

THE EFFECTS OF RESERPINE ON ARTIFICIALLY
INSEMINATED TURKEYS AND
SEMEN METABOLISM

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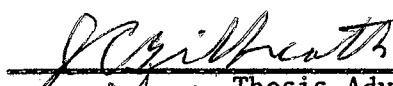
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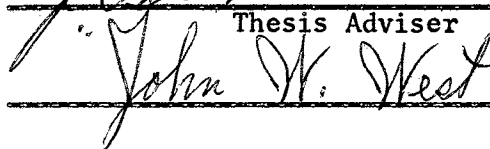
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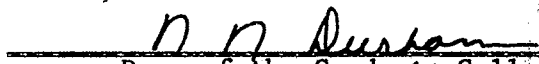
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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. GENERAL REVIEW OF LITERATURE	3
Tranquilizing Effects	3
Cardiovascular Effects	5
Control of Aortic Rupture	6
Body Weight and Feed Efficiency	9
III. GENERAL EXPERIMENTAL PROCEDURE	13
IV. PRESENTATION OF THE LITERATURE REVIEW, THE EXPERIMENTAL PROCEDURES, THE RESULTS AND SUMMARY ASSOCIATED WITH PHASE I AND PHASE II	18
Phase I. The Differential Effects of Reserpine on the Reproductive Performance of Breeder Turkeys Receiving Artificial Insemination	18
Review of Literature	19
Experimental Procedure	24
Results and Discussion	26
Summary	46
Phase II. The Effects of Reserpine on Metabolism of Whole Semen	49
Review of Literature	49
Experimental Procedure	52
Results and Discussion	56
Summary	61
V. GENERAL SUMMARY AND CONCLUSIONS	63
SELECTED BIBLIOGRAPHY	66

LIST OF TABLES

Table	Page
I. All-Mash Turkey Starter and Grower Ration	14
II. Turkey Breeder Ration	17
III. Female Body Weight	27
IV. Hen-Day Feed Consumption	30
V. Days to Sexual Maturity	33
VI. Analysis of Variance of Days to Sexual Maturity	33
VII. Percentage Egg Production	35
VIII. Percentage Fertility	37
IX. Percentage Hatch of Fertile Eggs	41
X. Percentage Hatch of Total Eggs Set	44
XI. The Effect of Reserpine (2.0 p.p.m.) on Sperm Counts in Turkey Semen	57
XII. The Effect of Reserpine Feeding (2.0 p.p.m.) on the Respi- ration of Turkey Spermatozoa After 1 and 2 Hours of Incubation at 40°C.	59
XIII. Total Microliters of Oxygen Consumed per 100 mg. of Testic- ular Tissue for Each Treatment at the End of Each Hour of Incubation	60

LIST OF FIGURES

Figure	Page
1. The Influence of Reserpine on Female Body Weight, Measured at 28-day Intervals	28
2. The Influence of Reserpine on Hen-Day Feed Consumption Measured at 28-day Intervals	31
3. The Influence of Reserpine on Percentage Egg Production, Measured at 28-day Intervals.	34
4. The Influence of Reserpine on Percentage Fertility, Measured at 14-day Intervals	38
5. The Influence of Reserpine on Percentage Hatch of Fertile Eggs, Measured at 14-day Intervals	42
6. The Influence of Reserpine on Percentage Hatch of Total Eggs Set, Measured at 14-day Intervals	45

CHAPTER I

INTRODUCTION

Within the past decade turkey producers have often suffered severe outbreaks of aortic rupture resulting in notable economic losses. In recent years a tranquilizer, reserpine, has been shown to be successful in the treatment and control of aortic rupture in turkeys.

Recent studies have reported a decline in the reproductive efficiency of turkeys fed reserpine. Jones et al. (1966) reported that reserpine produced a differential effect with respect to sex for fertility in naturally mated turkeys. In this study the reduction in fertility was greater in the female than in the male, although a reduction in fertility was observed in both sexes.

From these studies several new questions have arisen. Is the greater reduction in fertility of the female due to a decline in female libido produced by the tranquilizing effects of reserpine? Does reserpine alter the fertilizing ability of the turkey spermatozoa? This study was designed to investigate these questions.

In commercial turkey breeder operations artificial insemination has been substituted for natural mating. As a result, the low fertility which has often been observed in many naturally mated flocks has been partly alleviated. If artificial insemination were substituted for natural mating in studies of the effects of reserpine on reproductive performance, several advantages might be gained. First, any sociological

interactions between males and females during natural mating could possibly be excluded. Secondly, possible deviations in libido due to the effects of reserpine during natural mating could be excluded.

The studies reported in this thesis were designed to investigate the effect of reserpine on reproductive efficiency using artificial insemination and the effect of reserpine on the fertilizing ability of turkey sperm.

The results of this investigation will be presented in two different phases. Phase I will present the results of an experiment designed to examine the effects of reserpine on the reproductive performance of artificially inseminated breeder turkeys. Phase II will present the results of an experiment designed to investigate some effects of reserpine on semen metabolism.

CHAPTER II

GENERAL REVIEW OF LITERATURE

Tranquilizing Effects

Leonard Rauwolf, a German physician and botanist, organized an expedition to Asia and Africa in the early sixteenth century to study medicinal plants which had been mentioned by early Greek and Arab physicians. According to Schlittler and MacPhillamy (1954), the results of this expedition were published in 1882, and when a new genus was added to the plant family, Apocynaceae, it was named Rauwolfia in honor of Rauwolf.

In the genus, Rauwolfia, there exist 40 to 50 species. Most important, because of its medicinal properties, is R. serpentina. Siddiqui and Siddiqui (1931) reportedly isolated crystalline alkaloids from the roots of R. serpentina. Later Mueller et al. (1952) isolated an alkaloid from the roots of R. serpentina with a pronounced hypotensive and sedative action and named it reserpine.

Earl (1956) described the physical characteristics of reserpine as a pale yellow powder readily soluble in acetic acid, ascorbic acid and citric acid. The empirical formula of reserpine is $C_{33}H_{40}O_9N_2$.

Plummer et al. (1954) reported that reserpine exerted a calming and sedative action in a wide variety of animal species. A moderate hypotensive effect was produced in unanesthetized dogs after a single intravenous injection of 250.0 to 300.0 μ gs. per kgm. of reserpine. An

oral dosage of 25.0 μ gs. per kgm. daily was successful in producing a mild sedation after five to seven days. All dogs were readily aroused and retained good motor coordination following sedative doses of reserpine.

Schneider and Earl (1954) concluded that single doses of reserpine of 0.5 to 1.0 mg. per kg. administered intravenously, and 25.0 to 30.0 mgs. per kg. administered orally, consistently produced a state of tranquilization in normally hostile, jungle born, rhesus monkeys. They noted that no involuntary sleep was produced, although treated monkeys could be handled without the usual methods of restraint. The authors noted that the initial drug actions appeared 30 to 40 minutes after oral or intravenous administration of the drug.

The beneficial effects of reserpine in rearing ring-necked pheasants were recognized by Hewitt (1957). Reserpine fed to two-week old pheasants at 5.0 and 7.0 mgs. per kg. of feed reduced both picking and scalping. Hewitt and Reynolds (1957) observed that reserpine fed at the rate of 5.0 gms. per ton of diet reduced fighting among cocks which were in breeding condition. Hewitt (1959) noted that 40.0 mgs. of reserpine per male bird administered orally on the day prior to shipment resulted in easier handling.

The state of tranquility of mature White Leghorn capons which had received reserpine intravenously was observed by Sturkie (1959). Single doses ranged from 0.006 to 0.750 mg. per kg. of body weight and observations were taken for a period of 24 hours. Based on observations of their behavior it was concluded that 0.1 to 2.0 mgs. per kg. of body weight of reserpine was a tranquilizing dose.

Weiss (1960a) noted that no overt tranquilization was produced in

Single Comb White Leghorn hens which had been administered reserpine at the rate of 2.0 mgs. per kg. of feed for as long as 32 weeks.

Carlson (1956) fed 18-week old turkeys reserpine at levels of 0.5 and 1.0 mg. per kg. of feed for eight weeks. These levels were effective in suppressing the birds' desire to fight.

Ringer (1959) noted that no signs of reduced activity were observed in Broad Breasted Bronze turkeys fed 1.0 and 4.0 p.p.m. of reserpine in the diet.

Cardiovascular Effects

Since the discovery of the tranquilizing capabilities of reserpine, there have been numerous investigations designed to assess the cardiovascular effects of reserpine on various animal species. This review will encompass studies primarily concerned with the present-day commercial turkey.

Plummer et al. (1954) reported a marked decline in blood pressure of dogs after injections of 250.0 to 300.0 mgs. per kg. of reserpine. Following intravenous doses of reserpine at the rate of 0.5 to 1.0 mg. per kg. of body weight, Schneider and Earl (1954) reported that rhesus monkeys exhibited a marked reduction in rectal temperature. Heart rate dropped slowly over a period of hours after oral and intravenous administration.

In a study with White Leghorn capons, Sturkie (1959) reported a positive linear correlation between decrease in heart rate and increasing levels of reserpine at 0.01, 0.10 and 1.0 mg. per kg. dosages. With respect to blood pressure there existed the same situation, except that the slope of regression was smaller.

Gonzaga and Catalia (1963) indicated that reserpine at the oral level of 1.0 gm. per kg. of feed did not influence significantly the blood sugar level of laying White Leghorn hens.

A slight but consistent depression in body temperature, respiration rate, heart rate and blood pressure due to the administration of reserpine to Single Comb White Leghorns was observed by Weiss (1960a). In this study oral levels up to 2.0 mgs. per kg. of feed were administered to hens in their first year of production. In another experiment Weiss (1961) fed reserpine at the level of 1.6 mgs. per kg. of diet to one-year old White Leghorn hens for 14 weeks. This treatment had essentially no effect on blood pressure, pulse rate or respiration rate, but did depress body temperature a significant 0.6°C .

Reserpine at the levels of 0.1, 0.2, 0.3 and 0.4 p.p.m. in the diet significantly reduced blood pressure in Broad Breasted Bronze turkeys, according to Ringer (1959). In this study systolic blood pressure of control birds reached 297 mm. of Hg. compared to 230 mm. of Hg. for birds administered 0.4 p.p.m. of reserpine. Reserpine at 0.1 and 0.2 p.p.m. effectively lowered blood pressure in both male and female Broad Breasted Bronze turkeys at 27 weeks of age, according to Ringer (1960). In this study no sex differences in blood pressure were observed.

Control of Aortic Rupture

Ringer (1959) described a dissecting aneurysm or aortic rupture in turkeys as a rupture of the abdominal aorta located along the vertebral column of the back. Aortic rupture results in massive internal hemorrhage, and observed symptoms are birds gasping for air with blood being expelled from the mouth.

Barnett (1960) concluded that there are probably two factors that are of importance in creating an outbreak of massive internal hemorrhage. First, there is an apparent degeneration of the arterial walls. Secondly, the unusually high blood pressure of individual toms causes rupture of these weakened arteries. He concluded that the two possible ways of reducing mortality from aortic rupture would be by eliminating arterial degeneration and by reducing blood pressure.

These findings and assumptions agree with those of Ringer (1959) who concluded that aortic rupture was due to stress and high blood pressure.

The primary approach toward attempting to eliminate or eradicate aortic rupture has been that of reducing blood pressure by the use of reserpine. Couch (1959) reported that a loss of 15 toms per day, due to aortic rupture, at sixteen weeks of age, was reduced to zero by reserpine administered at the level of 1.5 mgs. per lb. of diet.

Barnett (1960) produced aortic rupture in a lot of control turkeys using beta-aminopropionitrile. The incidence of this artificially induced rupture was significantly reduced by feeding 0.8 and 1.6 mgs. of reserpine per lb. of diet. In a separate trial, a flock of 3300 turkeys suffered losses of 60 birds during a ten-day period due to aortic rupture. This flock was then randomly divided into two groups and reserpine administered to one group at levels of 0.45 mg. per lb. of diet for five days and 0.09 mg. per lb. of diet for two weeks. During the two weeks that reserpine was fed at the lower level, losses returned to three or four turkeys per day.

In another field trial a flock of 8500 turkeys, eight weeks of age, experienced losses from aortic rupture of 38 turkeys during a ten-day

period prior to treatment. A flock of 4000 turkeys which had experienced a mortality rate of 19 birds during the same period was used as a control. Reserpine was fed to the 8500 turkeys at the rate of 0.45 mg. per lb. of diet for 5 days and 0.09 mg. per lb. thereafter. Mortality was immediately reduced to zero, and recurrent losses after drug reduction did not occur, while losses in the control group remained static.

Waibel (1960) studied beta-aminopropionitrile-induced aortic rupture in two laboratory experiments using Broad Breasted Bronze and Lancaster White turkeys ranging from 0-5 weeks of age. In the first experiment, 0.5 and 1.0 p.p.m. of reserpine were fed continuously. The higher level appeared to reduce the severity of aortic rupture. In the second experiment 2.2 p.p.m. was fed only during the period of hemorrhaging and, in this case, the severity of aortic rupture was also reduced.

Losses as high as 15 turkeys per day in a flock of 1800 male turkeys, 12 weeks of age, were decreased to zero 72 hours after feeding 0.2 p.p.m. of reserpine in their diet, according to Morrison (1960). After removing these birds from two weeks of medication, aortic rupture reappeared. Treatment at 0.5 p.p.m. brought these recurrent losses under control. Another flock of 1500 Broad Breasted Bronze males, 26 weeks of age, experiencing aortic rupture losses of two to three males per day, was treated with reserpine at a level of 1.0 p.p.m. Within 24 hours all losses due to aortic rupture ceased. It was suggested by the author that reserpine administered at a level of 1.0 p.p.m. in the diet would bring severe outbreaks of aortic rupture under control within 24 to 48 hours.

Patrias (1960), summarizing ten years of field observations,

concluded that the problem of aortic rupture was increasing each year. Treatment levels of reserpine at 1.0 p.p.m. administered in the diet for three to seven days had controlled outbreaks of aortic rupture. Levels of 0.2 p.p.m. appeared adequate to prevent aortic rupture.

Body Weight and Feed Efficiency

Eränkő et al. (1957) injected young male rats subcutaneously with 0.04 mg. of reserpine daily for two months. Controls were given the same volume of physiological saline. In this study reserpine caused a pronounced retardation of growth, which was statistically significant ($P < .001$).

Hewitt (1957) fed reserpine at the rate of 5.0 and 7.0 p.p.m. in the diet to two-week old pheasants held in battery brooders for a period of two weeks. The groups fed reserpine ate slightly less food but gained slightly more weight than the controls.

Carlson and Morgan (1958) observed that reserpine at the level of 2.0 mgs. per lb. of feed apparently caused a slight reduction in growth rate of pheasants through eight weeks of age. These differences were largely overcome by the time the pheasants reached twelve weeks of age. When reserpine was added to the control diet at the level of 4.0 mgs. per lb. of feed, the rate of growth of the eight-week old males was decreased.

Couch (1959) administered reserpine orally at levels of 0.25, 0.50 and 1.0 mg. per lb. of feed to laying pullets and noted no apparent differences in feed conversion as compared to controls over a 168-day period.

Gilbreath (1959) administered reserpine orally at 2.0 mgs. per kg.

of feed to DeKalb 101 hybrid females under naturally occurring stress conditions. Treatment began when pullets were seven months of age and egg production was approximately 70 percent. Differences in body weight were not significant. A significant difference ($P < .05$) in feed consumption adjusted for hen-days was noted, with treated birds yielding lower consumption values.

Weiss (1960a) studied the effect of continuous treatment of reserpine on body weight of Single Comb White Leghorn pullets. Oral levels of reserpine up to 2.0 mgs. per kg. of feed did not affect body weight when fed for 32 weeks to hens in their first year of egg production.

Weiss (1960b), in an experiment where 30 Single Comb White Leghorn females were fed 0.2 mg. of reserpine per bird per day and exposed to 95°F. and 60 percent relative humidity for six weeks, noted that body weight was significantly greater for treated birds.

Eoff et al. (1961) observed no significant effects on feed conversion in White Leghorn pullets administered reserpine at the rates of 0.25, 0.50 and 1.00 mg. per lb. of feed.

Weiss (1961) fed reserpine at the level of 1.6 mgs. per kg. of feed to one-year old White Leghorn females for 14 weeks. This treatment had no effect on body weight.

Single Comb White Leghorn and New Hampshire chicks were fed various levels of reserpine by Burger et al. (1959). Reserpine fed to Leghorn chicks at the rate of 0.5 mg. per kg. of diet produced a slight but significant increase in growth. Reserpine fed to Leghorn chicks at levels of 5.0 to 500.0 mgs. per kg. of diet depressed growth and at 500.0 mgs. per kg. caused 96 percent mortality by the 24th day of age.

Carlson (1956), in a study of the effects of reserpine on 18-week

old growing turkeys, reported a differential response by sexes. Reserpine at levels of 0.5 and 1.0 mg. per kg. of feed depressed growth rate. It appeared that 0.5 mg. per kg. had little effect on growth rate of toms, but 1.0 mg. per kg. was definitely detrimental to growth. Both levels of reserpine decreased feed efficiency for both sexes.

"Reserpine mother liquor", a crude Rauwolfia preparation from which 90 percent of the reserpine content had been extracted, was administered to Broad Breasted Bronze poults by Burger et al. (1959). This preparation at levels of 0.5 mg. per kg. of diet produced a slight increase in growth and at 10.0 mgs. per kg. of diet produced a significant increase in growth by the 38th day of age.

Carlson (1960), in a study of reserpine with laying hens, noted that reserpine could be used at a level of 1.1 p.p.m. in the diet for a four-week period without interfering with growth and feed conversion.

Ringer (1960) reported that reserpine at levels of 0.1 and 0.2 p.p.m. fed continuously from one day through 24 weeks, to both sexes, did not influence the growth of Broad Breasted Bronze turkeys.

Anderson and Smyth (1960) studied the effects of continuous low level feeding of reserpine on the growth of large white turkeys. Growth and feed conversion at three and six weeks of age were significantly ($P < .01$) depressed at the 0.25 mg. per kg. level of reserpine. Increasing the dosage level at ten weeks to 0.5 mg. per kg. had essentially no effect on growth or feed conversion. In a treatment where reserpine was administered at the 0.5 mg. per kg. level from 10 to 24 weeks of age, growth was slightly retarded during the last four weeks.

Broad Breasted Bronze turkeys were fed levels of 0.0, 0.5, 1.0 and 2.0 mgs. of reserpine per kg. of diet, as reported by Rudolph et al.

(1962). Results indicated that graded levels of reserpine added to the diet caused a significant linear decrease in average body weight gain. Birds in this study were placed on treatment at 24 weeks of age.

Morrison (1962) administered reserpine in the diet at levels of 0.0, 0.2, 0.4 and 1.0 p.p.m. to Broad Breasted Bronze turkeys from eight to twenty weeks of age. Feed efficiency and rate of gain was depressed by all levels of reserpine.

Casey (1963) administered reserpine at levels of 0.0, 0.5, 1.0 and 2.0 p.p.m. in the diet to Broad Breasted Bronze turkeys 24 weeks of age. The body weights of females fed 0.5 p.p.m. of reserpine increased by 0.77 pound during the breeding season, as compared to a 0.5 pound increase of the control females. Females fed 2.0 p.p.m. of reserpine gained 0.1 pound. These differences were significant at the five percent level of probability. No significant differences were noted for hen-day feed consumption.

Jones (1964) indicated that reserpine administered in the diet at a level of 2.0 p.p.m. significantly reduced female body weight. It was also observed that during the latter half of the breeding season the untreated females had a higher percentage loss in body weight. Treated females had a significantly higher bird-day feed consumption than did the untreated females. A comparison of feed consumption and body weight of females showed that although untreated females had a higher mature body weight, the treated females had a higher daily feed consumption.

CHAPTER III

GENERAL EXPERIMENTAL PROCEDURE

This thesis reports the results of two separate experiments. The experimental procedures which were common to both experiments or phases are presented in this section. The procedure which is associated to a specific phase of the study will be presented in the respective phase.

The birds used in this investigation were large white turkeys purchased from a commercial hatchery.

On May 31, 1965, 100 male and 300 female poults were housed in four 12' x 16' brick brooder houses at the Turkey Research Station, Perkins, Oklahoma. The females were housed in equal numbers in three houses, with the 100 males being brooded in a single house.

From one day to 24 weeks of age, all birds received a series of all-mash starter rations fed ad libitum. The composition of these rations is shown in Table I. Three waterers of two and one-half gallon capacity each and four 36" metal feeders were furnished in each brooder house.

On June 2, 1965, all poults were desnooded. At four weeks of age all poults were vaccinated against Newcastle disease with a live virus vaccine utilizing the intranasal method of vaccination. Seven days later the poults in all four houses exhibited respiratory difficulties. They were immediately treated with a series of antibiotic products. After this series of treatments, all birds appeared to be fully

TABLE I

ALL-MASH TURKEY STARTER AND GROWER RATION

Ration number	SMT 601-3	SMT 602-3	GMT 603-3	GMT 604-3	GMT 605-3	GMT 607-3	GMT 608-3
Age fed	1-4 Weeks	5-6 Weeks	7-8 Weeks	9-12 Weeks	13-16 Weeks	17-20 Weeks	21-24 Weeks
Ingredients	Percent						
Ground yellow corn	29.15	32.30	43.05	53.65	58.16	73.19	39.00
Milo	----	----	----	----	----	----	38.70
Oat mill feed	5.00	5.00	2.40	2.00	1.80	1.20	1.00
Fat (tallow)	8.00	8.00	9.00	7.50	6.80	4.50	3.80
Corn gluten meal (60% protein)	3.50	3.50	2.50	2.10	1.90	1.30	1.10
Alfalfa meal (17% protein)	2.00	2.00	1.80	1.50	1.40	0.90	0.80
Fish meal (60% protein)	10.00	8.00	10.80	9.00	8.10	5.40	4.50
Blood meal (80% protein)	3.00	3.00	3.00	2.50	2.30	1.50	1.30
Meat and bone scrap (50% protein)	7.00	6.00	4.20	3.50	3.20	2.10	1.80
Soybean oil meal (50% protein)	24.00	22.70	16.00	12.00	10.50	6.00	4.50
Dried whey	2.00	2.00	1.80	1.50	1.40	0.90	0.80
Distillers solubles (CFS #3) ¹	3.00	3.00	1.80	1.50	1.40	0.90	0.80
Dicalcium phosphate	1.00	1.00	1.20	1.00	0.90	0.60	0.50
Calcium carbonate	1.00	2.00	1.20	1.00	0.90	0.60	0.50
dl-Methionine	0.10	0.10	0.10	0.10	0.09	0.06	0.05
VMC-60 ²	0.50	0.50	0.50	0.50	0.50	0.30	0.30
VC-60 A ³	0.25	0.25	----	----	----	----	----
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Histostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TM-10 (Terramycin supplement)	0.10	0.10	0.10	0.10	0.10	----	----

¹ Dried condensed fermented corn extractives--C.F.S. No. 3, Clinton Corn Processing Company, Clinton, Iowa.

² VMC-60-vitamin-mineral concentrate adds the following per pound of finished ration: vitamin A, 8,000 U.S.P. units; vitamin D₃, 1,200 I.C.U.; vitamin E, 6 I.U.; vitamin K, 3.0 milligrams; vitamin B₁₂, 0.008 milligrams; riboflavin, 4.0 milligrams; niacin, 32.0 milligrams; panthothenic acid, 8.0 milligrams; choline chloride, 500.0 milligrams; manganese, 27.7 milligrams; iodine, 0.86 milligrams; cobalt, 0.59 milligrams; iron, 21.8 milligrams; copper, 1.65 milligrams; and zinc, 22.7 milligrams.

³ VC-60A-vitamin concentrate adds the following per pound of finished ration: pyridoxine, 8.0 milligrams; biotin, 0.3 milligrams; thiamin, 12.0 milligrams; folic acid, 2.0 milligrams; inositol, 50.0 milligrams; para-amino-benzoic acid, 4.0 milligrams; and ascorbic acid, 10.0 milligrams.

recovered.

At five weeks of age all poults were moved from the brooder houses to a single 48' x 48' pole shed. Feed was supplied ad libitum from ten 36" metal trough feeders and three 8' bulk feeders. Water was available from ten automatic waterers each 20" in diameter.

Poults were vaccinated against fowl pox at 9 weeks of age using the "thigh stick" method of vaccination.

From 10 to 24 weeks of age, all turkeys in the pole shed were allowed to run on a single 150' x 250' bermuda grass range adjacent to the pole shed. During this time feed and water were provided ad libitum from four 8' bulk feeders and ten automatic waterers each 20" in diameter.

On November 16, 1965, the female turkeys were removed from the pole shed and assigned to breeding pens. All females utilized in the study were selected at random from the flock and placed into one of twenty-four shipping coops. When all coops contained five females, a table of random numbers was used to assign two coops to each of the twelve separate breeding pens.

Forty-eight males were randomly selected and divided into three equal groups of 16 males per group. These groups were then placed in a separate house where each group of males was assigned a 15' x 30' pen. The equipment in each pen consisted of a roosting area, one 3-gallon waterer and one 6' bulk feeder.

The breeding pens utilized for females were 50' x 100' in dimensions with a single 12' x 16' house in each pen. Each house contained a roosting area, six nests, one automatic waterer 24" in diameter and one hanging metal feeder.

All turkeys began receiving an all-mash breeder ration (Table II) at the time they were housed. Reserpine was not incorporated into the ration until the turkeys were 26 weeks of age.

The males began receiving artificial lighting at 26 weeks of age, when reserpine treatment began. The females were lighted at 28 weeks of age. In this study artificial lighting supplemented natural daylight so that both sexes received 14 hours of continuous light during each 24-hour period.

At 34 weeks of age, after the females had begun to lay, turkeys of both sexes were blood tested for Pullorum-Typhoid reaction. Laboratory analysis of these blood samples indicated one female reactor which was immediately replaced by a known non-reactor.

TABLE II
TURKEY BREEDER RATION

<u>Ingredients</u>	<u>% ration</u>	<u>Lbs. feed</u>
Fat (tallow)	9.4	94.0
Ground yellow corn	27.7	277.0
Ground yellow milo	20.0	200.0
Oat mill feed	6.5	65.0
Alfalfa meal (17% protein)	2.5	25.0
Wheat shorts	5.0	50.0
Soybean oil meal (50% protein)	7.5	75.0
Fish meal (74% protein)	6.5	65.0
Meat and bone scrap (50% protein)	4.0	40.0
Yeast culture	1.0	10.0
Distillers solubles	1.5	15.0
Dicalcium phosphate	2.9	29.0
Calcium carbonate	2.8	28.0
Salt	0.5	5.0
Dried whey	1.0	10.0
dl-Methionine	0.1	1.0
Fermacto	0.4	4.0
Lecithin	0.25	2.5
VMC-60	0.5	5.0
Vit. E (100,000 I.U./lb.)	17.0 gms/100 lbs	170.0 gms
NF-180	9.1 gms/100 lbs	91.0 gms
Histostat	22.7 gms/100 lbs	227.0 gms
	<u>100.05%</u>	<u>1000.5 lbs</u>

CHAPTER IV

PRESENTATION OF THE LITERATURE REVIEW, THE EXPERIMENTAL PROCEDURES, THE RESULTS AND SUMMARY ASSOCIATED WITH PHASE I AND PHASE II

Phase I. The Differential Effects of Reserpine on the Reproductive Performance of Breeder Turkeys Receiving Artificial Insemination

Studies from this station have indicated a decline in the reproductive efficiency of turkeys fed reserpine as a feed additive. Recent studies have revealed that reserpine exhibited a differential effect with respect to sex for fertility in naturally mated turkeys. In these studies the reduction in fertility was greater in the female, although a reduction in fertility was observed in both sexes.

From these studies new questions have arisen. For example, was this observed decline in fertility due to an unfavorable behavioristic effect between sexes due to the effects of reserpine? Secondly, is the greater reduction in fertility exhibited by the female due to an inhibition of the mating drive by the tranquilizing effects of reserpine?

This study was designed to investigate the effects of reserpine on reproductive performance of turkeys using artificial insemination.

The objectives were to determine:

1. the effect of reserpine on days to female sexual maturity,
2. the effect of reserpine on percentage egg production,
3. the differential effects of reserpine by sexes on percentage fertility,

4. the differential effects of reserpine by sexes on percentage hatch of fertile eggs, and
5. the differential effects of reserpine by sexes on percentage hatch of total eggs set.

Weight gains and feed consumption records were also recorded for females in this investigation.

Review of Literature

This study deals with several objectives; therefore, this specific review of literature will be divided into two parts. The effect of reserpine on egg production in turkeys will be reviewed in the first section. The second section will review the effects of reserpine on fertility and hatchability in turkeys, collectively referred to as reproductive traits.

Egg Production: Pheasant hens were fed reserpine at the rate of 5.0 gms. per ton of feed in one treatment and reserpine residue (SU-3446) at 25.0 gms. per ton in another treatment by Hewitt and Reynolds (1957). Both treatments were reported to reduce egg production.

In another study Hewitt (1959) reported that ring-necked pheasant hens receiving 7.0 mgs. per kg. of reserpine laid 17 percent fewer eggs than did the controls.

Greene et al. (1961) reported that 0.5 p.p.m. of reserpine caused a reduced level of egg production in ducks for three months with higher levels completely inhibiting egg production.

The effects of high environmental temperatures on egg production, as modified by reserpine, were measured by Van Matre et al. (1959). The results of this study indicated that reserpine fed to White Leghorn

females afforded them protection against a decrease in egg production.

Rood et al. (1958) incorporated reserpine in a commercial chick starter at the levels of 0.1 and 1.0 p.p.m. This was fed from the fourth through the seventh week of age to female White Plymouth Rock crosses. The groups fed 1.0 p.p.m. showed a significant difference ($P < .05$) in ovarian weights at 56 days of age, with treated females having the heavier weights. This difference was not apparent at 77 days of age.

Burger (1959) fed reserpine at levels of 0.0, 2.5 and 5.0 mgs. per kg. of feed, for a period of 14 days before stress, to White Leghorn x New Hampshire crossbred females. Seven-day egg production before stress showed no differences between treatments. A thermal stress of 104°F. for three hours was applied to all females. The treatment groups lost fewer females and survivors laid more eggs than did the control groups.

Couch (1959) reported that reserpine administered orally at the levels of 0.25, 0.5 and 1.0 mg. per pound of feed produced no differences in egg production of pullets as compared to the controls over a 168-day period.

Gilbreath (1959) reported that 2.0 mgs. of reserpine per pound of feed depressed egg production ($P < .10$) of DeKalb 101 hybrid females.

Reserpine fed at levels of 0.0, 2.5, 5.0 and 10.0 p.p.m. depressed egg production in Single Comb White Leghorns, according to Burger (1960).

Weiss (1960a) reported that oral levels of reserpine up to 2.0 mgs. per kg. of feed were compatible to sustained egg production when fed to White Leghorn females for as long as 32 weeks.

Parker (1960) observed that reserpine fed at a level of 0.5 p.p.m.

to White Leghorn females was effective in maintaining a higher level of egg production in temperatures above 90°F. Below this temperature, control groups exhibited higher egg production.

Carlson (1960) indicated that reserpine administered orally in the diet at 1.1 p.p.m. to laying turkey hens could possibly improve egg production.

Rudolph et al. (1962) administered reserpine in the diet to Broad Breasted Bronze turkeys at levels of 0.0, 0.5, 1.0 and 2.0 p.p.m. Treatment was started when the turkeys reached 24 weeks of age. The results indicated that graded levels of reserpine added to the diet caused a significant linear decrease in percentage egg production.

Greene et al. (1961) reported that 2.0 p.p.m. of reserpine fed in a practical turkey breeder ration to large white turkeys resulted in a marked reduction in egg production. This drop in egg production was first observed during the third month of lay.

According to Casey (1963) the egg production of Broad Breasted Bronze turkeys receiving 0.5 p.p.m. of reserpine in the diet was slightly higher than that of the controls during the entire breeding season. Reserpine fed at levels of 1.0 and 2.0 p.p.m. in the diet resulted in decreased egg production. In this trial reserpine treatment was initiated when the turkeys were 24 weeks of age. All three levels of reserpine significantly increased the number of days to onset of egg production.

In another trial, Casey et al. (1963) reported that age at sexual maturity and total egg production of Broad Breasted Bronze turkeys were not significantly influenced by reserpine. In this study reserpine was orally administered at levels of 0.5, 1.0 and 2.0 p.p.m. in the diet of

turkeys during the growing period (12 to 24 weeks of age).

Jones et al. (1966) administered levels of 0.0 and 2.0 p.p.m. of reserpine in the diet to Broad Breasted Bronze turkeys during the breeding season. Treated females required more time to reach sexual maturity than did the untreated females. Reserpine treatment also resulted in a significant reduction in percentage egg production.

Reproductive traits: Hewitt and Reynolds (1957) reported that reserpine and reserpine residue (SU-3446) reduced fertility and hatchability in naturally mated ring-necked pheasants. In this experiment reserpine was administered at the rate of 5.0 gms. per ton of ration and reserpine residue (SU-3446) was incorporated at the rate of 25.0 gms. per ton of ration.

Hewitt (1959) administered reserpine at the rate of 7.0 mgs. per kg. of feed to ring-necked pheasants and noted that fertility was 8.5 percent lower than that of the control birds.

Anderson and Smyth (1959) reported that Single Comb White Leghorns fed 0.5 mg. of reserpine per kg. of feed showed no differences in hatchability.

Greene et al. (1961) detected a marked reduction in fertility and hatchability due to reserpine administered in the diet at a level of 2.0 p.p.m. to turkeys during the breeding season. This effect on fertility and hatchability was noted in eggs produced during the first month of egg production.

Rudolph et al. (1962) indicated that levels of 0.5, 1.0 and 2.0 p.p.m. of reserpine fed to naturally mated Broad Breasted Bronze turkeys caused a significant linear decrease in percentage fertility. In this study reserpine treatment was begun when birds were 24 weeks of age.

There were no indications that the hatchability of fertile eggs was affected by reserpine.

Casey et al. (1963) observed a highly significant difference ($P < .01$) in percentage fertility of naturally mated Broad Breasted Bronze turkeys fed different levels of reserpine during the 12th through the 24th week of age. Fertility of eggs from hens receiving 1.0 p.p.m. of reserpine for 4 weeks, then 2.0 p.p.m. for eight weeks, was 14.07 percent less than that of non-treated females. In this study it was noted that reserpine administered during the 12-week growing period did not significantly affect the hatchability of fertile eggs of mature breeder turkeys.

Friars et al. (1964) administered 0.25 p.p.m. of reserpine in the ration to a small white strain of turkeys from the 9th week to the 19th week of age. In another study 1.0 p.p.m. of reserpine was administered in the diet during the 12th and the 15th weeks of age. No significant effect of treatment on fertility was noted. The death of embryos was recorded at different stages of incubation. Both continuous and intermittent reserpine treatment depressed the hatchability of fertile eggs.

Jones et al. (1966) administered reserpine in the diet at levels of 0.0 and 2.0 p.p.m. to Broad Breasted Bronze turkeys during the breeding season. Comparisons made for fertility indicated a marked reduction in percentage fertility of a naturally mated flock due to reserpine. There was also statistical evidence that reserpine was more detrimental to fertility when administered to females than when administered to the males. No statistically significant differences in percentage hatch of fertile eggs due to treatment were observed. Percentage hatch of total eggs set was reduced by reserpine treatment, with more detrimental

effects observed in treated females than in treated males.

Experimental Procedure

On November 30, 1965, the 12 pens containing 10 females each were randomly assigned to one of three treatments. Since previous studies had proven that these 12 breeding pens were homogeneous with respect to location effect, a completely randomized design was utilized.

Reserpine treatment was initiated when the turkeys were 26 weeks of age. Females in four of the breeding pens received 2.0 p.p.m. of reserpine in the breeder ration while the remaining eight pens of females received no reserpine.

One of the three pens of males received 2.0 p.p.m. of reserpine in the breeder ration while the remaining two pens received no reserpine treatment.

By treating only one-third of each sex, it was possible to create three treatment combinations. There were four pens of females in each treatment combination, and each pen served as an experimental unit. The three treatment combinations were as follows: treatment A, non-treated males x non-treated females; treatment B, non-treated males x treated females; and treatment C, treated males x non-treated females. Artificial insemination was used throughout the entire study.

Initial body weights were recorded for the females on a pen average basis when treatment began. Females were weighed at 28-day intervals throughout the study, and weights were recorded as a pen average. Feed consumption data were recorded on a pen basis each 28 days starting when treatment began. Feed consumption and body weights of males were not measured in this study.

Broody females were removed from the breeding pens when they were first observed and were not returned to their pens until they had returned to egg production.

Mortality records were maintained throughout the egg laying season in order to calculate the number of hen-days for each pen. Average hen-day feed consumption and percentage egg production were calculated for each pen by using hen-days per pen as the denominator.

Percentage egg production was computed every 28 days beginning January 1, 1966. The first egg was laid on January 2, 1966. Egg record sheets were placed in each house and daily egg production on a pen basis was recorded. Pen numbers were recorded on the eggs when they were collected.

Days to sexual maturity were calculated for each pen. In this study pens were designated as sexually mature when they attained 30 percent egg production. The number of days from hatching to 30 percent egg production was recorded as the number of days to sexual maturity.

All females were inseminated every two weeks throughout the breeding season beginning January 27, 1966, when egg production had reached 40 percent. Semen was collected by the abdominal massage method into small wax-lined glass vials. Immediately after collecting the semen, it was diluted in the ratio of one part semen to one part isotonic sodium chloride solution. This suspension was drawn into a multiple-injector inseminating gun manufactured by Robert Tyler, Dallas, Wisconsin. Plastic inseminating tubes were then filled with a constant volume of 0.025 ml. of semen-diluent suspension. Each hen was inseminated with this suspension using a separate tube for each female.

The collection-insemination operation for each treatment combination

was completed within 25 minutes. The sequence of inseminations was rotated throughout the breeding season to avoid bias for any given treatment combination.

Eggs were set at 14-day intervals beginning February 13, 1966 until the last setting on May 22, 1966.

Percentage fertility, percentage hatch of fertile eggs and hatch of total eggs set were calculated for eight two-week periods. Percentage fertility was determined by candling the eggs after 24 days of incubation. All eggs not readily identified as fertile by candling were broken out to determine if the eggs were actually infertile or if a dead germ was present. The dead embryos and poults were then totaled and counted as fertile eggs.

Results and Discussion

The effects of administering reserpine in the diet at the levels of 0.0 and 2.0 p.p.m. to large white turkeys is presented in this section. The reserpine treatment was begun at 26 weeks of age and was continued throughout the 24-week breeding season. A particular emphasis was given to the differential effects of reserpine exhibited in reproductive traits by sexes. The variables analyzed were: female body weight, hen-day feed consumption, days to sexual maturity, percentage egg production, percentage fertility, percentage hatch of fertile eggs and percentage hatch of total eggs set.

Female body weights were recorded at four-week intervals beginning at 26 weeks of age. These data were analyzed and are shown in Table III and Figure 1. The analysis of variance for female body weight over all period yielded a treatment-by-period interaction significant at the one

TABLE III
FEMALE BODY WEIGHT

Treatments	Periods							Over-all Period Treatment Means
	1	2	3	4	5	6	7	
A	17.58	19.39	20.03	19.37	19.10	18.77	18.80	19.00
B	17.11	18.01	18.00	18.38	18.57	18.50	18.36	18.28
C	17.68	19.66	20.23	19.65	18.91	19.01	19.72	19.26
C_{SS}^1 (AC vs B)	0.70	6.11	3.38	3.42	0.49	0.41	2.17	12.52
F value ²	2.49	26.25**	15.23**	11.16**	2.84	1.28	7.27*	10.47*

¹ Sum of squares for orthogonal comparison between non-treated females (Treatment A and Treatment C) and treated females (Treatment B) for female body weight.

² Sum of squares for comparison (AC vs B) with one df divided by error mean square with 9 df.

* Prob <.05

** Prob <.01

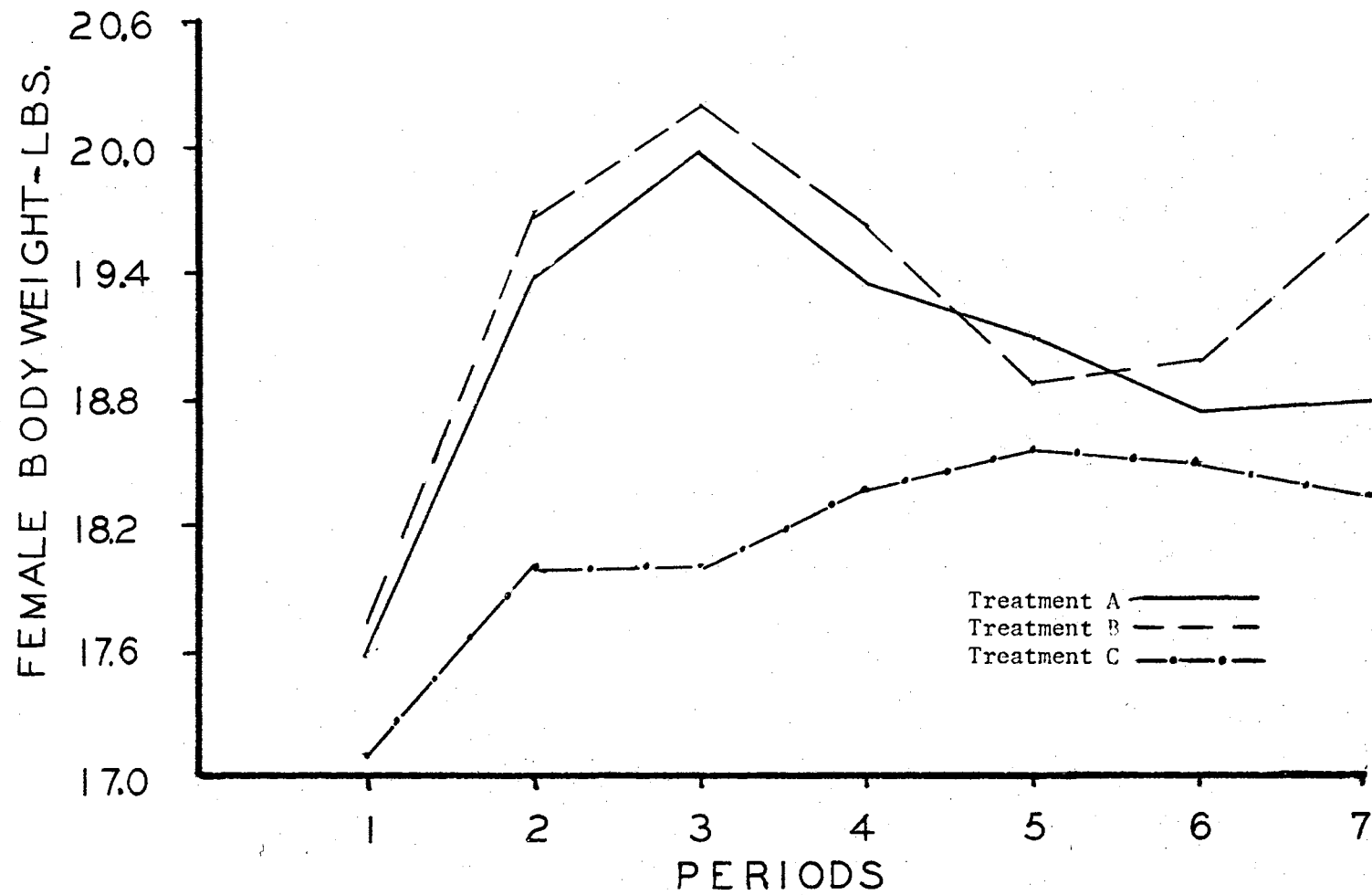


Figure 1. The Influence of Reserpine on Female Body Weight, Measured at 28-day Intervals. (Treatments A and C = Non-treated Females; Treatment B = Treated Females)

percent level of probability. This interaction is shown in periods five, six and seven of Figure 1. Over all periods the effect due to treatment was significant at the five percent level of probability, with the treated females exhibiting the lower body weights.

The difference within periods was analyzed using an orthogonal comparison in which the female body weights for treatments A and C were compared to treatment B. These results are summarized in Table III, wherein the comparison sum of squares for periods two, three and four were significant at the one percent level of probability. The comparison sum of squares over all periods was significant at the five percent level of probability.

These results indicate that reserpine treatment depressed female body weight early in the breeding season, with the most pronounced depression occurring from four to eight weeks after treatment was initiated.

It should be noted that while untreated females lost weight during the last half of the breeding season, treated females gained or at least maintained their body weights, as shown in Figure 1. This difference in body weight trends is probably responsible for the significant treatment-by-period interaction observed in this analysis. These results indicate that reserpine could possibly be beneficial in maintaining body weight late in the breeding season when warmer temperatures prevail.

A summary of average hen-day feed consumption is presented in Table IV and Figure 2. The analysis of variance over all periods yielded no significant treatment or treatment-by-period effects.

Using an orthogonal comparison, where non-treated females were compared to treated females within each period, several significant

TABLE IV
HEN-DAY FEED CONSUMPTION

Treatments	Periods						Over-all Period Treatment Means
	1	2	3	4	5	6	
A	0.6853	0.6083	0.5753	0.5253	0.4768	0.5305	0.4252
B	0.7300	0.6680	0.6860	0.5980	0.5618	0.5828	0.4783
C	0.6808	0.6078	0.6490	0.5238	0.5043	0.5395	0.4381
C_{SS}^1 (AC vs B)	0.0059	0.0096	0.01455	0.0144	0.0135	0.0061	0.06195
F value ²	4.67	2.29	1.66	1.22	6.43*	0.92	3.787

- ¹ Sum of squares for orthogonal comparison between non-treated females (treatment A and treatment C) and treated females (treatment B) for hen-day feed consumption.
² Sum of squares for comparison (AC vs B) with one df. divided by error mean square with 9 df.
* Prob. <.05

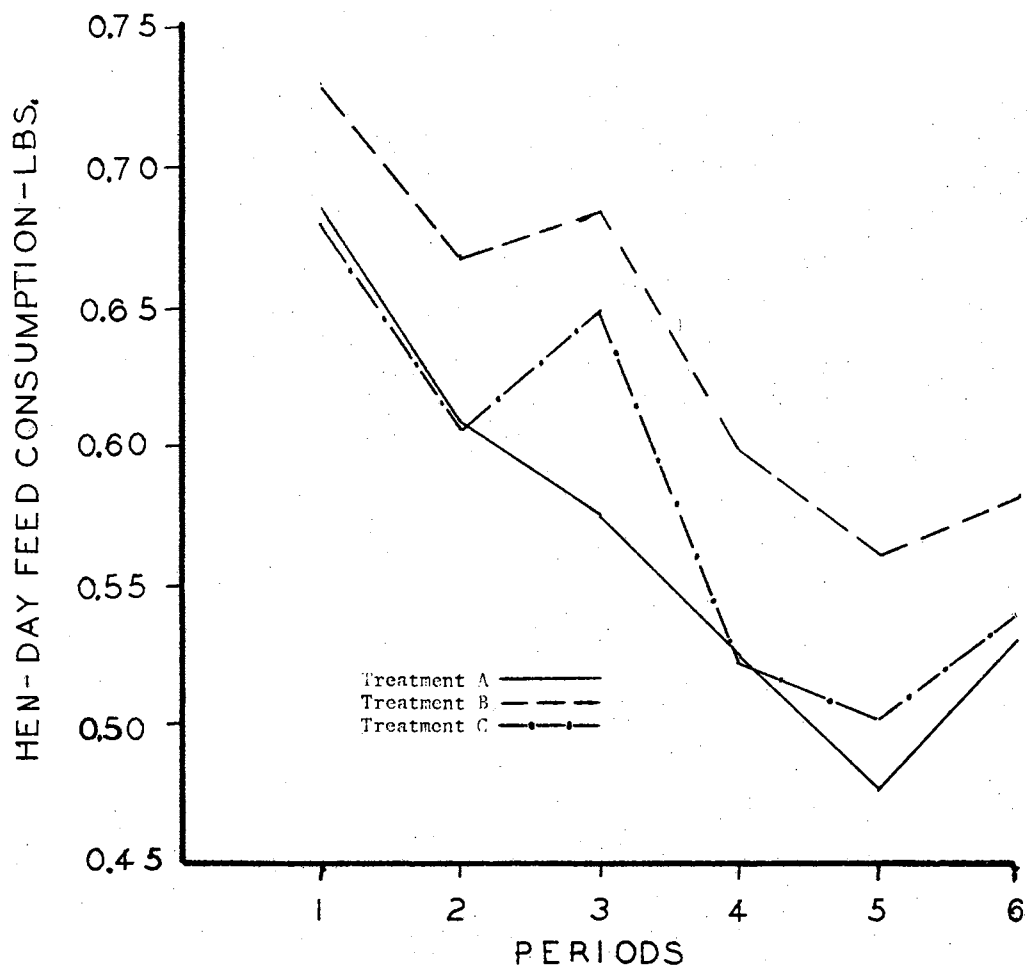


Figure 2. The Influence of Reserpine on Hen-Day Feed Consumption Measured at 28-day Intervals. (Treatments A and C = Non-treated Females; Treatment B = Treated Females)

comparison sums of squares were observed. The comparison sum of squares for period one was significant at the ten percent level of probability and for period five at the five percent level of probability. The comparison sum of squares for the over-all period treatment means was significant at the ten percent level of probability. In all cases the hen-day feed consumption was greater for the treated females than for the non-treated females. It should be noted that although treated birds consumed more feed their average body weights were less than the non-treated females.

The number of days to sexual maturity by pens is shown in Table V. A marked delay in the number of days to sexual maturity was observed for the reserpine treated pens. The analysis of variance for days to sexual maturity, shown in Table VI, yielded a treatment effect significant at the five percent level of probability. The orthogonal comparison between non-treated and treated females yielded a comparison sum of squares significant at the five percent level of probability. These results indicate that reserpine treated females required five to seven days longer to attain sexual maturity than did the non-treated females.

The analysis of variance for percentage egg production over all periods showed a treatment-by-period interaction significant at the five percent level of probability. This interaction is shown in periods three, four and five of Figure 3. The treatment effect over all periods was significant at the one percent level of probability, with treated females exhibiting the lowest level of egg production.

An orthogonal comparison, comparing non-treated females with treated females within each period, is shown in Table VII. The comparison sum of squares for periods one and two was significant at the one

TABLE V
DAYS TO SEXUAL MATURITY¹

Treatments	Pens				Treatment Average
	1	2	3	4	
A	227	225	225	224	225.25
B	227	232	226	228	228.25
C	221	225	225	222	223.25

¹ Age in days when 30% egg production was recorded for the pen.

TABLE VI
ANALYSIS OF VARIANCE OF DAYS TO SEXUAL MATURITY

Source	df.	S.S.	M.S.	F
Total	11	88.91667		
Treatment	2	50.66667		5.961*
[C (AC vs B)] ¹	(1)	(42.66667)	42.66667	10.039*
Remainder	(1)	(8.0000)	8.0000	
Error	9	38.25	4.25	

¹ Orthogonal comparison between non-treated females (treatment A and treatment C) and treated females (treatment B) for days to sexual maturity.

* Prob. < .05

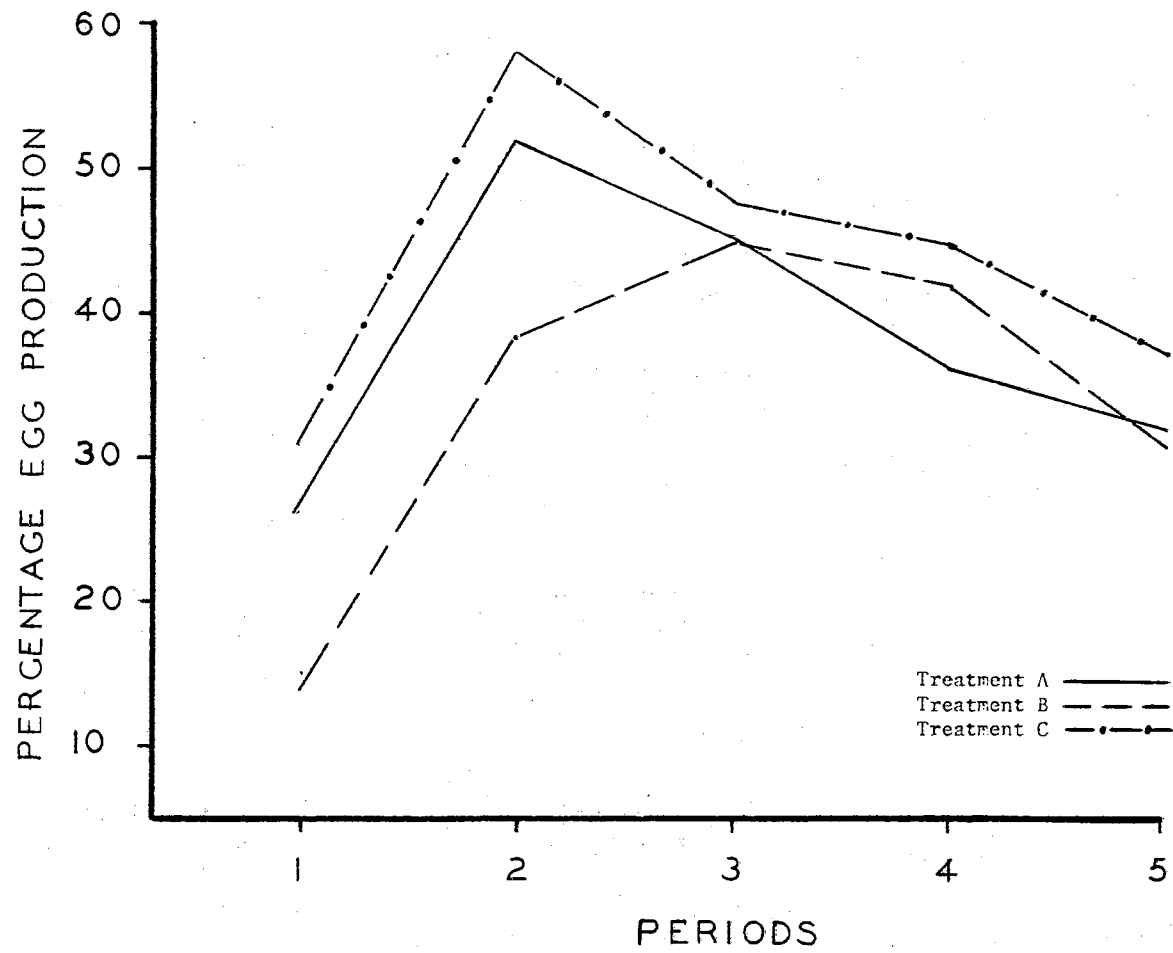


Figure 3. The Influence of Reserpine on Percentage Egg Production, Measured at 28-day Intervals. (Treatments A and C = Non-treated Females; Treatment B = Treated Females)

TABLE VII
PERCENTAGE EGG PRODUCTION

Treatments	Periods					Over-all Period Treatment Means
	1	2	3	4	5	
A	26.31	52.09	45.56	36.94	32.20	38.62
B	14.20	38.75	45.35	42.00	31.00	34.26
C	31.07	57.95	47.77	44.48	37.60	43.77
C_{SS}^1 (AC vs B)	560.28	705.90	4.59	4.48	40.61	641.58
F value ²	48.48**	23.42**	0.98	0.094	1.122	10.1101*

¹ Sum of squares for orthogonal comparison between non-treated and treated females for percentage egg production. (AC vs B)

² Sum of squares for (AC vs B) with one df divided by error mean square with 9 df.

* Prob <.05

** Prob <.01

percent level of probability. The comparison sum of squares for the over-all period treatment means was significant at the five percent level of probability.

These results indicate that reserpine treatment tends to depress percentage egg production in the first half of the breeding season, but differences due to treatment later in the breeding season appear to be negligible.

A noteworthy point is that the lower egg production exhibited by the treated females early in the breeding season could be due in part to the fact that these females required a greater number of days to reach sexual maturity.

Percentage fertility, percentage hatch of fertile eggs and percentage hatch of total eggs set were analyzed using the analysis of variance method for testing variance components. Since there were three treatment combinations with two degrees of freedom, only two orthogonal comparisons could be made. Therefore, in order to make all possible comparisons between treatment differences, Duncan's Multiple Range Test was used as described by Steel and Torrie (1960).

In the case of the variables mentioned above, the three comparisons made were: treatment A with treatment C, treatment C with treatment B and treatment A with treatment B. Using these comparisons, the response due to the effects of reserpine on one sex could be compared to the response when the other sex was not treated.

A summary of the data on percentage fertility is presented in Table VIII and Figure 4. The analysis of variance over all periods for percentage fertility showed a treatment-by-period interaction which was significant at the ten percent level of probability. These interactions

TABLE VIII
PERCENTAGE FERTILITY

Treatment	Periods								Over-all Period Treatment Means
	1	2	3	4	5	6	7	8	
A	55.85	69.65	60.97	39.20	13.16	20.88	9.60	0.86	33.77
B	55.91	62.56	67.72	51.37	28.21	6.93	8.64	7.67	35.19
C	52.77	73.58	65.06	59.20	31.75	31.37	13.42	10.00	41.83
Difference ¹ A-C	3.14	-3.93	-4.09	-20.00*	-18.59*	-10.49	-3.82	-9.137*	-8.06*
Difference ² C-B	-3.08	11.02*	-2.66	7.83	3.54	24.44*	4.78	2.33	6.64
Difference ³ A-B	0.06	7.09	-6.75	-12.17**	-15.05	13.95	0.96	-6.81	-1.42

¹ The average percentage fertility in treatment A (non-treated males x non-treated females) was compared to the average percentage fertility in treatment C (treated males x non-treated females) using Duncan's Multiple Range Test.

² The average percentage fertility in treatment C (treated males x non-treated females) was compared to the average percentage fertility in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

³ The average percentage fertility in treatment A (non-treated males x non-treated females) was compared to the average percentage fertility in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

* Observed treatment differences significant at the 5 percent level of probability.

** Observed treatment differences significant at the 1 percent level of probability.

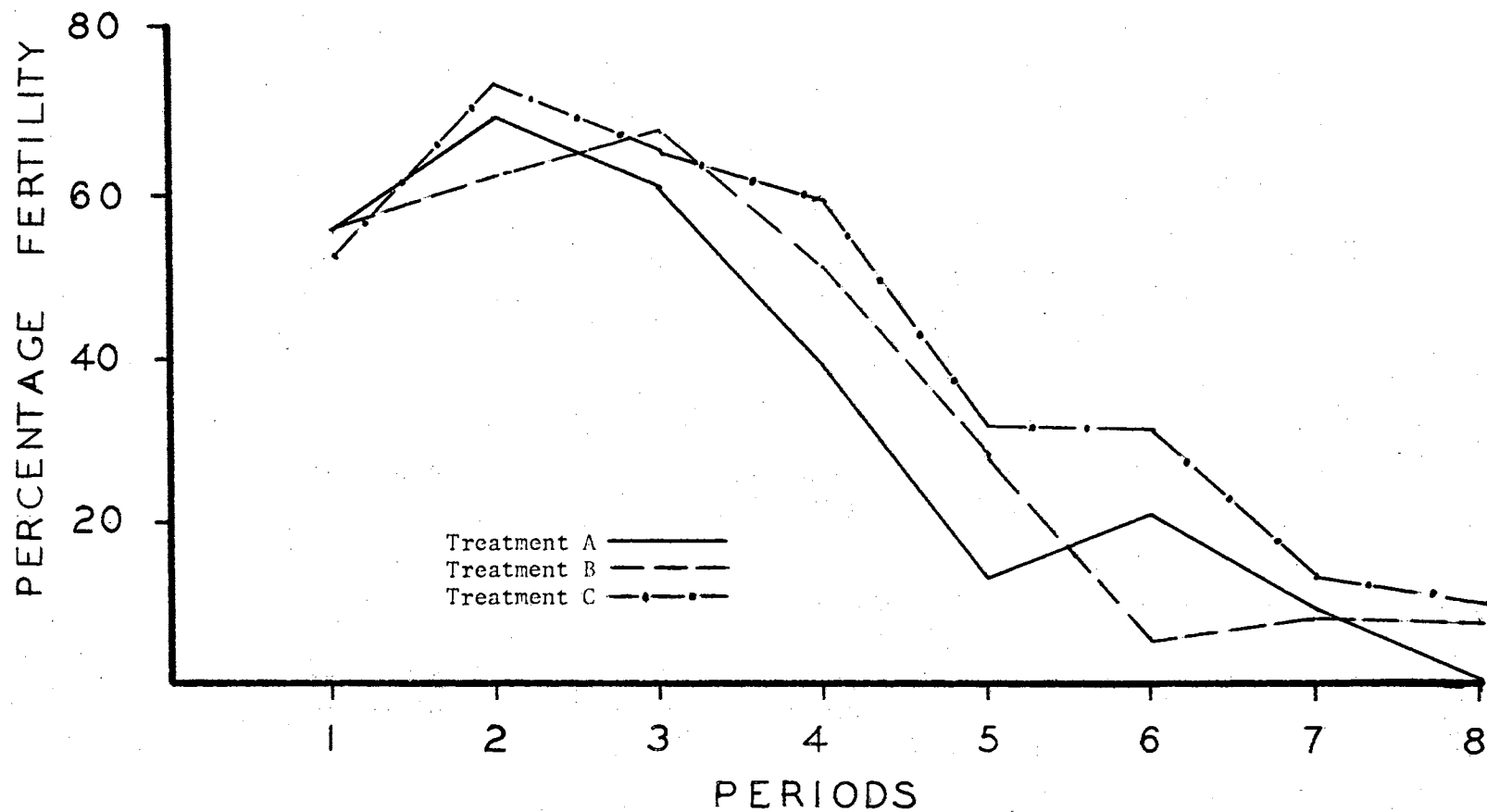


Figure 4. The Influence of Reserpine on Percentage Fertility, Measured at 14-day Intervals.
 (A = Male and Female Non-treated; B = Female Treated; C = Male Treated)

are shown to exist in all periods, except period four, in Figure 4.

Using the multiple range test, comparative differences were analyzed within each period. The difference between treatment A and treatment C was significant at the five percent level of probability in periods four, five and eight. In this comparison treatment C maintained higher percentage fertility in all periods except period one. The difference between the over-all period treatment means for treatment A and treatment C was significant at the five percent level of probability, with treatment C again exhibiting the highest percentage fertility.

The difference between treatment C and treatment B was significant at the five percent level of probability in periods two and six; and, in all periods except one and three, treatment C exhibited the highest fertility. The difference between the over-all period treatment means was not significant, although treatment C exhibited a larger mean than treatment B.

The difference between treatment A and treatment B was significant at the one percent level of probability in period four; and, in all periods except one, two, six and seven, treatment A exhibited the highest percentage fertility. The difference between the over-all treatment means was not significant, although treatment A maintained the highest percentage fertility. The large non-significant differences in periods five and six could be due to the large within-treatment variation experienced for these specific periods.

These results indicate that the only consistent differences in percentage fertility were between treatment A and treatment C, where non-treated females inseminated with semen from treated males exhibited a higher level of fertility than did non-treated females inseminated with

semen from non-treated males.

A summary of percentage hatch of fertile eggs is presented in Table IX and Figure 5. The analysis of variance over all periods yielded a treatment effect which was significant at the one percent level of probability. The treatment-by-period interaction for percentage hatch of fertile eggs was non-significant.

Comparative differences in percentage hatch of fertile eggs were analyzed using the multiple range test. The difference between treatment A and treatment C was significant at the five percent level of probability; and, in all periods except one and six, treatment C exhibited the highest level of hatchability. The difference between the over-all period treatment means for treatment A and treatment C was significant at the five percent level of probability, with treatment C again exhibiting the highest hatchability.

The difference between treatment C and treatment B was significant at the one percent level of probability in periods two and four and at the five percent level of probability, in period one. The difference between the over-all period treatment means was significant at the one percent level of probability. Treatment C exhibited the highest percentage hatch of fertile eggs in all periods as well as in the over-all period treatment means.

The difference between treatment A and treatment B was significant at the one percent level of probability in period one and at the five percent level of probability in period two. The difference between the over-all period treatment means was significant at the one percent level of probability. In all periods except five and eight and in the over-all period treatment means, treatment A maintained the highest percentage

TABLE IX
 PERCENTAGE HATCH OF FERTILE EGGS

Treatment	Periods								Over-all Period Treatment Means
	1	2	3	4	5	6	7	8	
A	56.85	40.08	33.15	42.20	20.00	73.75	21.25	25.00	39.03
B	19.35	25.48	24.28	28.28	22.41	37.50	4.17	39.58	24.63
C	52.29	47.88	45.85	65.44	56.06	50.59	41.67	43.75	49.94
Difference ¹ A-C	4.56	-7.80	-12.70	-23.24*	-36.06	23.16	-20.42	-18.75	-10.91*
Difference ² C-B	32.94*	22.40*	21.57	37.16**	33.65	13.09	37.50	4.17	25.31**
Difference ³ A-B	37.50**	14.60*	8.87	13.92	-2.41	36.25	17.08	-14.58	14.403**

¹ The average percentage hatch of fertile eggs in treatment A (non-treated males x non-treated females) was compared to the average percentage hatch of fertile eggs in treatment C (treated males x non-treated females) using Duncan's Multiple Range Test.

² The average percentage hatch of fertile eggs in treatment C (treated males x non-treated females) was compared to the average percentage hatch of fertile eggs in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

³ The average percentage hatch of fertile eggs in treatment A (non-treated males x non-treated females) was compared to the average percentage hatch of fertile eggs in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

* Observed treatment differences significant at the 5 percent level of probability.

** Observed treatment differences significant at the 1 percent level of probability.

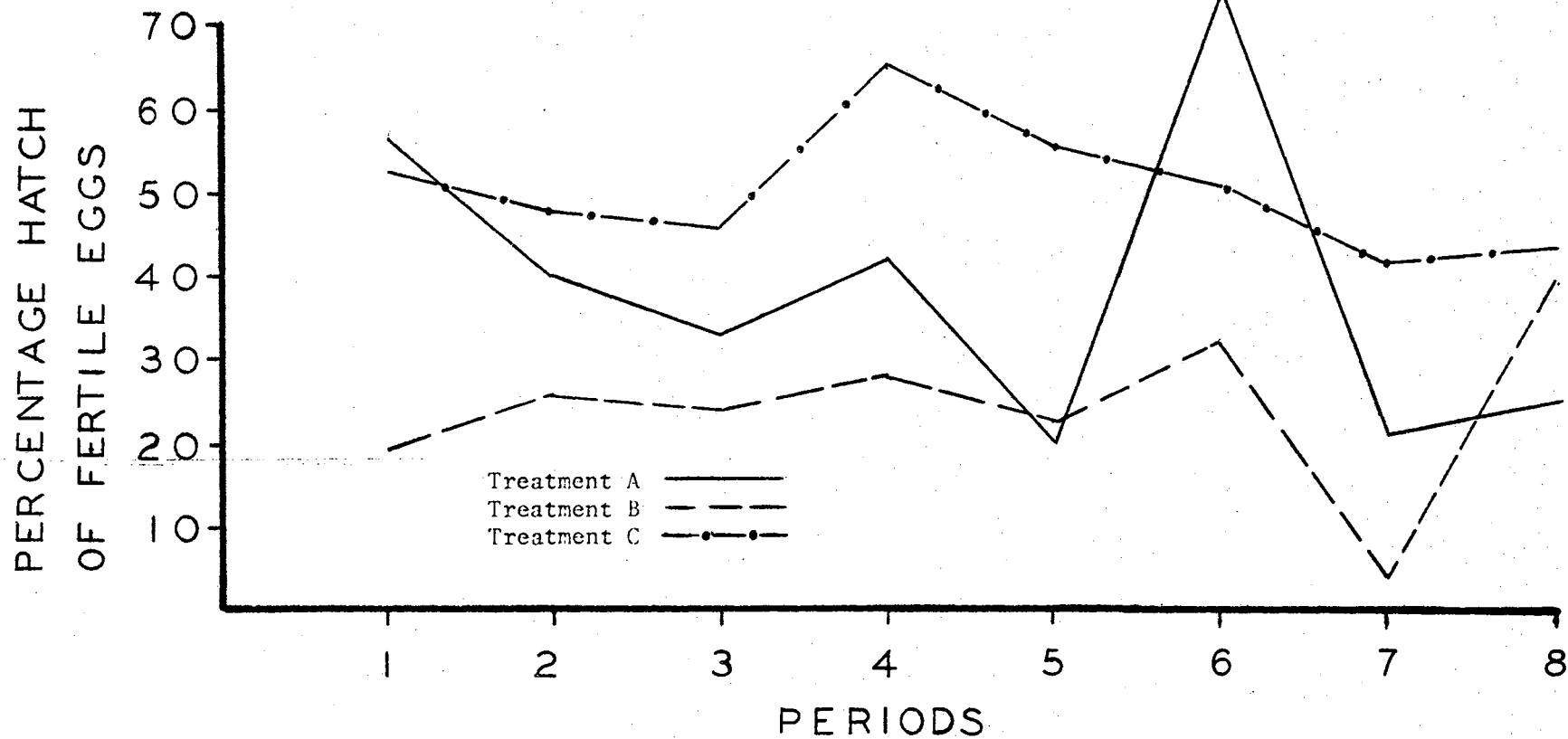


Figure 5. The Influence of Reserpine on Percentage Hatch of Fertile Eggs, Measured at 14-day Intervals.
 (A = Male and Female Non-treated; B = Female Treated; C = Male Treated)

hatch of fertile eggs.

These results indicate that treatment C (non-treated females inseminated with semen from treated males) exhibited a higher level of hatchability throughout the entire breeding season than either treatment A or treatment B. These results further indicate that treatment B (treated females inseminated with semen from non-treated males) maintained the lowest level of hatchability throughout the breeding season. Treatment A (non-treated females inseminated with semen from non-treated males) maintained an intermediate level of hatchability midway between treatment C and treatment A.

A summary of the data for the percentage hatch of total eggs set is presented in Table X and Figure 6. The analysis of variance over all periods yielded a treatment effect and a treatment-by-period interaction significant at the one percent level of probability. These interactions can be observed for all periods, except period six, in Figure 6.

Comparative differences for percentage hatch of total eggs set were analyzed using the multiple range test. The difference between treatment A and treatment C was significant at the five percent level of probability in periods four and five; and, in all periods except period one, treatment C exhibited the highest level of percentage hatch of total eggs set. The difference between the over-all period treatment means was significant at the one percent level of probability, with treatment C again exhibiting the higher level of percentage hatch of total eggs set when compared to treatment A.

The difference between treatment C and treatment B was significant at the one percent level of probability in period two and at the five percent level of probability in periods one, four and six. The

TABLE X
PERCENTAGE HATCH OF TOTAL EGGS SET

Treatment	Periods								Over-all Period Treatment Means
	1	2	3	4	5	6	7	8	
A	31.90	28.40	18.59	16.34	2.59	15.17	4.30	0.86	14.77
B	9.92	16.20	17.23	12.16	7.28	2.88	1.25	2.47	8.67
C	27.37	34.97	29.67	35.20	16.20	15.75	5.27	3.39	20.98
Difference ¹ A-C	4.53	-6.57	-11.08	-18.86*	-13.61*	-0.58	-0.97	-2.52	-6.21**
Difference ² C-B	17.45*	18.77**	12.44	23.04*	8.92	12.87*	4.02	0.91	12.31**
Difference ³ A-B	21.98**	12.20*	1.36	4.18	-4.69	12.29*	3.05	-1.61	6.10**

¹ The average percentage hatch of total eggs set in treatment A (non-treated males x non-treated females) was compared to the average percentage hatch of total eggs set in treatment C (treated males x non-treated females) using Duncan's Multiple Range Test.

² The average percentage hatch of total eggs set in treatment C (treated males x non-treated females) was compared to the average percentage hatch of total eggs set in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

³ The average percentage hatch of total eggs set in treatment A (non-treated males x non-treated females) was compared to the average percentage hatch of total eggs in treatment B (non-treated males x treated females) using Duncan's Multiple Range Test.

* Observed treatment differences significant at the 5 percent level of probability.

** Observed treatment differences significant at the 1 percent level of probability.

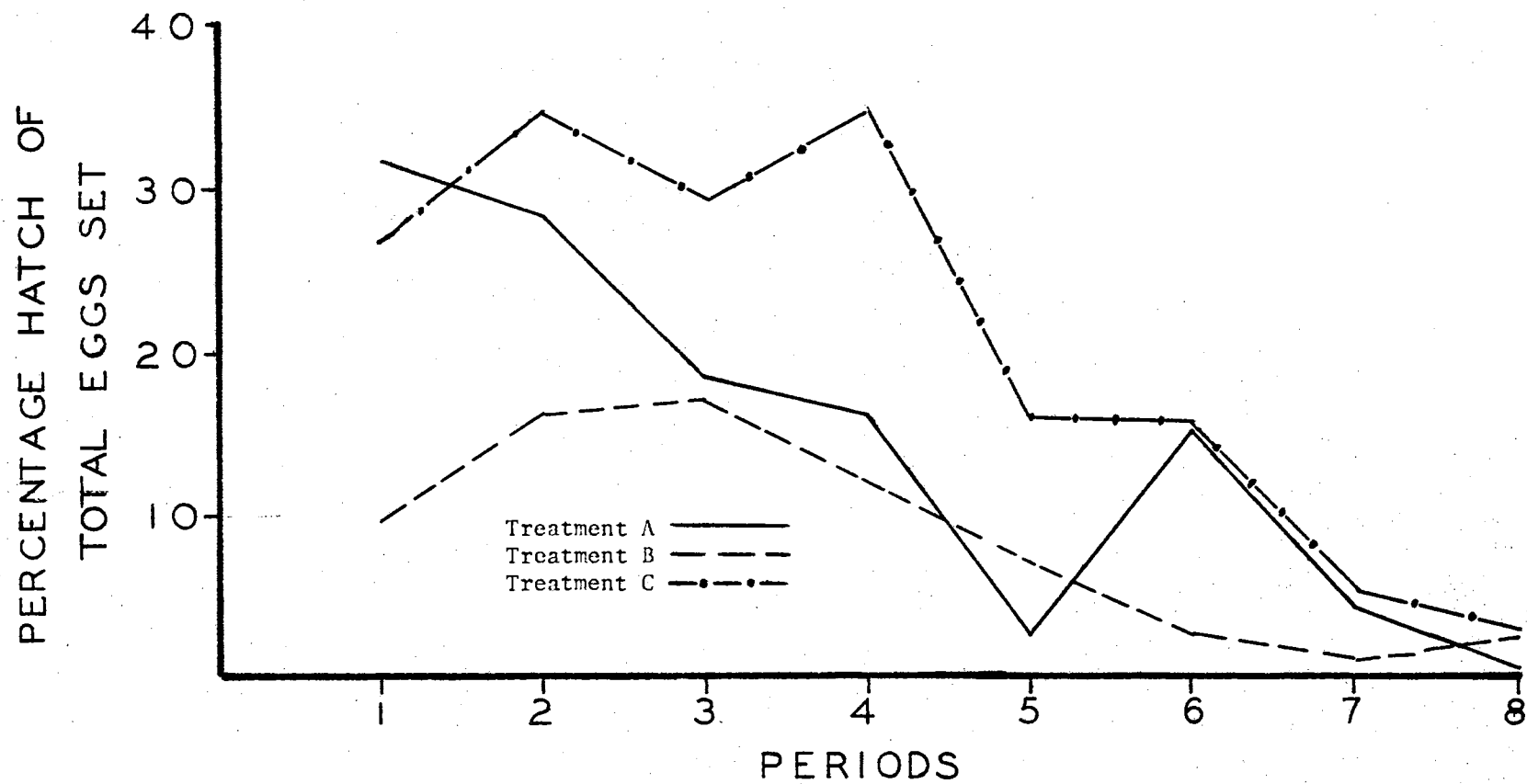


Figure 6. The Influence of Reserpine on Percentage Hatch of Total Eggs Set, Measured at 14-day Intervals. (A = Male and Female Non-treated; B = Female Treated; C = Male Treated)

difference between the over-all period treatment means was significant at the one percent level of probability. In all periods as well as in the over-all period treatment means, treatment C exhibited the higher level of percentage hatch of total eggs set when compared to treatment B.

The difference between treatment A and treatment B was significant at the one percent level of probability in period one and at the five percent level of probability in periods two and six. The difference between the over-all period treatment means was significant at the one percent level of probability. In all periods except periods five and eight and in the over-all period treatment means, treatment A maintained a higher level of percentage hatch of total eggs set than treatment B.

These results indicate that treatment C (non-treated females inseminated with semen from treated males) maintained a higher level of percentage hatch of total eggs set throughout the entire breeding season than either treatment A or treatment B. These results further indicate that treatment B (treated females inseminated with semen from non-treated males) exhibited the lowest level of percentage hatch of total eggs set throughout the breeding season. Treatment A (non-treated females inseminated with semen from non-treated males) maintained a level of percentage hatch of total eggs set which was significantly below treatment C throughout the breeding season.

Summary

This study, designed to investigate the effects of reserpine on the reproductive performance of breeding turkeys receiving artificial insemination, was conducted during the 1965-66 breeding season. Reserpine was administered to large white turkeys at the levels of 0.0 and 2.0

p.p.m., with one-third of each sex receiving the tranquilizer. Reserpine treatment was initiated when the turkeys were 26 weeks of age and was continued until the end of the 24-week breeding season.

The results indicated that reserpine treatments significantly depressed female body weight early in the breeding season, with the most pronounced depression occurring four to eight weeks after treatment was initiated. It was also observed that untreated females lost weight during the last half of the breeding season, while treated females gained or at least maintained their body weight.

The treated females had a significantly higher hen-day feed consumption than did non-treated females. A comparison of body weight and feed consumption of females showed that, although treated females consumed more feed, their average body weights were less than the non-treated females.

The reserpine treated females required five to seven days longer to attain sexual maturity than did the non-treated females. This difference was statistically significant.

In this study reserpine treatment significantly depressed percentage egg production in the first half of the breeding season. Differences due to treatment in the last half of the breeding season appeared to be negligible.

Comparisons made for percentage fertility indicated that non-treated females inseminated with semen from treated males had significantly higher fertility than did the controls.

Comparisons made for percentage hatch of fertile eggs indicated that non-treated females inseminated with semen from treated males had significantly higher hatchability than did the controls.

Comparisons made for percentage hatch of total eggs set indicated that again non-treated females inseminated with semen from treated males yielded significantly high percentages for hatch of total eggs set than did the controls.

Phase II. The Effect of Reserpine on the Metabolism of Whole Semen

Recent studies have indicated that reserpine administered in the diet of turkeys during the breeding season produced a marked decline in fertility. This observed reduction of fertility has been attributed to both sexes, although a greater reduction of fertility occurred in the female.

Although reports have been published on the general effects of reserpine on fertility in turkeys, a review of literature reveals that no work has been published on the possible changes in semen quality which may result from administering reserpine to mature turkey males. This study was designed to investigate some of the possible effects of reserpine on semen metabolism, as measured by oxygen consumption, in the domestic turkey. The objectives of this study were to determine the effect of reserpine fed to turkeys on:

1. the metabolism of whole semen as measured by oxygen consumption,
2. the sperm concentration of semen,
3. testes weight,
4. the percentage water in the testes,
5. the metabolism of testicular tissue as measured by oxygen consumption, and
6. body weight.

Review of Literature

Foote and Gray (1963) studied the effects of the tranquilizer, chlorpromazine, on the sperm production of dogs. Chlorpromazine was

administered orally to eight dogs at the rate of 4.4 mgs. per kg. of body weight every other day or daily for nine days. Results indicated that the motility of sperm collected from these dogs was unaffected, but sperm output was inconsistently higher in the treated group.

Prange and Bakewell (1966) reported that intravenous levels of 0.2 mg. of reserpine per kg. of body weight significantly increased the testes weight of mature male rats when administered for two weeks.

Eränkö et al. (1957) injected young male rats subcutaneously with 0.04 mg. of reserpine daily for two months. Controls were given the same amount of physiological saline. The animals were killed nine days after the last injection and the histological structure of the testes was studied in haematoxylin-eosin stained sections. Slides were classified according to the concentration of interstitial cells using two categories, "much" or "little". The results indicated that the reserpine treatment depressed the growth of the testes, as measured by the amount of interstitial tissue present ($P < .005$).

Khazan et al. (1960) injected five mature male pigeons, weighing between 250.0 and 300.0 gms. each, with 0.2 mg. of reserpine per kg. of body weight. This single injection caused a severe atrophy of the testes, with the testes from the treated pigeons weighing 20 to 50 percent less than the testes of the untreated pigeons. Histological examinations of testicular tissue sections revealed an impairment of spermatogenesis, due to reserpine treatment. Further microscopic examinations showed that spermatogenesis was arrested at the spermatid stage.

Hagen and Wallace (1961) injected one-month-old male chickens with 1.0, 2.0 and 4.0 mgs. of reserpine per kg. of body weight. These

injections were repeated at weekly intervals for three months. The results of this experiment indicated that the testes from reserpine treated chickens were only 10 percent of the weight of the testes from untreated chickens. Tissue sections from treated chickens exhibited extreme hypoplasia of the seminiferous tubules.

Anderson and Smyth (1960) studied the effects of continuous low level feeding of reserpine on the size and histology of the testes from large white male turkeys through 24 weeks of age. The results of this experiment indicated that reserpine administered at the level of 0.25 mg. per kg. of feed from the tenth through the twenty-fourth week of age could possibly stimulate testicular development. Microscopic examinations showed that the extent of spermatogenesis was positively related to testes size.

The detrimental effects of reserpine on fertility in turkeys have been reported by Rudolph et al. (1962), Casey et al. (1963) and Jones et al. (1966).

An assumption was made that the observed reduction in fertility due to reserpine treatment might be reflected in the metabolic activity of the semen. The idea that the rate of metabolism of semen reflects its fertilizing potential was reported by Bishop et al. (1954). In this study the metabolism of bull semen, as measured by oxygen uptake, was generally a reliable indicator of the fertilizing capacity of bull semen.

A number of subsequent investigations have in general confirmed this idea. Mercier and Salisbury (1946) studied the effect of season on the spermatogenic activity and fertility of dairy bulls used in artificial insemination. Results from this study indicated that the

metabolic activity of whole semen was significantly correlated to fertility.

Erb et al. (1952) and (1956) reported that the rate of semen metabolism was related to the fertility of dairy bulls representing several breeds.

Conflicting results have been observed in the relationship between the metabolic activity of semen and its fertilizing capacity in the class aves. Studies designed to investigate the effect of feeding thiouracil and thyroprotein on semen quality of chickens were reported by Shaffner and Andrews (1948) and Shaffner (1948). These reports indicated that semen metabolism was a reliable index for measuring the fertilizing ability of cock semen.

Bade et al. (1957) observed that oxygen uptake was not likely to furnish a means of distinguishing between turkey semen samples of different fertilizing capacities. It was concluded that the respiration rate of semen suspension was only a measure of the number of motile forms present.

Kosin (1958) noted that the respiration rate of whole turkey semen was a reliable measure of its fertilizing capacity, if adjusted for sperm concentration and the number of abnormal spermatozoa.

Experimental Procedure

Two of the three pens of sixteen males utilized in Phase I of this study were used as the source of donor birds for semen utilized in this phase. One pen of males received 2.0 p.p.m. of reserpine in a breeder ration. The control group received the same ration without reserpine.

Semen samples were obtained at the various ages by the abdominal

massage method. The initial collection was made when the toms were 36 weeks of age, followed by collections at 38, 40, 44, 46, 47 and 49 weeks of age. Since comparisons were to be made between treated and non-treated males, all measurements were made on pooled semen samples obtained from all of the sixteen males within each treatment. No attempts were made to adjust or balance an individual male's semen contribution to the pooled sample on different collection days.

The pooled semen samples for each treatment were collected into small wax-lined glass vials. The samples were collected in the morning and delivered to the laboratory within 25 minutes of the last collection. The collection period required approximately 35 minutes. The sequence of semen collection was rotated to avoid bias for any one pen of males.

A specimen of each sample of pooled semen was obtained for the sperm concentration determinations. Sperm concentrations were routinely determined by duplicate hemacytometer counts. All counts were made with a Spencer, Bright-Line hemacytometer. In each determination, a sample of well-mixed semen was drawn up to the 0.5 mark of the red blood cell pipette. The pipette was then filled to the 101 mark with cold water, (5°C.). The cold water was used to immobilize the spermatozoa while making the sperm counts. After agitating the pipette sufficiently to mix the semen and diluent, a drop of this suspension was applied to each of the counting chambers. Spermatozoa counts were made on five of the sixteen cells, and the mean value was then taken to calculate the sperm density as outlined by Allen and Champion (1955).

The remainder of each pooled semen sample was suspended in an avian Ringer's solution as was employed by Romanoff (1943). This suspension

was buffered at a pH of 7.3 in the ratio of one part of semen to 19 parts of Ringer's. Lardy and Phillips (1943) concluded that 7.25 was the optimum pH for the endogenous respiration of cock semen.

All metabolic determinations, in terms of oxygen consumption, were measured manometrically by the direct method using a Warburg differential respirometer as described by Umbreit et al. (1964). The gas phase was air and a shaking rate of 120 c.p.m. over a 2.5 cm. distance was used. The temperature of the water bath was maintained at 40°C. Kosin (1958) reported that favorable results were obtained by using a constant water bath temperature of 40°C.

Each treatment in each run was replicated five times. Readings were usually begun within 60 minutes after the last collection. As a standard procedure, each flask received 2.4 ml. of semen-Ringer's suspension in the main chamber. The center well received 0.4 ml. of 10 percent KOH in order to remove CO₂ from the flask during incubation. No flask was placed into the water until all flasks in the series were prepared. After an initial ten-minute equilibration period, readings were taken at fifteen minutes intervals for a period of two hours.

For purposes of statistical analysis, the rate of aerobic metabolism was expressed as microliters of oxygen consumed by 10⁸ spermatozoa per each fifteen minute period and the cumulated microliters of oxygen consumed per each fifteen minute period. Kosin (1958) reported that due to the continuous nature of the reactions, recording their progress as a series of independent readings taken at finite intervals appeared to be inappropriate. Assuming that the amount of oxygen consumed changes with time, then the variation among a group of cumulative readings for a given period is directly influenced by the amount of

variation expressed by this group of readings during the preceding period. An analysis of the amount of oxygen consumed for each fifteen minute period, although arbitrary, would reduce the effect of variation due to previous oxygen consumption. The initial readings were set at zero on all manometers, regardless of the treatment source of the semen sample.

An analysis of variance was used to analyze the results obtained within each trial and over all trials. For comparative purposes the cumulative microliters of oxygen consumed at the end of each hour of incubation were reported.

At 50 weeks of age each of the two groups of sixteen toms was divided into subgroups of eight males each. The males of one subgroup from each of the treatments were sacrificed, and two days later the remaining males were sacrificed. Live body weights were recorded before all males were sacrificed. From these males the weights of the paired testes were recorded. A 100.0 mg. sample of the larger testis of each pair was prepared for obtaining the metabolic determinations.

After homogenation, the prepared tissue sample was added to 2.0 ml. of avian Ringer's solution in each Warburg flask. The manometer readings obtained from the testicular tissue were adjusted for wet weight and estimated dry weight of the tissues as outlined by Umbriet et al. (1964). All incubation conditions were identical to those previously described for the semen samples. Due to a power failure during the first sequence of tissue incubation, the data obtained were not analyzed. The second trial was analyzed in a similar manner as that described for the semen measurements.

Results and Discussion

The effects of reserpine administered to large white turkey males, as measured by oxygen consumption of semen and testicular tissue, are presented in this section. Reserpine was administered in the diet at the rate of 0.0 and 2.0 p.p.m. from 26 through 50 weeks of age. The variables measured were sperm concentration, oxygen uptake of whole semen, testes weight, percentage water in the testes, oxygen uptake of testicular tissue and mature body weight.

The sperm count data for each of the trials are shown in Table XI. There were no statistically significant differences observed in the sperm counts between treatments within each trial. It was observed that the control birds had a slightly higher sperm count average than the reserpine treated males in all trials except trial 1 and trial 5.

The analysis of variance of the data for semen oxygen consumption within each trial indicated that differences due to treatment were significant at the one percent level of probability in all trials except trial 6. Second and third order interactions were not significant in this analysis. This analysis also indicated that time had a linear and cubic effect on oxygen consumption which was significant at the five percent level of probability. A quadratic effect for time was observed to be significant at the one percent level of probability.

The analysis of the results obtained over all trials indicated that the effect due to trial (age) was significant at the five percent level of probability. The effect of treatment and time interval were significant at the one percent level of probability. The second order interactions between age, time interval and treatment as well as the third

TABLE XI
 THE EFFECT OF RESERPINE FEEDING (2.0 p.p.m.)
 ON SPERM COUNTS IN TURKEY SEMEN

Period	Age (wks)	Control sperm count $\times 10^4/\text{mm}^3$	Reserpine Treatment sperm count $\times 10^4/\text{mm}^3$
1	36	900 \pm 86.3*	946 \pm 93.9
2	38	912 \pm 80.1	880 \pm 85.4
3	40	1093 \pm 97.9	1042 \pm 125.7
4	44	1008 \pm 94.8	960 \pm 90.3
5	46	745 \pm 67.1	815 \pm 80.0
6	47	1010 \pm 86.8	970 \pm 89.9
7	49	1078 \pm 98.8	1020 \pm 102.4

* Mean \pm Standard Error

order interactions were not significant in this study.

Table XII shows the cumulated oxygen consumption in microliters consumed per 10^8 spermatozoa at the end of one and two hours for each treatment in each trial. The first hour oxygen consumption values of endogenous respiration for the control birds in this study closely approximated those values reported for the turkey by Bade et al. (1956) and Kosin (1958). Table XII indicates that semen obtained from reserpine treated males exhibited a depression in aerobic respiration when compared to the respiration of semen from non-treated males. The difference in cumulated oxygen consumption seemed to increase with time.

An analysis of the testes weight was performed on the paired testes weights adjusted and unadjusted for body weight. This analysis yielded no significant differences between testes weights of treated and non-treated males.

The average percentage of water determined in the testicular tissue was 83.3 percent in the control males and 85.5 percent in the reserpine treated males. This observed differences of 2.2 percent was significant at the five percent level of probability.

Table XIII shows the observed cumulated microliters of oxygen consumed per 100.0 mg. of testicular tissue over a time interval of three hours. The analysis of variance of cumulated oxygen consumption per 100.0 mg. of testicular tissue, adjusted for wet weight, yielded no statistically significant differences due to reserpine treatment.

The analysis of variance for cumulated oxygen consumption per 100.0 mg. of testicular tissue, adjusted for dry weight, indicated that differences after two and three hours of incubation were significant at the ten percent level of probability. The values shown in Table XIII

TABLE XII

EFFECT OF RESERPINE FEEDING (2.0 p.p.m.) ON THE RESPIRATION OF TURKEY SPERMATOOZA AFTER 1 AND 2 HOURS OF INCUBATION AT 40°C.

Period No.	Age	Hours of Incubation			
		1st hour		2nd hour	
		Control	Reserpine	Control	Reserpine
		- μ l/10 ⁸ sperm-		- μ l/10 ⁸ sperm-	
1	36	9.4	5.2	15.3	8.0
2	38	8.0	7.2	14.7	12.2
3	40	10.5	10.0	19.3	17.3
4	44	9.3	8.3	16.5	14.6
5	46	8.6	7.4	14.7	12.5
6	47	9.9	8.9	14.9	13.9
7	49	9.4	8.4	16.3	12.6
Ave.		9.3	7.9	15.9	13.0

TABLE XIII

TOTAL MICROLITERS OF OXYGEN CONSUMED PER 100 mg.
OF TESTICULAR TISSUE FOR EACH TREATMENT AT
THE END OF EACH HOUR OF INCUBATION

Time (hrs)	Wet Weight			Dry Weight		
	Control	Reserpine	Diff.	Control	Reserpine	Diff.
	- μ l/100 mg.-			- μ l/100 mg.-		
1 hr.	0.30	0.32	0.02	1.98	2.44	0.42
2 hr.	0.55	0.56	0.01	3.38	4.20	0.82
3 hr.	0.67	0.75	0.08	4.49	5.55	1.04

indicate that the testicular tissue from reserpine treated males was consistently higher in oxygen consumption. The difference observed between the two treatments, when adjusted for wet weight, were not significant due to a large amount of variation within the treatments.

The average body weight for the control males was 30.3 lbs. and 33.6 lbs. for the reserpine treated males. The difference observed between these two treatments was significant at the five percent level of probability.

Summary

A series of trials was conducted to determine the oxygen metabolism of turkey semen collected from two groups of donor males. The control group received a standard breeder ration without reserpine. The treated group received 2.0 p.p.m. of reserpine in the same breeder ration.

Oxygen uptake was measured by the methods of Warburg and was based on the pooled semen from each group. As a general rule, 0.1 ml. of semen-Ringer suspension was added to 2.3 ml. of avian Ringer's solution buffered at a pH of 7.3.

In this study no significant differences were observed in sperm counts between treatment groups. Aerobic respiration was significantly depressed in semen produced from birds which were treated with reserpine. No significant differences due to treatment were noted for testes weights. Differences observed between the two treatments, with respect to percentage water contained in the testicular tissue, were significant at the five percent level of probability. Further differences were noted in the oxygen consumed per 100.0 mg. of testicular tissue. These differences were significant at the ten percent level of probability

when adjusted for dry weight. Differences in body weight between treated and non-treated males were significant at the five percent level of probability, with treated males weighing more than non-treated males. The observed differences in body weight tend to exhibit the weight-sparing effect of reserpine that has been shown to exist in a high temperature environment.

CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

Two separate experiments were conducted to investigate some of the effects of reserpine administered orally in the diet at the level of 2.0 p.p.m. to large white turkeys. Phase I presents the results of an experiment designed to examine the effects of reserpine on reproductive performance of artificially inseminated breeder turkeys. Phase II presents the results of an experiment designed to investigate some of the effects of reserpine on semen metabolism, metabolism of testicular tissue, and sperm concentration.

The results of Phase I indicate that reserpine treatment significantly depressed female body weight early in the breeding season. In the latter part of the breeding season, when higher temperatures prevailed, non-treated females lost weight while treated females gained or at least maintained their body weight. This weight-sparing effect of reserpine in warm weather was again exhibited in Phase II when treated males which were administered reserpine continuously from the twenty-sixth week to the fiftieth week of age outweighed non-treated males. This greater body weight of the treated males during warm weather was significant at the five percent level of probability.

The results of Phase I further indicate that reserpine treatment caused a significant increase in hen-day feed consumption of females. A comparison of body weight and feed consumption of females showed that

although treated females consumed more feed, their average body weights were less than that of the non-treated females.

Reserpine treatment increased the number of days to sexual maturity. The difference between treated and non-treated females for days to sexual maturity was significant at the five percent level of probability.

Comparisons were made between three treatment combinations with respect to the reproductive traits; percentage fertility, percentage hatchability, and percentage hatch of total eggs set. In all comparisons treatment C (non-treated females inseminated with semen from treated males) exhibited significantly higher values for all reproductive traits than did the other treatments. Treatment B (treated females inseminated with semen from non-treated males) exhibited the lowest percentage values for the reproductive traits. Treatment A (non-treated females inseminated with semen from non-treated males) exhibited intermediate percentage values for the reproductive traits, which in most cases were midway between treatment C and treatment B.

The results of Phase II showed no significant differences in sperm counts between treatment groups of males. Aerobic respiration was significantly depressed in semen produced from males which were treated with reserpine. This difference was significant at the one percent level of probability. No significant differences due to treatment were noted for testes weights. Differences observed between the treated and non-treated males, with respect to percentage water present in the testicular tissue, were significant at the five percent level of probability. Further differences were noted in the oxygen consumed per 100.0 mg. of testicular tissue. These differences were significant at the ten percent level of probability when adjusted for dry weight.

It should be noted that while semen from treated males exhibited the lowest rate of aerobic respiration, non-treated females inseminated with semen from the same donor birds exhibited a significantly higher percentage fertility, percentage hatch of fertile eggs, and percentage hatch of total eggs set than did the other treatment combinations.

The results of the experiments in this study indicate that the rate of metabolic activity, as measured by oxygen uptake, appears to be negatively associated with fertility. A high respiration rate could result from excellent environmental conditions and could possibly exhaust endogenous substrates prematurely. These considerations could be very important for the avian spermatozoa, in which fertilizing capacity implies the maintenance of functional life during several days or even several weeks in the oviduct of the female.

The results of the experiments in this study tend to support the conclusion of Ogasawara and Lorenz (1964) wherein semen exhibiting the lowest respiration rate tends to yield the highest percentage fertility, percentage hatchability, and percentage hatch of total eggs set.

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