period 6) feed was withheld from the females for various lengths of time (up to 24 hours) near the end of each production period in order to induce a release of pesticide from the adipose tissue.

Experiment 2. Effects of p, p'-DDE and p, p'-DDT on the Performance of Coturnix Quail

Two-hundred day-old Coturnix quail of mixed sex were divided into 4 groups of 50 chicks each. The chicks were fed a ration with either 0, 100, or 300 ppm of p, p'-DDE or 100 ppm of p, p'-DDT. All received the soybean meal-glucose monohydrate diet (Table 1) until

2 weeks of age and the corn-soybean meal diet thereafter.

At 8 weeks of age the birds were wing-banded and transferred into the finisher battery. Males were housed with females at all times at a ratio of 1:1 with twelve birds per pen. At 25% egg production (10 weeks) the 4 populations were subdivided so that half of each population continued on the ration containing 3.0% of calcium (1403 m) while the other half received the ration containing 0.5% of calcium (1409 m). All eight treatments were duplicated (Figure 2).

Collection of data was similar to that in Experiment 1. In addition, fertility and hatchability experiments were conducted once each 28-day period beginning at 10 weeks of age. Eggs were saved for eight days prior to incubation. Livability of chicks from pesticide treated parents was observed during production period 5. Chicks were housed in the normal fashion and fed a control feed with no pesticide for 6 weeks. The experiment was terminated after 30 weeks.

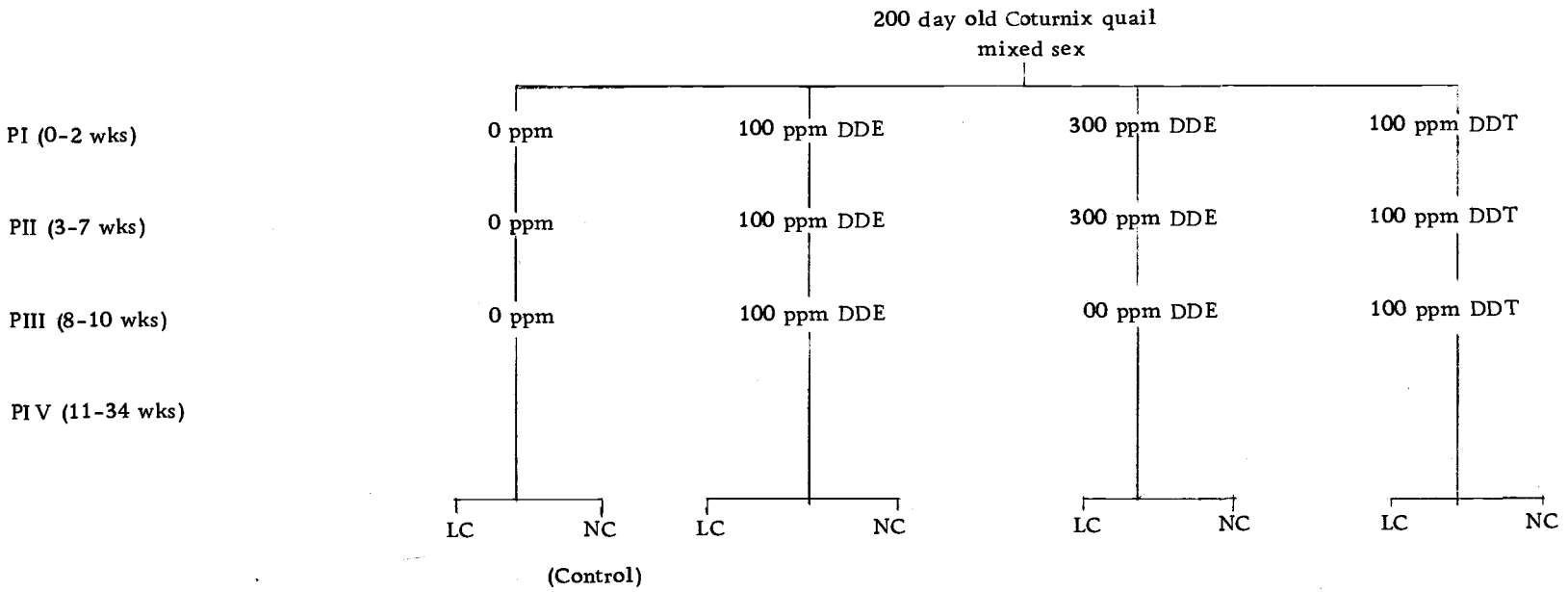

Figure 2. Experimental design for experiment 2.
Legend: LC - 0.5% calcium, ration 1409 m;
NC - 3.0% calcium, ration 1403 m.

IV. RESULTS

Experiment 1. Effect of 50 and 100 ppm of p,p'-DDE on the Performance of Coturnix Quail

During the developing period body weights of quail of mixed sex were significantly ($P < 0.01$) increased by the pesticide treatment. Quail treated with 50 ppm of DDE averaged 49 g. per bird as compared to the controls which averaged 44 g. per bird at 2 weeks of age (Table 2). However, this growth response diminished gradually and no difference in body weight was observed by 10 weeks of age. This growth response was not observed in two later trials (Table 3).

A summary of the production results involving the averages of eight 28-day production periods is presented in Tables 4 and 5. No differences were evident when data were averaged and compared involving no pesticide or pesticide treatment during the developing period. In view of this, tables and figures, unless otherwise noted, involve combining like treatments during the production period without regard to prior pesticide treatment (Figure 1).

During the production periods, females fed the low calcium diet (0.5 percent of calcium) averaged 138 g. per bird, which was significantly ($P < 0.01$) heavier than those fed the normal calcium diet (3.0 percent of calcium) which averaged 124 g. per bird irrespective of pesticide treatment (Table 6). DDE did not appear to affect

Table 2. Effect of DDE on body weight during the developing period in experiment 1.

Treatment[1] DDE	Av. body weights (g./bird)								
	Developing Period[2]			Production Periods[3]					
	2 wk.	4 wk.	6 wk.	Prel.[4]	1[5]	2	3	4	5
0 ppm (control)	44	83	109	122	127	132	129	133	134
100 ppm[6]	49**	89	111	120	129	130	129	130	132

1 200 birds per treatment.

2 Mixed sex.

3 Females only.

4 Ca 10 wks. of age.

5 Period equivalent to 28 days.

6 50 ppm DDE during first 2 weeks of the developing period.

** Difference from controls is significant at the $P < .01$ level.

Table 3. Effect of DDE on body weight during the developing period of Coturnix quail.

Treatment[1] DDE	Av. body weights[2] - (g./bird) 2 wk.	4 wk.	6 wk.
0 ppm (control)	43	85	110
50 ppm	44	84	110
100 ppm	41	84	109

1 50 birds per treatment.

2 Mixed sex.

Table 4 Effects of DDE during the developing period on the performance of females in production during experiment 1.

Treatment[1]		Egg Production	Egg Weight	Specific Gravity	Broken Eggs	Body Weight	Feed Consumption	Female Mortality
Developing period[2]	Production period[3]	(%)	(g)	$(1+10^{-4})$	(%)	(g./bd)	(g./bd/day)	(%)
0 ppm	0 ppm/3.0% Ca (Control)	43	9.7	691	2	121	18	42
0 ppm	0 ppm/0.5% Ca	41	10.0	559**	12**	137**	21	25
0 ppm	100 ppm/3.0% Ca	60*	10.0	673	2	122	24	67
0 ppm	100 ppm/0.5% Ca	36	9.7	546**	13**	132*	20	46
100 ppm[4]	0 ppm/3.0% Ca	52	9.9	681	3	128	22	50
100 ppm[4]	0 ppm/0.5% Ca	41	9.6	541**	8**	138**	20	33
100 ppm[4]	100 ppm/3.0% Ca	60*	10.5	689	3	124	20	67
100 ppm[4]	100 ppm/0.5% Ca	41	10.2	541**	16**	136**	20	67

1 24 females per treatment.

2 Developing period - one day old to 8 weeks of age.

3 Production period - figures are averages for eight 28-day periods.

4 50 ppm DDE during first 2 weeks of the developing period.

* Difference from controls significant at the $P < .05$ level.

** Difference from controls significant at the $P < .01$ level.

Table 5. Effects of DDE during the developing period on the performance of sexually mature males in experiment 1.

Treatment[1]		Body Weight (g/bd)	Feed Consumption (g/bd/day)	Male Mortality (%)
Developing Period[2]	Production Period[3]			
0 ppm	0 ppm/0.5% Ca (control)	114	14	15
0 ppm	100 ppm/0.5% Ca	102*	13	88
100 ppm[4]	0 ppm/0.5% Ca	113	14	30
100 ppm[4]	100 ppm/0.5% Ca	100*	14	95

1 20 males per treatment.

2 Developing period-one day old to 8 weeks of age.

3 Production period-figures are averages for eight 28-day periods.

4 50 ppm DDE during first 2 weeks of the developing period.

* Difference from controls is significant at the $P < .05$ level.

Table 6. Effects of DDE and calcium on female body weight during egg production in experiment 1.

Treatment[1]	Av. body weights by period[2] (g./bird)									
	Prel.[3]	1	2	3	4	5	6	7	8	Av.
0 ppm DDE/3.0% Ca (control)	122	123	124	120	123	126	124	130	131	
100 ppm DDE/3.0% Ca	121	123	123	121	124	123	114	124	131	
Av.		123	124	120	124	125	119	127	131	124
0 ppm DDE/0.5% Ca	122	131	139	138	143	142	140	140	144	
100 ppm DDE/0.5% Ca	120	135	138	137	137	140	129	138	134	
Av.		133*	139**	138**	140**	141**	134**	139*	139	138**

1 48 birds per treatment

2 Period equivalent to 28 days.

3 Ca 10 weeks old.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

female body weight (Figure 3).

All males involved during the production periods were fed the same calcium level (0.5 percent). Males receiving 100 ppm of DDE gradually lost weight beginning with the second period and by period 4 body weights of males on control feed averaged 117 g. per bird while DDE treated males were significantly ($P < 0.05$) lower and averaged only 99 g. per bird (Figure 4, Table 7).

Egg production appeared quite variable in all treatments and the average for the eight periods did not show any significance which could be attributed to the pesticide treatment except those birds treated with DDE and fed the normal calcium level which produced significantly ($P < 0.05$) more eggs than those fed the control diet. Average egg production for birds treated with 100 ppm of DDE and fed the normal calcium ration was 59 percent while birds fed the control diet averaged 47 percent (Table 8). On the other hand, egg production dropped significantly ($P < 0.01$) during period 1 when birds were initially fed the low calcium diet. Egg production for those birds averaged only 39 percent as compared to 53 percent for birds fed the normal calcium ration irrespective of pesticide treatment. Females fed the lower calcium ration gradually increased in egg production but tended to lay fewer eggs than birds fed the normal calcium diet (Figure 5).

DDE, at 100 ppm, did not cause quail to lay eggs with any

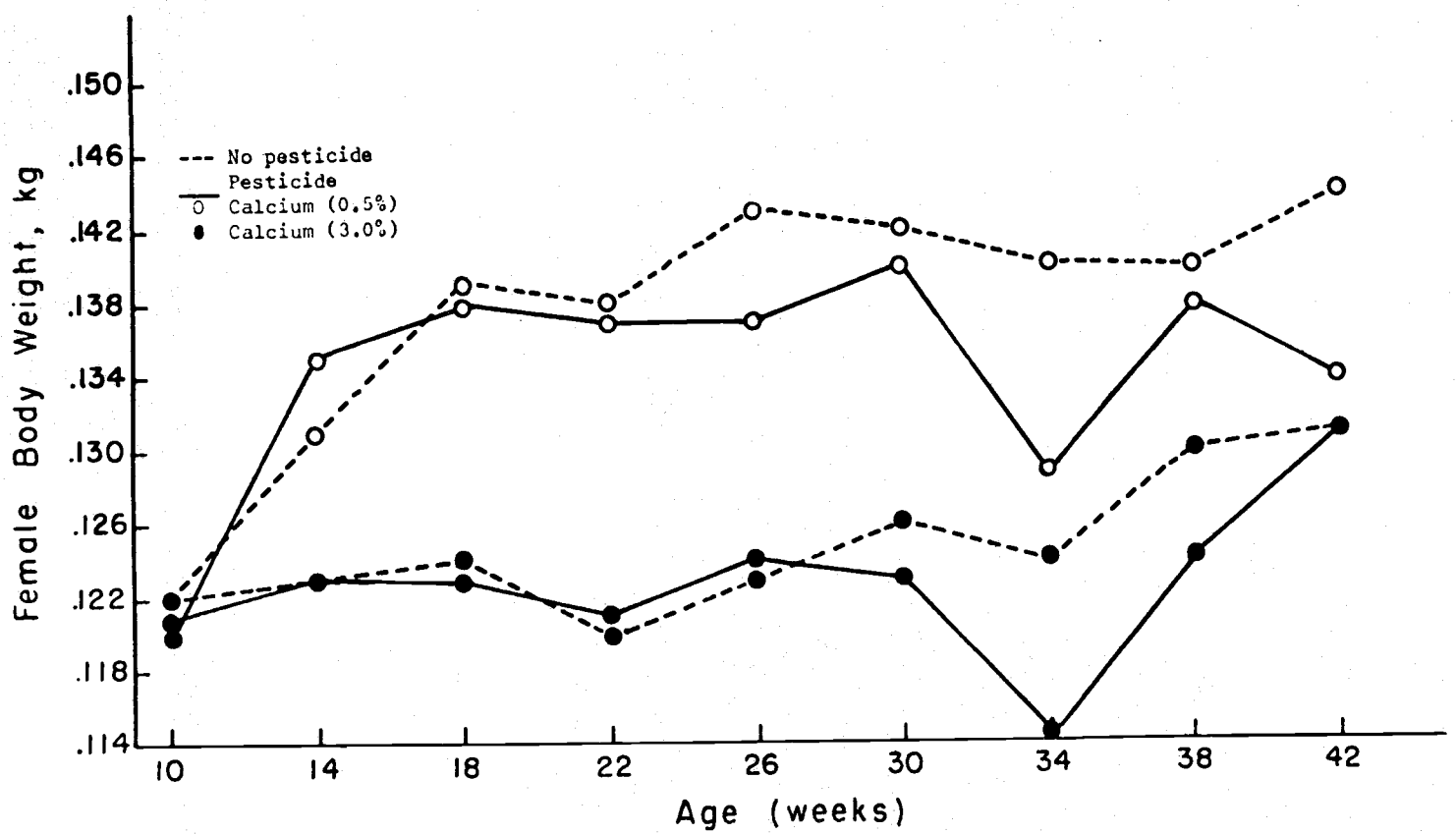

Figure 3. Effects of DDE and calcium on female body weight in experiment 1.

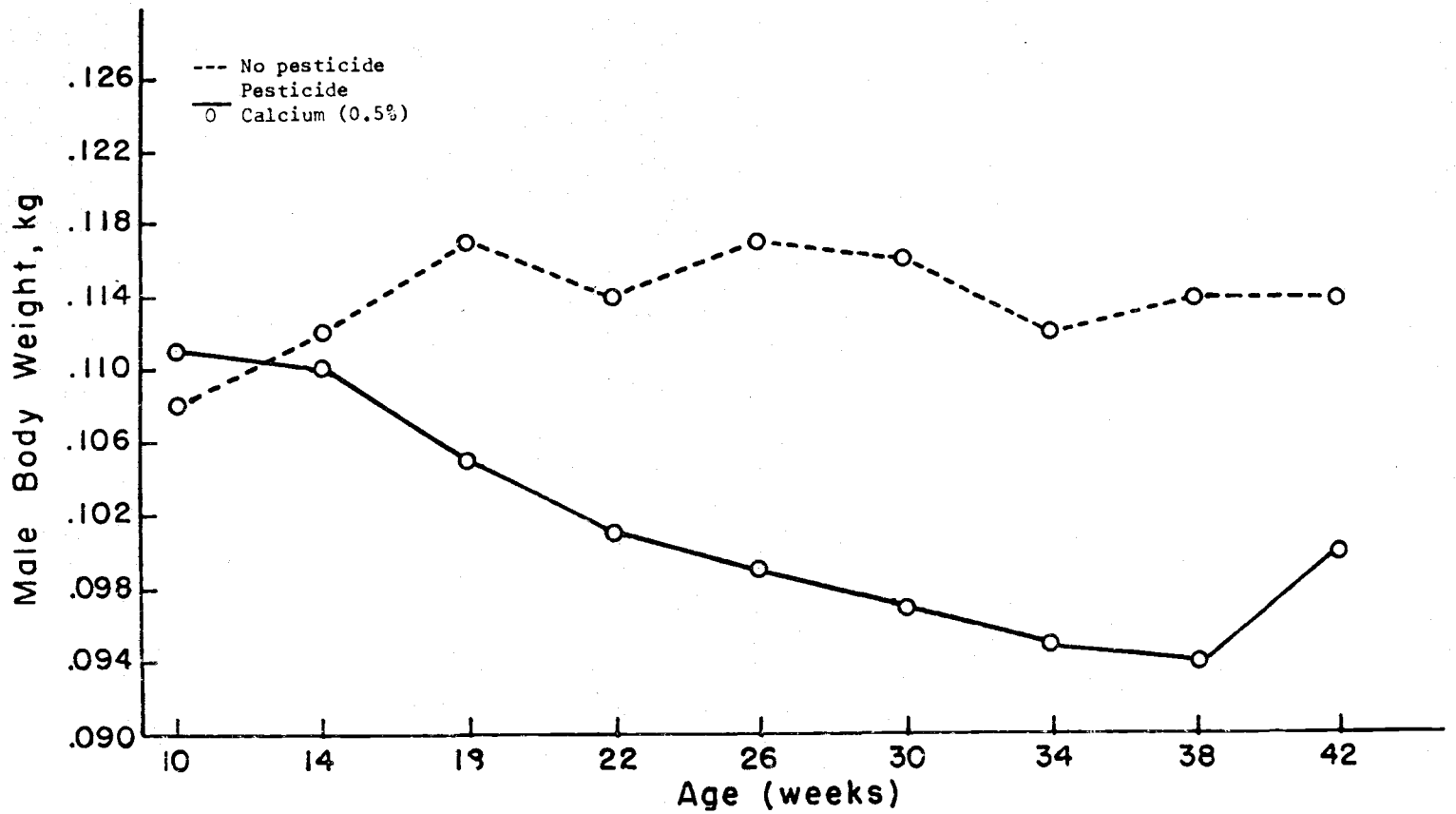

Figure 4. Effect of DDE on male body weight in experiment 1.

Table 7. Effect of DDE on male body weight in experiment 1.

Treatment[1] DDE	Av. body weight by period[2] (g./bird)								
	Prel.[3]	1	2	3	4	5	6	7	8
0 ppm (control)	108	112	117	114	117	116	112	114	114
100 ppm	111	110	105	101	99*	97	95	94	100*

1 40 birds per treatment.

2 28 day periods.

3 Ca 10 weeks old.

* Difference from controls is significant at the $P < .05$ level.

Table 8. Effects of DDE and calcium on egg production in experiment 1.

Treatment[1]	Av. egg production per period[2] (%)									
	Prel.[3]	1	2	3	4	5	6	7	8	Av.
0 ppm DDE/3.0% Ca (control)	27	46	45	53	55	52	50	46	50	47
0 ppm DDE/0.5% Ca	34	29**	34	44	41	43	43	43	49	40
100 ppm DDE/3.0% Ca	25	47	65*	66	67	67*	68*	64*	66	59
100 ppm DDE/0.5% Ca	21	28**	36	44	38*	40	43	42	48	38

1 48 females per treatment.

2 Period equivalent to 28 days.

3 3.0% Ca for all treatments.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

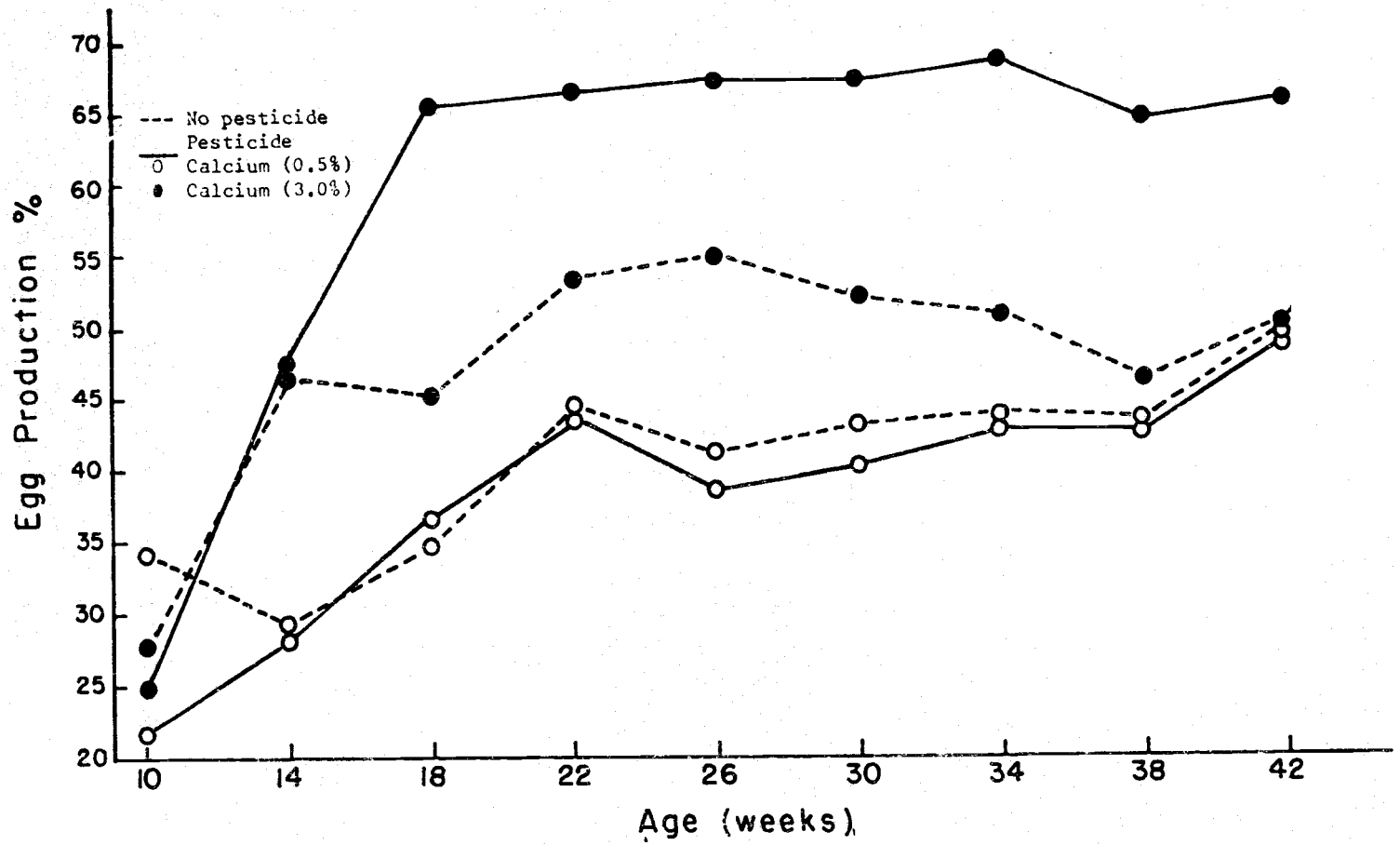

Figure 5. Effects of DDE and calcium on egg production in experiment 1.

decrease in eggshell thickness as indicated by specific gravity of whole eggs (Figure 6). However, females fed the low calcium diet laid eggs with significantly ($P < 0.01$) lower specific gravity which averaged 1.0537 as compared with birds fed the normal calcium level, irrespective of pesticide treatment, which averaged 1.0687 (Table 9).

Although egg breakage was quite variable, females fed the lower calcium level laid more cracked and soft-shelled eggs than birds fed the normal calcium level irrespective of pesticide treatment. Quail fed the low calcium ration produced eggs that averaged 12.3 percent cracked or shoft-shelled. This was significantly ($P < 0.01$) higher than birds fed the normal calcium ration which averaged 2.4 percent irrespective of pesticide treatment (Table 10). In general, pesticide treatment did not significantly ($P < 0.05$) affect egg breakage; however, birds fed the low calcium ration and treated with DDE consistently produced the greatest number of cracked eggs (Figure 7). During production period 4 this breakage was 21.3 percent and proved significantly ($P < 0.01$) higher than any other treatment (Table 10).

There were no meaningful differences in feed consumption or egg weight for any of the calcium or pesticide treatments (Table 4). Due to the experimental design, no meaningful fertility data was collected (Table 11).

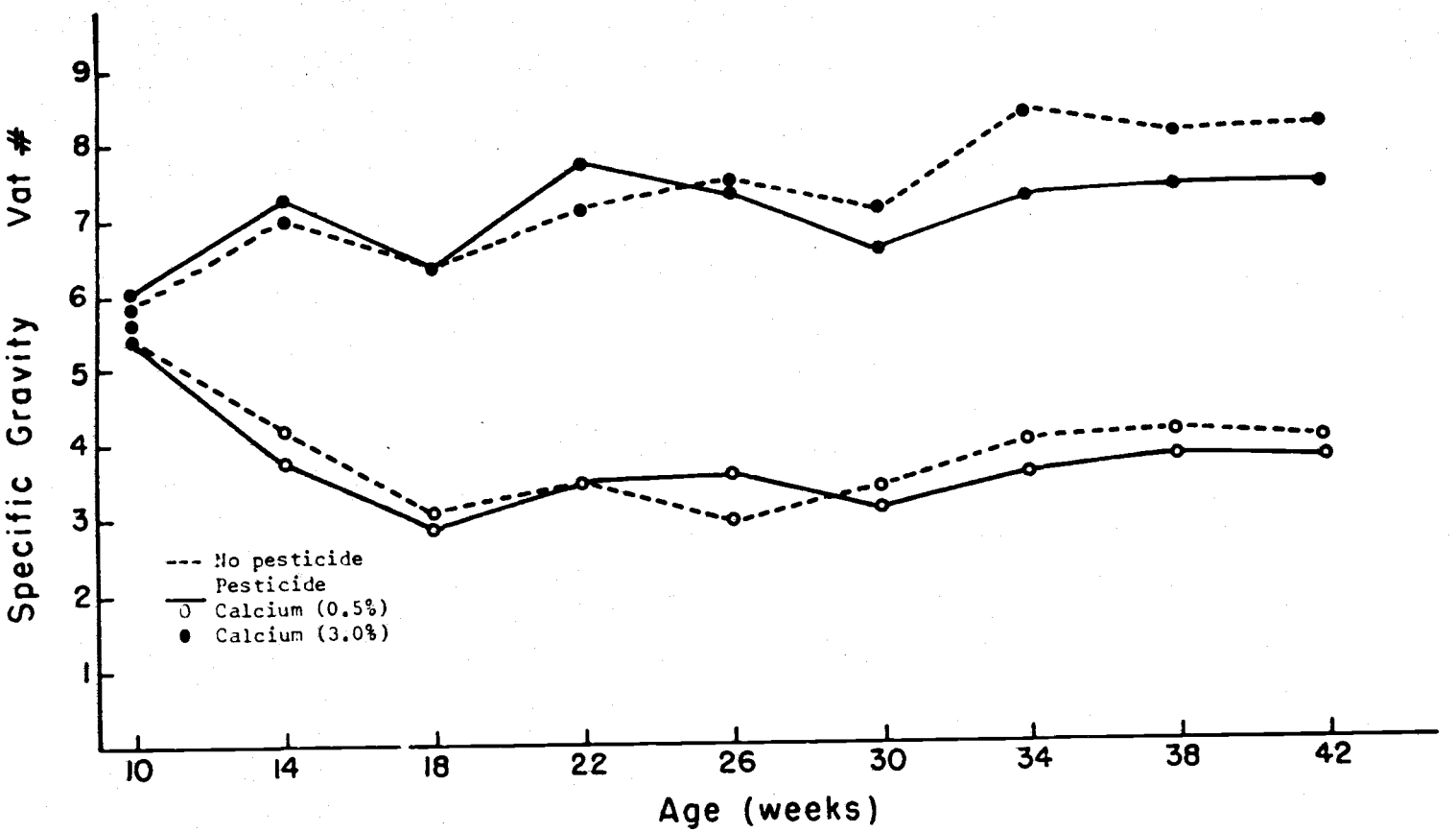

Figure 6. Effects of DDE and calcium on the specific gravity of eggs in experiment 1.

Legend: Vat #1 equals 1.056 specific gravity with 0.004 increasing intervals thereafter.

Table 9. Effects of DDE and calcium on specific gravity of eggs in experiment 1.

Treatment[1]	Specific gravity by periods[2] $(1+10^{-4})$									
	Prel.[3]	1	2	3	4	5	6	7	8	Av.
0 ppm DDE/3.0% Ca (control)	631	681	655	668	688	668	720	712	718	
100 ppm DDE 3.0% Ca	640	681	652	712	795	664	691	693	692	
Av.		681	654	690	692	666	706	702	705	687
0 ppm DDE/0.5% Ca	624	576	519	538	511	528	552	555	552	
100 ppm DDE/0.5% Ca	620	545	514	536	536	519	537	542	540	
Av. **		560	516	537	524	524	544	548	546	537

1 48 females per treatment.

2 Period equivalent to 28 days.

3 No Ca stress.

** All averages significantly lower ($P < .01$) than 3.0% Ca treatments.

Table 10. Effects of DDE and calcium on cracked and soft-shelled eggs in experiment 1.

Treatment[1]	% cracked by period[2]								
	1	2	3	4	5	6	7	8	Av.
0 ppm/3.0% Ca (control)	1.1	1.2	2.0	5.2	4.1	3.2	2.7	1.4	2.6
100 ppm/3.0% Ca	2.7	0.0	1.6	1.5	2.3	5.0	3.3	2.4	2.4
Av.	1.9	0.6	1.8	3.4	3.2	4.1	3.0	1.9	2.5
0 ppm/0.5% Ca	3.3	9.6	10.0	11.2	15.3	12.6	11.2	16.4	11.2
100 ppm/0.5% Ca	9.1	15.8	14.3	21.3	16.4	18.0	14.5	18.0	15.9
Av. **	6.2	12.7	12.2	16.2	15.8	15.3	12.8	17.2	13.6

1 48 females per treatment.

2 Period equivalent to 28 days.

** All averages significantly lower ($P < .01$) than 3.0% Ca treatments.

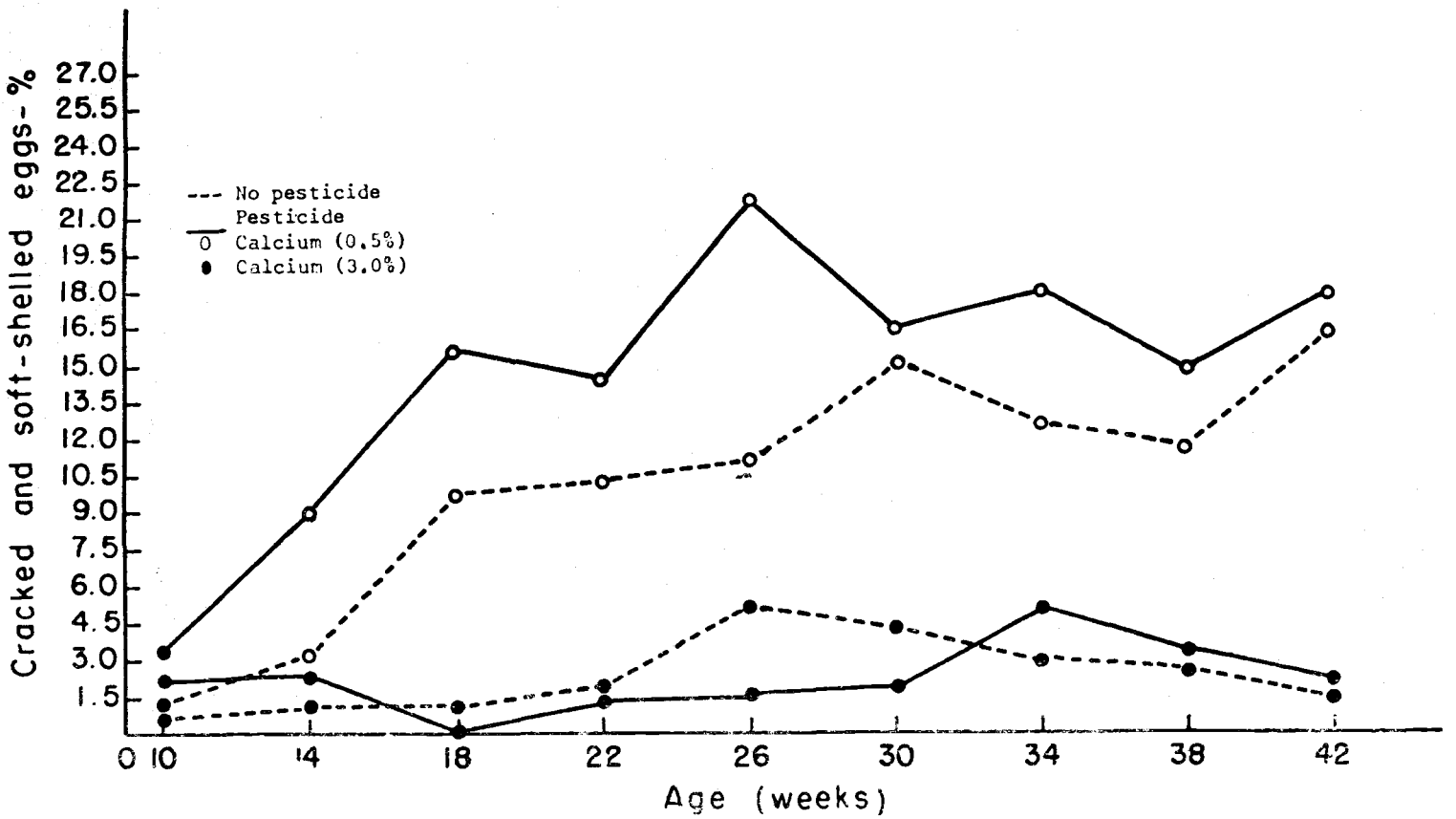

Figure 7. Effects of DDE and calcium on the incident of cracked and soft-shelled eggs in experiment 1.

Table 11. Effect of length of mating period on fertility of eggs of Coturnix quail.

Group[1]	Time Male With Female[2] (hours)	No. eggs set[3]	Fertility (%)	Hatchability[4] (%)
1	1	65	0	-
2	2	70	4	86
3	3	85	13	81
4	6	81	21	92
5	9	76	12	84

1 Four males were mated to twelve females in each group. All groups were replicated and fed a control diet.

2 Feed was withheld during mating time. The 6 hour mating was split into 2 consecutive days of 3 hours each, likewise the 9 hour mating was split into 3 consecutive days of 3 hours each.

3 Eggs were saved for eight days after each mating.

4 Percent based on number of fertile eggs.

Female mortality was higher with those birds fed the normal calcium level as compared with those fed the low calcium level. Birds fed the low calcium ration and no pesticide had 25 percent mortality by period 8 whereas females on normal calcium with no pesticide showed 42 percent (Table 4). DDE, at 100 ppm, did not seem to affect female mortality until period 6, however, by the end of period 8 birds fed DDE averaged 62 percent mortality while females not treated with DDE averaged only 38 percent (Figure 8). Male mortality was much higher with DDE treatment, 91 percent, as compared with 22 percent for the controls by period 8 (Table 5 and Figure 9).

Experiment 2. Effects of 100 and 300 ppm of p, p'-DDE and 100 ppm of p, p'-DDT on the Performance of Coturnix Quail

There were no differences in body weights of quail fed any of the pesticide treatments during the developing period as seen in Table 12. However adult females fed 300 ppm of DDE gradually lost weight which was significantly ($P < 0.05$) lower than the controls by period 5 (Table 13). The other pesticide treatments did not seem to affect female body weight. The two calcium levels had no sigificant effect ($P < 0.05$) effect on female body weight, however, those birds fed 100 ppm of DDT and the 3 percent calcium diet tended to weigh less than the controls (Table 13).

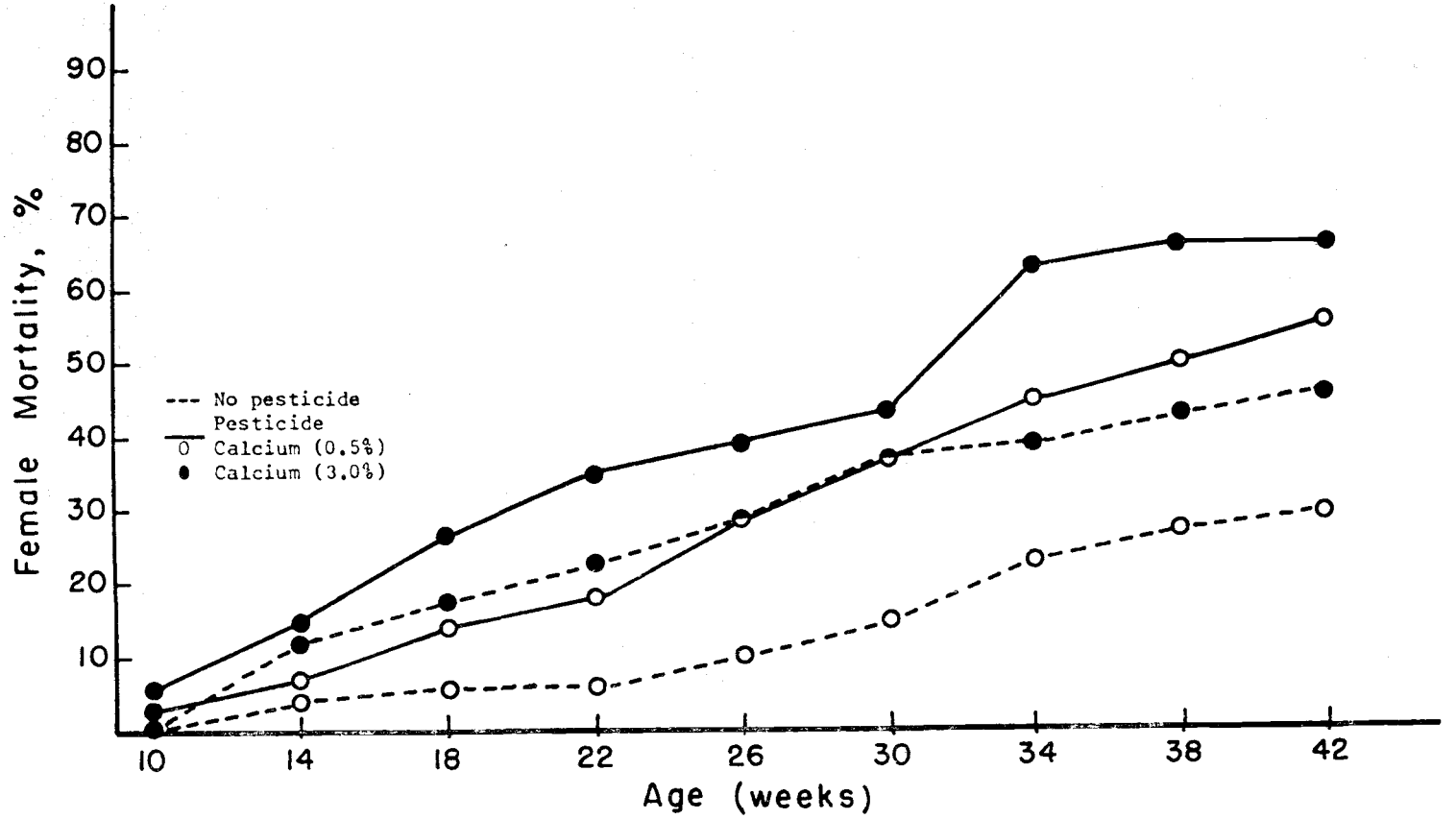

Figure 8. Effects of DDE and calcium on the mortality of females in experiment 1.

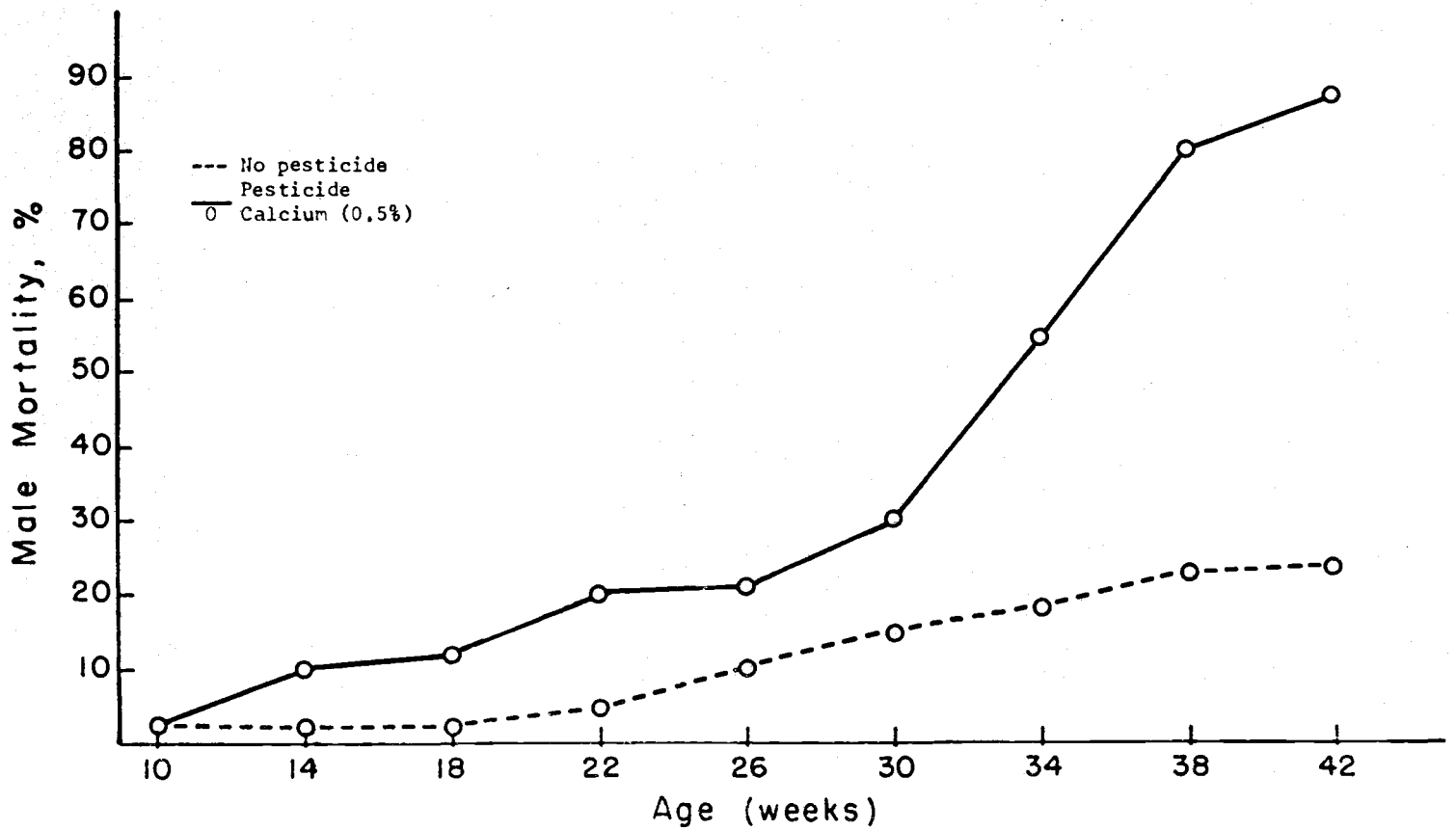

Figure 9. Effect of DDE on the mortality of males in experiment 1.

Table 12. Effects of DDE and DDT on body weight during the developing period in experiment 2.

Treatment[1]	Av. body weights (g./bird)								
	Developing period[2]					Production period[3]			
	2 wks	4 wks	6 wks	Prel.[4]	1[5]	2	3	4	5
0 ppm (control)	42	85	105	120	124	135	123	127	134
100 ppm DDE	42	86	106	121	132	136	134	130	132
300 ppm DDE	43	87	103	120	126	117	122	112	110*
100 ppm DDT	42	85	104	119	124	125	126	130	127

1 24 birds per treatment.

2 Mixed sex.

3 Females only.

4 Ca 10 weeks of age.

5 4 week periods.

* Difference from controls is significant at the $P < .05$ level.

Table 13. Effects of DDE, DDT and calcium on female body weight during egg production in experiment 2.

Treatment[1]		Av. body weights by periods[2] (g./bird)						
		1	2	3	4	5	6	Av.
0 ppm/3.0% Ca (control)		126	136	123	126	135	132	130
0 ppm/0.5% Ca		123	134	123	128	134	131	129
	Av.	124	135	123	127	134	132	130
100 ppm DDE/3.0% Ca		132	134	132	133	138	138	134
100 ppm DDE/0.5% Ca		132	138	136	128	125	127	131
	Av.	132	136	134	130	132	132	132
300 ppm DDE/3.0% Ca		115	114	119	101	111	110	112
300 ppm DDE/0.5% Ca		138	119	125	124	108	96	118
	Av.	126	116	122	112	110*	103*	115
100 ppm DDT/3.0% Ca		120	116	120	126	120	124	121
100 ppm DDT/0.5% Ca		128	134	132	134	132	133	132
	Av.	124	125	126	130	126	128	126

1 12 birds per treatment.

2 Period equivalent to 28 days.

* Difference from controls is significant at the $P < .05$ level.

In contrast to experiment 1 one-half the male population in experiment 2 were fed the 3 percent calcium ration with or without the pesticide treatment. There were no significant differences ($P < 0.05$) between calcium levels and body weights but males fed the lower calcium diet and treated with 0 or 100 ppm of DDT tended to be heavier than males given the same treatment with the normal calcium level. On the other hand, males treated with 100 ppm of DDE and fed the lower calcium level and those treated with 300 ppm of DDE and fed either calcium level gradually lost weight with a significant ($P < 0.05$) difference noted for the latter during period 3 as compared with males fed a control diet (Table 14).

The egg production, as in experiment 1, was quite variable within treatments. Pesticide treatment had no significant ($P < 0.05$) effect on egg production, however, those birds treated with 300 ppm of DDE produced fewer eggs than those fed the control diet. Egg production for those females fed the 3 percent calcium diet averaged 25 percent for the 300 ppm DDE treated birds as compared to 52 percent for the controls (Table 15). Calcium treatment had no significant ($P < 0.05$) effect on egg production, in contrast to experiment 1, but birds fed the lower calcium level consistently produced fewer eggs than birds fed the normal calcium level.

A level of 100 and 300 ppm of dietary DDE and 100 ppm of dietary DDT did not result in any thinning of eggshells as indicated

Table 14. Effects of DDE, DDT and calcium on male body weight in experiment 2.

Treatment[1]		Av. body weight by period[2] (g./bird)						
		1	2	3	4	5	6	Av.
0 ppm/3.0% Ca (control)		106	113	109	114	114	109	111
0 ppm/0.5% Ca		114	123	116	118	120	124	119
	Av.	110	118	112	116	117	116	115
100 ppm DDE/3.0% Ca		114	118	113	114	105	101	111
100 ppm DDE/0.5% Ca		107	111	108	102	096	094	103
	Av.	110	114	110	108	100*	098**	107
300 ppm DDE/3.0% Ca		109	104	100	099	099	097	101
300 ppm DDE/0.5% Ca		103	100	092	085	084	086	92
	Av.	106	102	096*	092*	091*	091**	096
100 ppm DDT/3.0% Ca		100	114	114	104	112	113	110
100 ppm DDT/0.5% Ca		108	115	117	113	122	118	116
	Av.	109	114	116	108	117	116	113

1 12 birds per treatment.

2 Period equivalent to 28 days.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

Table 15. Effects of DDE, DDT and calcium on egg production in experiment 2.

Treatment[1]	Av. egg production per period[2] (%)							
	Prel.[3]	1	2	3	4	5	6	Av.
0 ppm/3.0% Ca (control)	25	48	64	60	58	49	63	52
0 ppm/0.5% Ca	27	36	39	48	38	40	49	40
100 ppm DDE/3.0% Ca	24	42	62	52	72	70	77	57
100 ppm DDE/0.5% Ca	26	33	48	43	44	27	16	34
300 ppm DDE/3.0% Ca	23	34	31	20	28	18	22	25
300 ppm DDE/0.5% Ca	27	38	46	31	44	45	30	37
100 ppm DDT/3.0% Ca	24	42	60	65	74	83	78	61
100 ppm DDT/0.5% Ca	25	38	40	52	44	38	41	40

1 12 females per treatment.

2 Period equivalent to 28 days.

3 3.0% Ca for all treatments.

by specific gravity measurements of whole eggs (Table 16). However, birds fed the low calcium diet layed eggs with significantly ($P < 0.01$) thinner eggshells. There was no pesticide × calcium interaction. Specific gravity averaged 1.0539 for eggs from birds fed the low calcium ration as compared with birds receiving the normal calcium diet whose eggs averaged a specific gravity of 1.0675 irrespective of pesticide treatment (Table 16).

Although egg breakage was quite variable in both experiments, birds fed the lower calcium level layed more cracked and soft-shelled eggs than those fed the normal level. Females fed the low calcium ration produced eggs that averaged 16.4 percent cracked and soft-shelled (Table 17). This was significantly ($P < 0.01$) higher than birds fed the normal calcium diet which produced eggs that averaged 2.9 percent. There was no significant ($P < 0.05$) difference in numbers of cracked and soft-shelled eggs produced by birds treated with pesticide as compared with birds not treated with pesticide and fed the same calcium level. There was, however, a trend for birds treated with 100 ppm of DDT and fed the lower calcium ration to produce the greatest number of cracked eggs (Table 17).

There were no meaningful differences in feed consumption or egg weight for any of the calcium or pesticide treatments (Table 18). Fertility of eggs was also not significantly ($P < 0.05$) affected by any of the treatments except those birds treated with 300 ppm of

Table 16. Effects of DDE, DDT and calcium on specific gravity of eggs in experiment 2.

Treatment[1]		Specific gravity by periods[2]						
		Prel.[3]	1	2	3	4	5	Av.
					$1 + 10^{-4}$			
0 ppm/3.0% Ca		670	672	673	677	679	666	
100 ppm/3.0% Ca		669	671	704	701	698	691	
300 ppm DDE/3.0% Ca		675	610	650	675	608	640	
	Av.		663	678	688	672	674	675
0 ppm/0.5% Ca		668	507	539	560	486	560	
100 ppm DDE/0.5% Ca		670	560	588	554	466	500	
300 ppm DDE/0.5% Ca		666	522	551	580	491	573	
100 ppm DDT/0.5% Ca		674	634	535	503	535	543	
	Av. **		556	553	549	495	544	539

1 12 females per treatment.

2 Period equivalent to 28 days.

3 No Ca stress.

** All averages significantly lower ($P < .01$) than 3.0% Ca treatments.

Table 17. Effects of DDE, DDT and calcium on cracked and soft-shelled eggs in experiment 2.

Treatment[1]	% cracked by period[2]						
	1	2	3	4	5	6	Av.
0 ppm/3.0% Ca (control)	0.0	2.6	3.2	6.0	12.0	1.0	4.1
100 ppm DDE/3.0% Ca	0.6	1.7	4.8	7.5	5.0	2.5	3.7
300 ppm DDE/3.0% Ca	1.2	0.0	0.0	8.0	5.0	0.0	2.4
100 ppm DDT/3.0% Ca	1.7	0.8	0.5	2.5	1.0	1.5	1.3
Av.	0.9	1.3	2.1	6.0	5.8	1.2	2.9
0 ppm/0.5% Ca	6.2	11.4	19.2	30.5	23.0	6.5	16.1
100 ppm DDE/0.5% Ca	4.5	17.8	18.6	22.0	30.0	6.0	16.5
300 ppm DDE/0.5% Ca	4.6	15.4	14.4	18.5	8.5	0.0	10.2
100 ppm DDT/0.5% Ca	4.4	18.8	26.0	38.5	16.0	20.5	20.7
Av.	4.9*	18.6**	19.6**	27.4**	19.4**	8.2*	16.4

1 12 females per treatment.

2 Period equivalent to 28 days.

* Difference from 3.0% Ca treatment is significant at the $P < .05$ level.

** Difference from 3.0% Ca treatment is significant at the $P < .01$ level.

Table 18. Effects of DDE, DDT and calcium on egg weight and feed consumption during experiment 2.

Treatment[1]		Egg Weight[2] (g.)	Feed Consumption[2] (kg./bd/day)
0 ppm/3.0% Ca (control)		10.2	.019
100 ppm DDE/3.0% Ca		10.2	.019
300 ppm DDE/3.0% Ca		10.2	.018
100 ppm DDT/3.0% Ca		9.0	.020
	Av.	9.9	.019
0 ppm/0.5% Ca		10.5	.021
100 ppm DDE/0.5% Ca		10.7	.020
300 ppm DDE/0.5% Ca		10.0	.018
100 ppm DDT/0.5% Ca		9.6	.020
	Av.	10.2	.020

1 12 females per treatment.

2 Average for six 28-day production periods.

DDE and fed the lower calcium level. The latter produced eggs of significantly ($P < 0.01$) lower fertility by period 3 (Table 19). Birds treated with 300 ppm of DDE and fed the low calcium ration averaged 60 percent fertility for the four production periods as compared to 98 percent fertility for the controls.

When like calcium diets were grouped regardless of pesticide treatment birds fed the low calcium diet produced eggs of significantly ($P< 0.05$) lower hatchability than birds fed the normal calcium diet. The former, not treated with any pesticide, averaged 42 percent hatchability of eggs while birds fed a control diet produced eggs that averaged 72 percent hatchability (Table 20). There were no significant ($P <0.05$) differences in hatchability from those birds fed the low calcium ration with no pesticide as compared with those fed the same calcium diet with pesticide added. However, birds fed the normal calcium level, treated with pesticide, tended to lay eggs with lower hatchability as compared to birds fed the control diet. An exception to this trend was those birds treated with 100 ppm of DDT. Livability of chicks fed a normal diet from parent stock which had been fed any of the treatments in experiment 2 was unaffected (Table 21).

In contrast to experiment 1, female mortality in experiment 2 was higher with the low calcium ration, 46 percent, as compared with birds fed the normal calcium ration, 38 percent, and no pesticide (Table 22).

Table 19. Effects of DDE, DDT and calcium on fertility of eggs in experiment 2.

Treatment[1]	Fertility (%) by periods[2]				
	a[3] 1	a[3] 2	a[3] 3	a[3] 4	Av.
0 ppm/3.0% Ca (control)	(72)99	(46)98	(26)96	(39)98	98
0 ppm/0.5% Ca	(40)82	(27)92	(24)95	(18)94	88
100 ppm DDE/3.0% Ca	(72)92	(50)86	(29)78	(45)92	87
100 ppm DDE/0.5% Ca	(51)92	(38)95	(31)84	(33)90	90
300 ppm DDE/3.0% Ca	(41)98	(24)88	(7)100	(6)100	96
300 ppm DDE/0.5% Ca	(30)77*	(24)76*	(16)50**	(11)39**	60*
100 ppm DDT/3.0% Ca	(45)98	(50)83	(35)95	(48)90	92
100 ppm DDT/0.5% Ca	(53)92	(47)85	(43)96	(51)62	84

1 12 females per treatment.

2 Period equivalent to 28 days.

3 Figures in parentheses refer to number of eggs set.

* Difference from control is significant at the $P < .05$ level.

** Difference from control is significant at the $P < .01$ level.

Table 20. Effects of DDE, DDT and calcium on hatchability of eggs in experiment 2.

Treatment[1]	Hatchability[2](%) by periods[3]				
	a[4] 1	a[4] 2	a[4] 3	a[4] 4	Av.
0 ppm/3.0% Ca (control)	(72)84	(46)84	(26)60	(39)60	72
0 ppm/0.5% Ca	(40)52	(27)38	(24)24	(18)52	42*
100 ppm DDE/3.0% Ca	(74)52	(38)54	(29)30	(45)53	48
100 ppm DDE/0.5 Ca	(51)64	(38)54	(31)46	(33)38	50
300 ppm DDE/3.0% Ca	(41)48	(24)53	(7)57	(6)67	56
300 ppm DDE/0.5% Ca	(30)30	(24)36	(16)50	(11)33	37*
100 ppm DDT/3.0% Ca	(45)63	(50)72	(35)74	(48)67	69
100 ppm DDT/0.5% Ca	(53)42	(47)24	(43)39	(51)34	35*

1 12 females per treatment.

2. Hatchability data based on all fertile eggs.

3 Period equivalent to 28 days.

4 Figures in parentheses refer to number of eggs set.

* Difference from control is significant at the $P < .05$ level.

Table 21. Livability of chicks fed a control diet from parent stock treated with pesticides and two calcium levels for 32 weeks.

Treatment[1]	No. Birds	Body Weight[2] (g./bird)	Mortality[3] (%)
0 ppm/3.0% Ca (control)	40	107	3.2
0 ppm/0.5% Ca	25	104	4.0
100 ppm DDE/3.0% Ca	35	103	5.3
100 ppm DDE/0.5% Ca	28	106	7.4
300 ppm DDE/3.0% Ca	12	104	4.8
300 ppm DDE/0.5% Ca	10	101	6.5
100 ppm DDT/3.0% Ca	36	106	2.3
100 ppm DDT/0.5% Ca	30	105	6.4

1 Treatment of parent stock for 32 weeks.

2 Average weight at 6 weeks of age; mixed sex.

3 Accumulative mortality from 0-6 weeks of age.

Table 22. Effects of DDE, DDT and calcium on mortality in experiment 2.

Treatment[1]	Male[2] Mortality	Female[2] Mortality
0 ppm/3.0% Ca (control)	0	38
0 ppm/0.5% Ca	0	46
100 ppm DDE/3.0% Ca	0	42
100 ppm DDE/0.5% Ca	16	84
300 ppm DDE/3.0% Ca	52	90
300 ppm DDE/0.5% Ca	70	70
100 ppm p, p'DDT/3.0% Ca	30	43
100 ppm p, p'DDT/0.5% Ca	24	43

1 12 males and 12 females per treatment.

2 Accumulative mortality up to 32 weeks of age.

Mortality caused by pesticide treatment was quite variable and apparently not associated with calcium treatment. Mortality was highest, 90 percent, among birds treated with 300 ppm of DDE and fed the normal calcium ration, however, it was nearly as high, 84 percent, among females treated with 100 ppm of DDE and fed the low calcium diet. DDT, at 100 ppm, did not seem to affect female mortality (Table 22).

Male mortality was highest with those birds treated with 300 ppm of DDE and averaged 70 and 52 percent mortality for the low and normal calcium diets, respectively. Males seemed to be more sensitive to DDT than to DDE toxicity at the same levels, averaging 27 percent mortality for the former and 8 percent mortality in the latter at 100 ppm of dietary treatment by period 5 (Table 22).

V. DISCUSSION

In attempting to understand how, or indeed if, DDT or other chlorinated organic residues in the environment could be responsible for the thin eggshell phenomenon, workers have been seriously handicapped by their inability to duplicate in the laboratory the degree of shell thinning found in the environment. Nevertheless, there are now many well-documented temporal and spatial inverse relationships between shell thickness and DDE content of the egg for the peregrine falcon (Cade et al., 1971), prairie falcon (Fyfe et al., 1969; Enderson and Berger, 1970; Fimreite et al., 1970), great blue heron (Vermeer and Reynolds, 1970), brown pelican (Risebrough et al., 1970; Blus et al., 1971, 1972) and the double-crested cormorant (Anderson et al., 1969), but not for the common tern, Sterna hirundo (Switzer et al., 1971). A positive correlation between egg content of pp'-DDE and shell thickness has been observed for the eggs of the moorhen, Gallinula chloropus (Fowler et al., 1971) thus demonstrating the importance of species variability.

In the present experiment egg weight and eggshell thickness, as determined by specific gravity measurements, were not affected by any of the pesticide treatments. Although the specific gravity of an entire egg and its relationship to shell thickness has been recognized for many years (Arscott and Bernier, 1961); it

was surprising that this rapid, nondestructive method was only referred to in one other experiment of this type (Hunt and Foster, 1972).

Risebrough et al. (1970) observed that gallinaceous species tend to be more resistant than birds in other orders to decreases in shell thickness after exposure to organochlorine compounds. Quail seem rather resistant with no changes in shell thickness being reported after treatment of 100 ppm of either pp'-DDT or pp'-DDE by Cecil et al. (1971) and small changes or changes only after severe treatment reported by Bitman et al. (1969), Stickel and Rhodes (1970), Peakall and Lincer (1970) and Tucker and Haegele (1970). Half the number of birds in the present experiment were stressed by a low calcium diet; however, no pesticide X calcium interaction was observed. In contrast to these experiments, Coturnix quail maintained for 21 days on a diet containing 225 ppm technical grade DDT layed eggs with shells of greatly reduced thickness both during and soon after treatment, the maximum thickness reduction for a shell being 60 percent (McFarland et al., 1971). In the latter experiment one might argue that the compound responsible for shell thinning is a component of technical grade DDT. Significant shell thinning was reported by Smith et al. (1970) after feeding 10 ppm of technical DDT to White Leghorn chickens for 2 months. In another experiment Sauter and Steele (1972) reported significant shell thinning in White Leghorn chickens after feeding 0.1 ppm technical DDT for ten weeks.

On the other hand, no egg shell thinning of White Leghorn chickens was found by Davison and Sell (1972) after feeding 200 ppm pp -DDT for 12 weeks or by Cecil et al. (1972) after feeding 50 ppm of either pp'-DDT, op'-DDT or pp'DDE for 28 weeks followed by 300 ppm for 12 weeks. However, the trend isn't consistent and others have reported no effect on egg shell thickness when feeding White Leghorn hens 20 ppm of technical grade DDT for 15 weeks (Hunt and Foster, 1972). Further work is needed before one can ascribe potent shell thinning properties to a minor component of technical DDT.

Stephen et al. (1970) fed chickens a mixture of 20 ppm pp'-DDT plus 20 ppm pp'-DDE in the diet and considered that any decreases observed in percentage shell calcium were due to decreased calcium consumption, rather than to a direct effect of the treatment. However, apart from Stephen et al. (1970), reports of decreased feed consumption of DDT-contaminated diets have been apparent only when the DDT level was high. No rejection was reported at 20, 250 or 300 ppm, respectively, when fed to chickens (Hunt and Foster, 1972; Noakes and Benfield, 1965; Lillie et al., 1972), at 400 ppm by pheasants (Genelly and Rudd, 1956) or at 500 ppm by bobwhite quail (Linduska and Springer, 1951), but a diet containing 700 ppm of DDT was rejected by Coturnix quail (Cross et al., 1962). In many of the experiments which reported shell thinning after administering the insecticide in the diet feed consumption data were not given. Cooke

(1973) illustrated the effect of witholding food on the shell thickness of eggs from chickens. He found that the mean shell thickness decreased 16 percent after the birds were fasted 24 hours and 22 percent after a 48 hour fast. In the present experiment feed consumption was not affected by any of the pesticide treatments.

Reduced food intake cannot account for the great majority of observed decreases in shell thickness. Field data indicates that birds in the order Falconiformes are very sensitive to DDE contamination and eggshell thinning. Limited studies by which falcons have been deliberately exposed to insecticides seem to substantiate the field data (Porter and Wiemeyer, 1969; Enderson and Berger, 1970; Wiemeyer and Porter, 1970). Coturnix quail eggs in the present experiment averaged 263 ppm total residue of DDE (Appendix Table 1) and experienced no shell thinning; whereas eggs of the American sparrowhawk, Falco sparverius, contained only 32 ppm of DDE and had shells ten percent thinner than controls (Wiemeyer and Porter, 1970). Other species that have demonstrated shell sensitivity to DDT and its metabolites are the ring doves in the order Columbaforms (Haegele and Hudson, 1973; Peakall, 1970), mallard ducks in the order Anseriformes (Lehner and Egbert, 1969; Heath et al., 1969; Longcore et al., 1971) and recently a single study using the screech owl, Otus asio, in the order Styrigiformes showed a high shell sensitivity with 13 percent thinning for those birds treated with a diet

containing only 10 ppm of DDE (McLane and Hall, 1972).

Incidence of decreased shell thickness and increased shell breakage in the field has been reported by Ratcliffe (1967, 1970), by Hickey and Anderson (1968), by Berger et al. (1970), Keith et al. (1970) and by Risebrough et al. (1970). If the shell is thin it is more likely to be cracked when laid (McNally, 1965). When captive ducks were exposed to DDE (Heath et al., 1969; Longcore et al., 1971) shell thickness was reduced and there was an increased incidence of cracked shells. Bitman et al. (1969) found that quail treated with pp'-DDT and a low calcium diet produced more broken eggs, 15.6 percent as against 11.1 percent in the control group (low calcium without addition of DDT). It was noted that in each group certain quail were responsible for most of the broken eggs In the present experiment birds treated with the low calcium diet produced eggs with shells that were significantly ($P < 0.01$) thinner with a higher incidence of cracks. Although the overall means were not significant between birds treated with pesticide in the low calcium diet and birds fed the low calcium diet without the addition of pesticide, there was a trend for those birds treated with 100 ppm of DDE (exper. 1) and 100 ppm of DDT (exper. 2) to produce the highest number of cracked eggs. As noted by Bitman et al. (1969) certain quail seemed more susceptible than others within each group to egg breakage. Due to the design of the pens with slanted floors and egg traps the percentage of cracked eggs recorded in the

present experiment was probably less than would have been recorded if the eggs were left within the cages.

In the present study hatchability of fertile eggs was significantly ($P < 0.05$) lower in those birds fed the low calcium diet as compared with the controls, irrespective of pesticide treatment. This result seemed in conflict with Romanoff and Romanoff (1949) who stated that thin-shelled eggs of low specific gravity apparently contain enough calcium to satisfy the needs of the developing embryo and generally have normal hatchability. Specific gravity units were not given so possibly the calcium content of the eggshells in the present study was below that needed for normal development. Possibly the Coturnix quail has a higher calcium requirement than White Leghorn chickens. Nelson et al. (1964) reported higher hatchability when levels of 2.5 to 3.0% calcium were used in the diets of Coturnix quail.

Statistically there were no significant ($P < 0.05$) differences in hatchability of fertile eggs from those birds fed the low or normal calcium diets without pesticide addition as compared with those birds treated with pesticide and given the same calcium diet. It should be noted, however, that chicks from parents fed the normal calcium diet and treated with 300 ppm of DDE were observed to have difficulty in hatching out from the shell and birds fed this diet tended to have lower hatchability as compared to controls. This is in agreement with Stickel and Rhodes (1970) who artifically incubated eggs from Coturnix

quail treated with DDT and found reduced hatchability for eggs from the highest dosed group but the difference was not significant. Hatchability of quail was significantly affected at 200 ppm DDT (Dewitt, 1955) and was reduced to zero at 500 ppm DDT (Cross et al., 1962) Reduced hatchability from eggs with thin but sound shells was not due to parental breakage because eggs were all artificially incubated. Mortality would be expected, as observed in the present experiment, mainly during the latter stages of incubation due to the increased rate of absorption of the yolk with pesticide residues. Possibly difficulty in pipping was caused by a release of the stored pesticide into the chicks system due to the physical exertion of escaping from the shell.

Livability of chicks from parents fed any of the diets in the present study was normal when these chicks were brooded under ideal artificial means and fed a control diet. It was noted, however, that if by accident stress was introduced by way of extreme temperatures or by feed withdrawal for any length of time during the first few days of life, generally some chicks would die from pesticide treated parents, whereas chicks from controls would not. Poonacha et al. (1973) noted in their studies that when Coturnix quail were exposed to either starvation or to lower temperatures, the DDT residues stored in the body fat were thought to be released into various other tissues such as liver, brain, kidney, etc. causing toxicity. Haegele and Hudson (1973) criticized the typical pesticide

reproductive studies with precocial species because of the use of mechanical incubators for incubation of eggs and mechanical brooders for survival of hatched young. They stated that such investigations do not take into account the full role of the adults. For successful nesting of wild birds adult behavior is of great importance in the incubation of eggs and survival of the young. On the other hand, Cooke (1973) stated that data on artificial incubation of eggs with thin shells in pesticide studies are valuable because such eggs are no longer vulnerable to fits of aberrant behavior by the adult bird. Possibly by using a pilot animal that has genetically retained broodiness and combining both natural and artificial incubation of eggs and rearing of young one could measure the degree the parental behavior affects successful nesting.

The LC_{50} of pp'-DDT and pp'-DDE for young Coturnix quail is 400 to 700 ppm and 1,200 to 1,400 ppm respectively when fed treated feed for five days followed by untreated feed for three days (Heath et al., 1969). In the present long term experiments the effect of sublethal doses of pesticide was complicated by other types of stress and mechanical injury causing death which could not be attributed directly to the pesticide treatment. In experiment 1 the females fed the control diet experienced much higher mortality than those birds fed the low calcium diet. Death was attributed primarily to prolapse of the uterus during egg laying. Birds fed the low calcium

diet, causing them to lay eggs with thinner shells, rarely experienced this type of mortality. However, in experiment 2 this difference due to calcium treatment was not observed. Also, females in experiment 1 fed the low calcium diet had increased body weight gains as compared to controls irrespective of pesticide treatment. The former tended to lay fewer eggs and body weight gain was caused primarily by an increase of adipose tissue. Again, females in the second experiment did not show this increase of body weight due to calcium treatment.

Females experienced an increased mortality in both experiments when treated with the higher levels of pesticide for a prolonged period of time. Mortality was quite variable, however, within each pesticide treatment and was not consistently related to either the normal or low calcium diets. A few birds seemed particularly resistant to pesticide toxicity within each group and upon termination of the experiments those survivors contained large amounts of visible body fat whereas most of those dying during dosage contained little or none. Chemical analysis of the adipose tissue from the heavy resistant birds and the thin, susceptible ones showed 3,800 ppm and 7,500 ppm of DDE, respectively, demonstrating a diluting effect in the former. Similar results of DDT poisoning were reported for Coturnix quail, starlings, and cowbirds, (Gish and Chura, 1970; Harvey, 1967; Stickel and Stickel, 1969).

Males in experiment 1 had a high mortality (88 percent) when treated with 100 ppm of DDE. All males in experiment 1 were fed the low calcium ration which was considered adequate (Arscott, 1969). On the other hand, males in experiment 2 fed the same diet experienced only 16 percent mortality. The difference in mortality of males with the same pesticide level was probably due to the difference in the two experimental designs. In experiment 1, males were housed together, 20 birds per pen, whereas in experiment 2 six males were housed with six females. Marsh (1971) stated that male Coturnix quail, if separated from females, will continue attempts to breed with the resulting agitation. The former plus the higher density of birds per pen in experiment 1 may have caused the higher mortality observed even amongst the controls.

Males in experiment 1 were more sensitive to DDE toxicity than females fed the same level. Eggs have been shown to be a mechanism for ridding the body of chlorinated hydrocarbon residue (Azevedo et al., 1965; Genelly and Rudd, 1956; Wurster et al., 1965). Cecil et al. (1972) working with White Leghorn chickens found that 34 percent of the daily intake of pp'-DDT and 42 percent of pp'-DDE was excreted in the eggs whereas only very small amounts of pesticide residue was found in the feces. In the present experiment (experiment 1) during onset of lay (six weeks of age) eggs contained an average of 255 ppm of DDE (Appendix Table 1).

The egg-laying capability of the females may have been a factor in reducing their mortality due to pesticide toxicity. As observed with the females, certain males seemed quite resistant to pesticide toxicity within each treatment and most of these resistant birds had a higher accumulation of adipose tissue as compared to the controls (Figure 10). This accumulation of fat could possibly be caused by the effect of DDT and DDE on the thyroid gland of birds. Jefferies and French (1971) found that pigeons fed low dose rates of DDT were in a hyperthyroidal condition, whereas those fed high dose rates were in a hypothyroidal state. Glick (1972) observed that feeding 500 ppm of DDT for five weeks to White Leghorn males significantly increased liver weights and depressed comb size. Coturnix quail when treated with 150 ppm of DDE were found to be in a hypothyroidal condition with significantly increased weights of the thyroid glands; however, the histological picture of the glands was rather variable from bird to bird (Richert and Prahlad, 1972).

Thyroidectomy in chickens causes severe effects on growth and reproduction (Sturkie, 1965) and also shell thinning (Taylor and Burmester, 1940). Undoubtedly changes in the thyroids of the birds exposed to pesticide treatments in the present experiments were responsible for some of the variability on frequently observed sub-lethal effects, such as egg production, body weight, fertility and mortality.

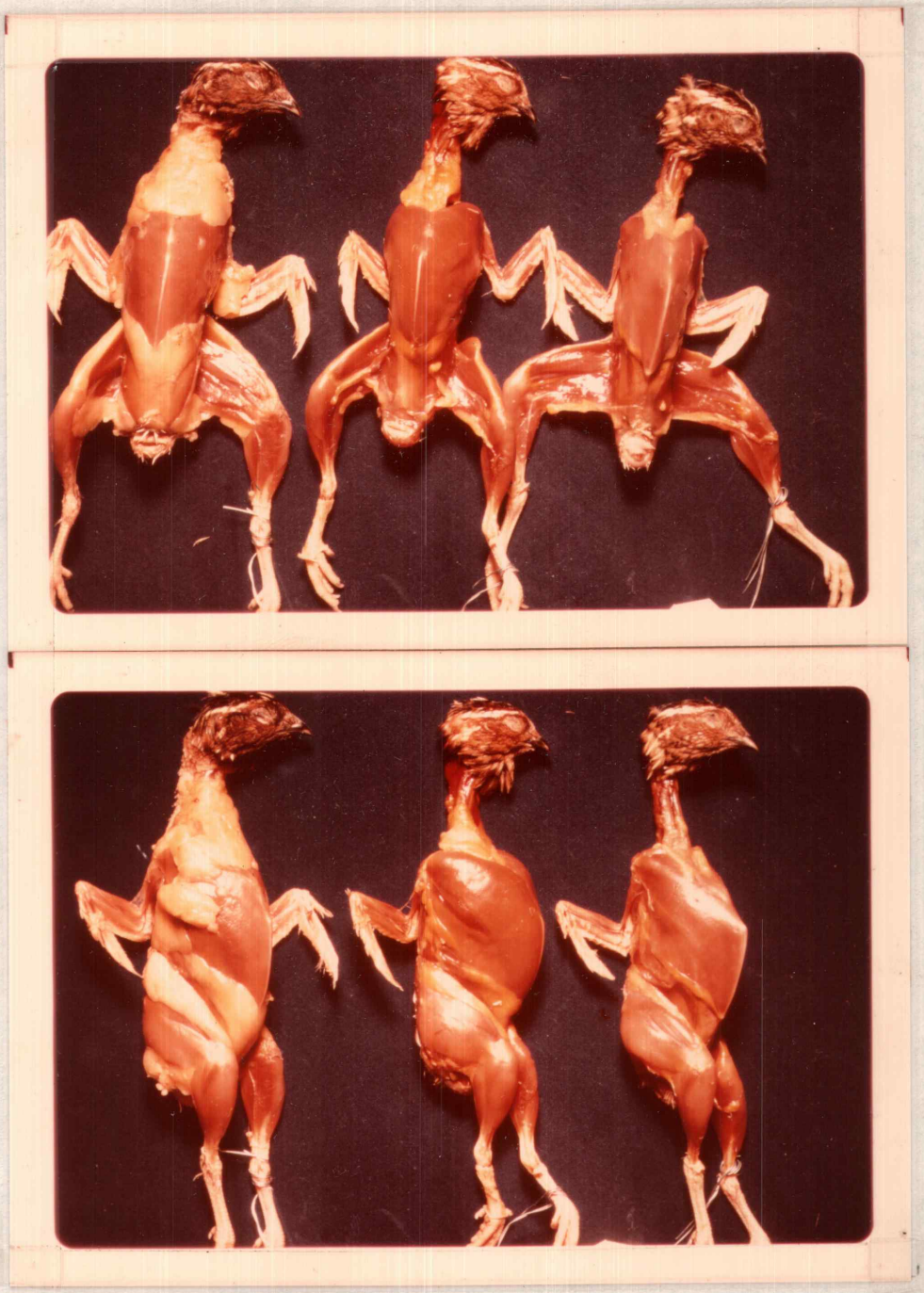

Figure 10. Effect of DDE on adipose tissue distribution of Coturnix quail. Birds on the right and the left were treated with 100 ppm of DDE for 42 weeks. Bird in center was fed the control diet.

In the present study fertility data was incomplete for experiment 1 due to the experimental design where males were kept separate from females. Males were introduced into female pens for various lengths of time but the resulting data was inconsistent probably due to the aging effects of sperm. Sittman and Abplanalp (1965) found that the duration of fertility in Coturnix quail appears to be slightly shorter than ducks and only about one-half and one-fourth, respectively, of the persistence of fertility in chickens and turkeys. Fertility data was collected in experiment 2 where males were housed with females at all times. Results indicate a trend towards reduced fertility of eggs from those birds treated with 300 ppm of DDE and fed the low calcium diet. This reduction probably was not caused by the direct action of DDE on the reproductive organs but as a secondary effect of the loss of body weight. Arscott et al. (1972) found that feeding White Leghorn males 100 ppm of pp'-DDT and 100-200 ppm of p'p'-DDE showed no significant effects on their reproductive performance, as characterized by semen volume, packed cell volume, fertility and hatchability. However it was noted that males fed 100 ppm of p, p'-DDT had a significant reduction in body weight. This reduction in body weight was accompanied by a drop in semen volume. Parker and Arscott (1964) have stated that whenever White Leghorn cockerels lose from 11 to 16 percent in body weight semen production increases. Essentially similar results have been noted with

Rhode Island Red males following feed restriction (Parker and McSpadden, 1941). Thus the decrease noted in fertility of eggs in the present study could possibly be attributed to the substantial loss in male body weight in those birds fed the higher pesticide contaminated diets.

Cooke (1973) stated that from North America a flood of papers has recently demonstrated inverse relationships between shell thickness and DDE content of the eggs of primarily Falconiformes and that some of these workers have confidently presented significant correlation coefficients despite the values for egg residues clearly not being normally distributed. He further stated that sometimes researchers studying thin shells have perhaps not been sufficiently cautious or critical about new evidence and that much field evidence blaming organochlorines is circumstantial, however, even in these instances the general trend that highly contaminated eggs have thin shells is apparent.

It is strange that very few of the field researchers make any mention of their possible interference on the reproductive cycle of raptorials. Falconiformes are not at all tolerant of disturbance during their nesting cycle. Any studies that involve repeated visits to the nesting ledge each year, from egg-laying to fledging (and this for several years in succession) will in themselves result in declines of the segment of the population so studied (Beebe, 1970). Cade

when studying peregrine falcons on the Colville River in Alaska stated that if he made a second survey trip down the river, the nest failures on the upper part of the river that he visited first, when the birds had eggs, proved to be much higher than the nest failures that occurred lower on the river where he visited eyries that had young (Hickey, 1969). Others have stated that close observation of reproductive cycles of raptorials have caused, at least in some instances, reproductive failures (Craighead and Craighead, 1969; Reynolds, 1970; Seidensticker and Reynolds, 1971). It would seem advisable, therefore, that amongst the flood of research projects that are carried out upon supposedly endangered North American raptorials that the researcher would be aware of the implications of his presence upon the breeding behavior of the bird in question and not focus all his attentions upon finding the highest level of DDE contamination.

VI. SUMMARY AND CONCLUSION

The objectives of this research were to investigate the effects of moderate to high levels of p, p'-DDE and p, p -DDT on the performance of Coturnix quail fed a normal and calcium deficient diet and to further assess the value of this species as a suitable pilot animal in pesticide research.

The following conclusions were drawn from this study:

1. DDE intake during the developing period only did not appear to affect the reproductive performance of adult Coturnix quail.
2. Egg shell thickness, as measured by specific gravity, and number of cracked eggs was not significantly affected by any of the pesticide treatments.
3. Egg shells from quail fed the lower calcium level were markedly thinner and contained more cracks.
4. There was no pesticide X calcium interaction in relation to egg shell thinning.
5. In general, egg production was quite variable and was not significantly affected by any of the treatments but it was reduced by the lower calcium diet and by the highest DDE treatment (300 ppm).
6. Onset of lay, egg weight and feed consumption were not affected by either pesticide or calcium treatments.

7. Male and female birds tended to weigh more when fed the lower calcium diet as compared with the controls.
8. Both sexes gradually lost body weight when treated with DDE for a prolonged period of time; males to a greater degree than females. However, a few birds within each pesticide treatment gained weight in the form of excess body fat.
9. Female mortality was normally quite high when fed a control diet and was attributed to prolapse of the uterus. The low calcium diet tended to have some sparing effect upon the incidence of prolapse.
10. Male and female mortality was increased by the long-term pesticide treatments and in general, males seemed more sensitive to pesticide toxicity than females.
11. Male fertilizing capacity tended to be reduced by birds treated with 300 ppm of DDE due to the loss of body weight.
12. Hatchability of fertile eggs was reduced by the lower calcium diet. Chicks from parents treated with 300 ppm of DDE were observed to have difficulty in pipping from the shell; however, a significant hatchability reduction due to pesticide treatment was not apparent.
13. Livability of hatched chicks was unaffected by either calcium or pesticide treatments when brooded under normal conditions.
14. Coturnix quail, a galliforme, seem unsuited as a pilot animal

in pesticide research where one would primarily attempt to observe egg shell thinning caused by DDT. However, other metabolic studies such as the effect of DDT upon the thyroid gland, resistance within treatment and the relationship between stress and susceptibility to pesticide toxicity of Coturnix quail need to be further researched.

BIBLIOGRAPHY

Anderson, D. W., and J. J Hickey. 1969. Significance of chlorinated hydrocarbon residues to breeding pelicans and cormorants, The Canadian Field-Naturalist 83:91-112.

Arscott, G. H. 1969. Personal communication, Department of Poultry Science, Oregon State University, Corvallis, Oregon.

Arscott, G. H., and P. E. Bernier. 1961. Application of specific gravity to the determination of eggshell thickness, Agricultural Science, No. 2, School of Agriculture, Oregon State University, Corvallis, Oregon.

Arscott, G. H., W. A. Robson, and I. J. Tinsley. 1972. Effect of DDE and DDT on reproductive performance of adult White Leghorn male chickens, Nutritional Reports International 6:307-311.

Azevedo, J. A., Jr., E. G. Hunt, and L. A. Woods, Jr. 1965. Physiological effects of DDT on pheasants, California Fish and Game 51:276-293.

Beebe, F. L. 1971. The myth of the vanishing peregrine. A study in the techniques of the manipulation of public and official attitudes. 7619 East Saawich Road, Box 37, Saawichton, British Columbia, Canada.

Berger, D. D., D. W. Anderson, J. D. Weaver, and R. W. Risebrough. 1970. Shell thinning in eggs of Ungava peregrines, The Canadian Field-Naturalist 84:265-267.

Bitman, J. 1969. Hormonal and enzymatic activity of DDT, Agricultural Science Review 7:6-12.

Blus, Lawrence J., Robert G. Heath, Charles D. Gish, Andre A. Belisle, and Richard M. Prouty. 1971. Eggshell thinning in the brown pelican: implication of DDE, Bioscience 21:1213-1215.

Blus, Lawrence J., Charles D. Gish, Andre A. Belisle, and Richard M. Prouty. 1972. Logarithmic relationship of DDE residues to eggshell thinning, Nature 235:376-377.

Brown, A. W. A. 1951. The chlorinated hydrocarbons - DDT, Insect control by chemicals, New York: John Wiley and Sons, Inc.

Burlington, H. and V. F. Lindeman, 1950. Effect of DDT on testes and secondary sex characters of White Leghorn cockerels. Proceedings of the Society of Experimental Biology, New York, 74:48-51.

Cade, T. J., C. M. White, and J. R. Haugh. 1968. Peregrines and pesticides in Alaska, The Condor 70:170-178.

Cade, T. J., J. L. Lincer, C. M. White, D G. Roseneau, and L. G. Swartz. 1971. DDE residues and eggshell changes in Alaskan falcons and hawks, Science 172:955-957.

Cameron, G. R. and F. Burgress. 1945. The toxicity of 2, 2-bis (p-Chlorphenyl) 1, 1, 1-Trichlorethane (DDT), British Medical Journal 1:865-871.

Cecil, H. C., J. Bitman, and S. J. Harris. 1971. Effects of Dietary p, p'-DDT and p, p'-DDE on egg production and eggshell characteristics of Japanese quail receiving an adequate calcium diet, Poultry Science 50:657-659.

Cecil, Helene C., George F. Fries, Joel Bitman, Susan J. Harris, R. J. Lillie, and C. A. Denton. 1972. Dietary p, p'-DDT, o, p'-DDT or p, p'-DDE and changes in eggshell characteristics and pesticides accumulation in egg contents and body fat of caged White Leghorns, Poultry Science 51:130-138.

Cooke, A. S. 1973. Shell thinning in avian eggs by environmental pollutants, Environmental Pollution 4:85-152.

Craighead, J. J., and F. C Craighead, Jr 1969. Hawks, owls, and wildlife, New York: Dover Publications, Inc.

Cross, D. L., H. L. King, and D. L. Haynes. 1962. The effects of DDTin the diet of Japanese quail, Quarterly Bulletin Michigan State University Agricultural Experiment Station 44:688-696.

Davison, K. L., and J. L. Sell. 1972. Dieldrin and p, p'-DDT effects on egg production and eggshell thickness of chickens, Bulletin of Environmental Contamination and Toxicology 7:9-18.

DeWitt, J. B. 1955. Effects of chlorinated hydrocarbon insecticides upon quail and pheasants, Agricultural and Food Chemistry 3: 672-676.

DeWitt, J. B 1956. Chronic toxicity to quail and pheasants of some chlorinated insecticides, Agricultural and Food Chemistry 4: 863-866.

Enderson, J. H. and D. D. Berger. 1968. Chlorinated hydrocarbon residues in peregrines and their prey species from Northern Canada. The Condor 70:149-153.

Enderson, J. H., and D. D. Berger. 1970. Pesticides: eggshell thinning and lowered production of young in prairie falcons, BioScience 20:355-356.

Ernst, R. A. 1967. The physiological effects of selected pesticides on the Japanese quail (Coturnix coturnix japonica) and embryogenesis of the domestic fowl, Dissertation Abstracts 27:2936B.

Fimreite, N., R. W. Fyfe, and J. A. Keith. 1970. Mercury contamination of Canadian prairie seed-eaters and their avian predators, Canadian Field Naturalist 84:269-276.

Fowler, J. F., L. D. Newsom, J. B. Graves, F. L. Bonner, and P. E. Schilling. 1971. Effect of dieldrin on egg hatchability, chick survival and egg-shell thickness in purple and common gallinules, Bulletin Environmental Contamination and Toxicology 6:495-501.

Fyfe, R. W., J. Campbell, B. Hayson, and K. Hodson. 1969. Regional population decline and organochlorine insecticides in Canadian prairie falcons, Canadian Field Naturalist 83:191-200.

Genelly, R. E., and R. L. Rudd. 1956. Effects of DDT, toxaphene and dieldrin on pheasant reproduction, Auk 73:529-539.

Gill, J. A , B. J. Verts, and A G. Christensen. 1970. Toxicities of DDE and some other analogs of DDT to pheasants, Journal of Wildlife Management 34:223-226.

Gish, C. D., and N. J. Chura. 1970. Toxicity of DDT to Japanese quail as influenced by body weight, breeding condition, and sex, Toxicology and Applied Pharmacology 17:740-751.

Glick, B. 1972. The immunobiological influence of Mirex and DDT, Poultry Science 51:1861.

Gunther, Francis A. 1945. Dichlorodiphenyltrichloroethane. I. Solubility in various solvents, Journal of American Chemical Society 67:189-190.

Haegele, M. A., and Rick H. Hudson. 1973. DDE effects on reproduction of ring doves, Environmental Pollution 4:53-57.

Harvey, J. M. 1967. Excretion of DDT by migrating birds, Canadian Journal of Zoology 45:629-634.

Heath, R. G., J. W. Spann, and J. F. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies, Nature 224:47-48.

Hickey, J. J. 1969. Peregrine falcon populations: their biology and decline, Madison, Milwaukee, and London: University of Wisconsin Press, p. 421.

Hickey, J. J., and D. W. Anderson. 1969. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds, Science 162:271-273.

Hill, K. R., and G. Robinson. 1945. A fatal case of DDT poisoning in a child, British Medical Journal, pp. 845-847.

Howes, J. R. 1964. Environmental factors affecting ovulation in Coturnix quail, Journal of the Alabama Academy of Science 35: 20-21.

Hunt, E. G., J. A. Azevedo, L. A. Woods, and W. T. Castle. 1968. The significance of residues in pheasant tissues resulting from chronic exposure to DDT. In: Chemical Fallout:4-6. Morton W. Miller, Springfield.

Hunt, J. R., and T. S. Foster. 1972. Effects of dietary chlorinated pesticides on shell quality, Poultry Science 51:1861.

Jefferies, D. J. 1967. The delay in ovulation produced by p, p'-DDT and its possible significance in the field, Ibis 109:266-272.

Jefferies, D. J., and M. C. French. 1971. Hyper- and hypothyroidism in pigeons fed DDT: an explanation for the thin eggshell phenomenon, Environmental Pollution 1:235-242.

Jones, F. J. S., and D. D. B. Summers. 1968. Relation between DDT in diets of laying birds and viability of their eggs, Nature 217:1162-1163.

Keith, J. O., L. A. Woods, and E. G. Hunt. 1970. Reproductive failure in brown pelicans on the Pacific Coast, Transcript North American Wildlife Conference 35:56-64.

Lehner, P. N., and A. Egbert. 1969. Dieldrin and eggshell thickness in ducks, Science, New York, 224:1218-1219.

Lillie, R. J., C. A. Denton, H. C. Cecil, J. Bitman, and G. F. Fries. 1972. Effect of p, p'-DDT, o, p'-DDT and p,p'-DDE on the reproductive performance of caged White Leghorns, Poultry Science 51:122-129.

Linduska, J. P., and P. F. Springer. 1951. Chronic toxicity of some new insecticides to bobwhite quail, U. S. Dept. Interior, Spec. Sci. Rep. Wildlife 9 (Mimeograph)

Longcore, J. R., F. B. Samson, and T. W. Whittendale. 1971. DDE thins eggshells and lowers reproductive success of captive black ducks, Bulletin Environmental Contamination and Toxicology 6:485-490.

Marsh, A. F. 1971. Quail Manual, Garden Grove, California: Marsh Farms.

McFarland, L. Z., R. L. Garrett, and J. A. Nowell. 1971. Normal eggshells and thin eggshells caused by organochlorine insecticides viewed by the scanning electron microscope, Proceedings of Annual Scanning Electron Microscope Symposium 4:377-384.

McLane, M. A. R., and L. C. Hall. 1972. DDE thins screech owl eggshells, Bulletin of Environmental Contamination and Toxicology 8:65-68.

McNally, E. H. 1965. The relationship of eggshell weight to cracked eggs, Poultry Science 44:1513-1518.

Metcalf, R. L. 1955. Organic Insecticides. Their chemistry and mode of action, New York: Interscience Publishers, Inc.

Mrak, E. M. 1960. Report of agricultural chemicals and recommendations for public policy, Governor Edmund G. Brown's Special Committee on Public Policy Regarding Agricultural Chemicals. Sacramento, California.

Nelson, F. E., J. K. Lauber, and L. Mirash. 1964. Calcium and phosphorus requirements for the breeding Coturnix quail, Poultry Science 43:1346.

Noakes, D. N., and C. A. Benfield. 1965. Tissue accumulation of DDT and its metabolites in the domestic fowl, Journal of Science Food Agriculture 16:693-697.

O'Brien, R. D. 1967. Insecticides: Action and metabolism, New York: Academic Press, Inc.

Parker, J. E., and B. J. McSpadden. 1941. Factors influencing fertility in domestic fowls, Proceedings Association of Southern Agricultural Workers 42:214.

Parker, J. E., and G. H. Arscott. 1964. Energy intake and fertility of male chickens, Journal of Nutrition 82:183-187.

Peakall, D. B. 1970. Pesticides and the reproduction of birds, Scientific American 222:72-78.

Peakall, D. B. 1970. p, p'-DDT: effect on calcium metabolism and concentration of estradiol in the blood, Science 168:592-594.

Peakall, D. B., and J. L. Lincer. 1970. Effect of chlorinated hydrocarbons on active transport of calcium in the avian oviduct, American Zoologist 10:515.

Poonacha, K. B., B. C. Wentworth and A. B. Chapman. 1973. Genetic resistance to DDT in the Japanese quail. Coturnix coturnix japonica. Poultry Science 52:841-846.

Porter, R. D., and S. N. Wiemeyer. 1969. Dieldrin and DDT: effects on sparrow hawk eggshells and reproduction, Science 165:199-200.

Poulson, T. L., and W. B. White. 1969. The cave environment, Science 165:971-981.

Ratcliffe, D. A. 1967. Decrease in eggshell weight in certain birds of prey, Nature 215:208-210.

Ratcliffe, D. A. 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds, Journal of Applied Ecology 7:67-115.

Reynolds, R. T. 1970. Personal communication, Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.

Richert, E. P., and K. V. Prahlad. 1972. Effects of DDT and its metabolites on thyroid of the Japanese quail, Coturnix coturnix japonica, Poultry Science 51:196-200.

Risebrough, R. W., J. Davis, and D. W. Anderson. 1970. Effects of various chlorinated hydrocarbons, Oregon State University Environmental Health Science, Series No. 1, pp. 40-53.

Romanoff, A. L., and A. J. Romanoff. 1949. The avian egg, New York: John Wiley and Sons, Inc., London: Chapman and Hall, Limited.

Rudd, R. L. 1964. Pesticides and the living landscape, the University of Wisconsin Press.

Sauter, E. A., and E. E. Steele. 1972. The effect of low level pesticide feeding on the fertility and hatchability of chicken eggs, Poultry Science 51:71-76.

Seidensticker, J. C., and H. V. Reynolds. 1971. The Wilson Bulletin 83:408-417.

Sittman, D., and H. Abplanalp. 1965. Duration and recovery of fertility in Japanese quail (Coturnix coturnix japonica), British Poultry Science 6:245-250.

Smith, S. I., C. W. Weber, and B. L. Reid. 1970. Dietary pesticides and contamination of yolks and abdominal fat of laying hens, Poultry Science 49:233-237.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Sixth edition. Ames, Iowa State University Press.

Stephen, B. J., J. D. Garlich, and F. E. Guthrie. 1970. Effect of DDT on induction of microsomal enzymes and deposition of calcium in the domestic chicken, Bulletin of Environmental Contamination and Toxicology 5:569-576.

Stickel, L. F., and W. H. Stickel. 1969. Distribution of DDT residues in tissues of birds in relation to mortality, body condition, and time, Ind. Medical Surg. 38:44-53.

Stickel, L. F., and L. I. Rhodes. 1970. The thin eggshell problem, Oregon State University Environmental Health Science, Series No. 1, pp. 31-35.

Sturkie, P. D. 1965. Avian Physiology, London: Bailliere, Tindall and Cassell.

Switzer, B., and V. Lewis. 1971. Shell thickness, DDE levels in eggs, and reproductive success in common terns (Sterna hirundo), in Alberta, Canadian Journal of Zoology 49:69-73.

Taylor, L. W., and B. R. Burmester. 1940. Effect of thyroidectomy on production, quality, and composition of chicken eggs, Poultry Science 19:326-331.

Tucker, R. K., and H. A. Haegele. 1970. Eggshell thinning as influenced by method of DDT exposure, Bulletin of Environmental Contamination and Toxicology 5:191-194.

Tucker, R. K. and H. A. Haegele. 1971. Comparative acute oral toxicity of pesticides to six species of birds. Toxicology and Applied Pharmacology 20:57-65.

Vermeer, K., and L. M. Reynolds. 1970. Organochlorine residues in aquatic birds in the Canadian prairie provinces, Canadian Field Naturalist 84:117-130.

Weihe, M. 1967. Effects of DDT on reproduction in hens. Acta pharmacologia et toxicologia 25:54.

West, T. F., and G. A. Campbell. 1952. DDT and newer persistent insecticides, New York: Chemical Publishing Co., Inc.

Wiemeyer, S. N., and R. D. Porter. 1970. DDE thins eggshells of captive American kestrels, Nature 227:737-738.

Wurster, D. H., C. F. Wurster, Jr., and W. N. Strickland. 1965. Bird mortality following DDT spray for Dutch elm disease. Ecology 46:488-499.

APPENDIX

Appendix Table 1. Analysis[1] of feed, eggs, and whole birds for DDE (ppm) during experiment 1.

Period	Control			100 ppm of DDE		
	Feed	Eggs	Birds	Feed	Eggs	Birds
1	0.13	0.15	0.08	94.8	255.0	333.25
4	0.08	1.50	2.07	94.6	291.5	246.1
7	0.05	1.64	2.24	98.7	287.7	302.05

[1] Procedure:

Feeds - 10 gram samples extracted overnight in soxhlet apparatus with 1:1 acetone-hexane. High level samples diluted to gas chromatographic concentrations and analyzed, controls partitioned into hexane and diluted through florisil clean-up column and fractions collected for DDE.

Eggs - four eggs taken from sample and contents homogenized. One gram homogenized sample extracted in acetone, partitioned into hexane. High level samples diluted to gas chromatographic concentrations and analyzed, controls eluted through florisil clean-up column and fractions collected for DDE analysis.

Birds - Whole birds were extracted with 500 ml acetone, then with 300 ml acetone. High level samples diluted to gas chromatographic concentrations and analyzed. Aliquot taken of control extracts and partitioned into hexane and analyzed.

All pesticide analysis done on Electron Capture gas chromatograph.