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Influence of Some Stressors on Peripheral Blood Response to Estrogen Stimulation in the Female Mallard Duck

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INFLUENCE OF SOME STRESSORS ON PERIPHERAL BLOOD RESPONSE TO ESTROGEN STIMULATION IN THE FEMALE MALLARD DUCK

by

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INTRODUCTION

Presentation of the Problem

It has been shown that the reproductive state of the female mallard duck, Anas platyrhyncos, bears a very definite relationship to the levels of serum calcium and total protein which increase at the approach of the breeding season (Decker, 1965). These changes in the levels of calcium and protein were attributed to increasing blood titers of estrogen. Exogenous administration of 17-"B"-estradiol has also been observed to increase the levels of serum calcium and protein (Hofman, 1966; Smoes, 1967). Due to this relationship between these blood chemical constituents and the reproductive state of the duck, the breeding condition of this bird can be determined from the level of these constituents. However, these observations are limited in their application since they have not taken into account the effect of stress. Therefore, the present study was undertaken to investigate the influence of "stress" on the response of the blood chemical constituents of the female mallard duck to endogenous estrogens and exogenous 17-"B"-estradiol and to determine whether the level of these constituents still reflect the level of circulating estradiol under stressful conditions. The stressors employed in this research were confinement, cold and exogenous ACTH.

Literature Review

The breeding period of the domestic fowl is characterized by high levels of serum calcium and protein (Sturkie, 1965). The increase in serum calcium begins at the onset of the breeding season and undergoes a marked elevation just preceding egg-shell formation. This hypercalcemia is maintained until shell formation is completed (Riddle and Reinhart, 1926). Riddle and Dotti (1936) produced similar effects on the calcium content of normal and castrated pigeons by the administration of estrogen. In birds the blood calcium is present in two forms, the diffusable (non-ionized and ionized calcium) and the non-diffusable form (bound calcium) (Sturkie, 1965; Urist, 1959). The increase in the serum calcium is principally in the bound fraction but the mechanism is not completely understood. Kyes and Potter (1934), in working with pigeons, observed that during ovarian follicular development the endosteal cavity of the long bones of birds filled with bony trabeculae. Laudauer et al. (1941) reported that it is possible to produce this type of new bone formation in pigeons by estrogen injections and that this newly formed trabeculae becomes calcified. This medullary bone serves as a calcium reserve for the formation of the egg shell (Hurwitz, 1964). Urist (1959) observed that estrogen acts in conjunction with parathormone from the pituitary gland in increasing the blood calcium levels by causing the reabsorbtion of the calcified medullary bone. Greenburg et al. (1936) reported that estrogen stimulates the appearance of phosphoproteins with a high calcium binding capacity, presumably to bind the newly released calcium.

In addition to raising serum calcium levels, estrogen also increases the total protein (Sturkie, 1965; Hofman, 1966). Hofman (1966) reported that daily injections of estradiol increased the total protein level from 4.8 g% to 7.6 g%. This increase in the total protein was due to an increase in phosphoprotein, lipoprotein, and the globulin fraction. Hofman (1966) observed that the globulin fraction increaed from 2.0 g% to 5.1 g% where as the albumin fraction decreased from 2.8 g% to 2.6 g% after estradiol treatment in the non-breeding female mallard duck. The fractions analyzed in this research were albumin and globulin. Proteins from these two fractions, in addition to mucin, are present in the egg albumen. Sturkie (1965) and Hofman (1966) reported that albumin to globulin ratio could be used to indicate the reproductive state of the bird because the value of this ratio depended upon the estrogen titer. Hofman (1966) reported that the ratio of the albumin to globulin (A/G ratio) in the non-breeding (prenuptial) female mallard duck was about 1.4 as compared th the A/G ratio of about .6 in the breeding mallard duck.

Besides affecting the blood chemistry, estrogen also induces growth of the avian oviduct. Administration of 17-B"-estradiol has been shown to increase the size of the oviduct in immature pullets (Lorenz et al., 1962) and in mature female mallard ducks by Hofman (1966). Because the serum levels of calcium, total protein and the A/G ratio depend upon the amount of estrogen present, the levels of these blood constituents can be employed as indices of the reproductive state of the bird.

It has been shown that unfavorable environmental conditions such as overpopulation and cold result in a decrease in reproduction

(Christian, 1955, 1956; Wilson, McNally and Ota, 1957). Christian (1955, 1956) demonstrated, in working with mice, that overpopulation resulted in a decrease in the reproductivity. Siegel (1959b) found that the total egg production was significantly lowered in white leghorn pullets at 1.33 feet square per bird for 196 days than those allotted 4 feet square per bird. The stressors involved in the work conducted by Siegel (1959b) were both competition and social interaction from crowding. On the other hand, limited work conducted by Smoes (1967) indicated that confinement alone did not necessarily suppress reproduction in semidomesticated mallard ducks.

Christian (1960) attributed the decrease in reproductivity in the white mouse to an increase in adrenocortical activity as a result of increase secretion of ACTH and a decrease in the pituitary secretion of gonadotropins. As evidence of increased ACTH secretion, he noted the cellular hyperplasia of the zonae fraciculata-reticularis of the adrenal gland. Flickinger (1961) noted that the adrenals of grouped birds were significantly larger than those of paired birds. The increase in the adrenal glands was associated with the number of birds present. Siegel (1958a) also found adrenocortical hyperplasia in young male and female chickens when subjected to increased population densities. He agreed with Christian that the decrease in reproduction under conditions of overpopulation may result from stress stimulation of corticotropin release with a concomitant decrease in gonadotropin secretion. From the works of Christian and Siegel, it can be concluded that the pituitary gland is the endocrine organ which initiates this stress response by releasing ACTH causing adrenocortical hyperplasia.

Jarrett (1965) speculated that ACTH stimulated the release of adrenal androgens which suppressed the release of LH from the pituitary gland. Wilson et al. (1958) reported the presence of increased levels of androgens in female mice which had an ACTH-producing pituitary tumor. He attributed the presence of excess androgens to the action of ACTH on the adrenal gland. Mason et al. (1958) found a significant increase in the secretion of androgens after administration of ACTH to human subjects. Varon and Christian (1963) reported that androgens could suppress the release of LH from the pituitary gland in immature female mice. Zarrow, Greenman and Peters (1961) reported that certain androgens inhibited the estrogen effect on the oviduct of the chick. Flickinger (1966) has suggested an approach other than the inhibition of LH from the pituitary gland in reproductive suppression. He proposed that the increased levels of adrenal corticoids may interfere with the reproductive responses to gonadotrophic hormones or gonadal hormones (estrogen) among birds.

Cold is a third type of factor which is able to suppress reproduction. Wilson, McNally and Ota (1957) reported that air temperatures lower than 9 degrees centigrade depressed egg production in chickens. Cold initiates stress response characterized by ACTH release in young chickens (Chancellor and Glick, 1960). Chowers et al. (1964) noted that in dogs a sudden lowering of the preoptic temperature increased cortisol levels and proposed that cold initiated a defense mechanism which activates the pituitary-adrenal system. Cortisol is the primary adrenal corticoid in mammals. The stimulatory effect of low temperatures on adrenocortical function in the rat is well established (Katsh et al.,

1954; Levin, 1945; Sayers and Sayers, 1959). The stimulatory effect is probably mediated through the adenohypophyseal release of ACTH, since hypophysectomy abolished the response (Long, 1947).

Since the inhibitive effect of these environmental stressors is in part via the release of ACTH from the pituitary gland, it was of interest to determine the effect of exogenous ACTH on the response of both the blood chemical constituents and the reproductive tract of birds treated with estrogen. Christian (1964) and Jarrett (1965) reported that administration of ACTH in mice decreased both ovarian and uterine weights. Flickinger (1966) observed a similar effect on the ovary and oviduct of the chicken and noted that the suppressive influence of ACTH was proportional to the dose administered. Urist and Deutch (1960) reported that injection of ACTH caused hyperplasia of the adrenal gland. Gonadal atrophy associated with adrenocortical hyperplasia in ACTH-injected birds resembled the endocrine adaptation resulting from increased social pressure among birds (Flickinger, 1965). Jarrett (1965) and Brimblecomb (1954) in working with mice have found that the adrenal gland must be present for the response to ACTH. On the other hand, Christian (1964) has suggested that ACTH might exert its effect extra-adrenally. This hormone is specific for the adrenal gland but it is known to exert a number of extra-adrenal responses (Turner, 1961). He reported that ACTH prevented ovulation in the intact and adrenalectomized corticoid-maintained birds and concluded that the action of ACTH on the ovary is independent of the adrenal glands.

METHODS AND MATERIALS

Experimental Animals

The animals used in this research were semidomesticated female mallard ducks all of which were approximately nine months old at the outset of the study. The ducks were obtained from a commercial supplier (Whistling Wings, Hanover, Illinois).

Prior to their use as experimental animals, the ducks were housed on a farm in Portage, Michigan. Throughout each of the four experiments, the ducks were maintained in Wood Hall (Western Michigan University) under constant conditions of light and temperature. The photoperiod was 11 hours light and 13 hours dark for the first two experiments and 12 hours light and 12 hours dark for the last two. The ducks not placed in the cold room were exposed to fluorescent lights with an intensity of 25 foot candles and those placed in the cold room were exposed to a 150 watt bulb with an intensity of 25 foot candles. The birds not exposed to cold were maintained at a temperature of 18-25 degrees centigrade. Those placed in the cold room were exposed to a temperature of minus 9.7 degrees centigrade. Two ducks were placed in each cage. These stainless steel cages measured 36" X 25" X 28" and were fitted with wire matting small enough to facilitate walking yet large enough for the fecal matter to pass through. Food and watter were given ad libitum. The cages were equiped with a 28" X 6.5" X 5" water trough. The food consisted of Napiana pellets (see Table 1 of Appendix A for composition).

Blood Sampling and Serum Analysis

Blood was collected from the metatarsal vein into a sterile vial according to the procedure of Decker (1965) as modified by Hofman (1966). The duck was suitably restrained and placed on a specially designed table. The area distal to the knee joint was cleansed with alcohol and allowed to dry. Pressure was then applied to the vein where it crosses the joint, resulting in an enlargement of the vein. The vein was then punctured with a heparinized hypodermic needle and about 2 milliliters of blood was allowed to flow down the leg into the sterile plastic vial. The blood was placed in a refrigerator to allow for complete clotting. The time necessary for clotting vaired from 15 minutes to 45 minutes according to the speed of collection. The clotted blood was centrifuged at 3,000 rpm for five minutes after which the supernatant serum was removed and immediately frozen for analysis at a later date.

Calcium concentration was determined with a Turner fluorometer using a method derived from the works of Wallack and Steik (1963) and Kepner and Hercules (1963). A commercial standard, Versatol (General Diagnostics Company) with known concentrations of blood constituents was analyzed with each serum analysis to insure the continued accuracy of the procedure.

The total protein was read directly in grams per hundred milliliters with an American Optical T.S. meter. The analysis of albumin and globulin fractions of the total protein was carried out by electrophoretic separation of these constituents on cellulose acetate strips

followed by analysis of the strips with a Photovolt Densitometer equipped with an integrator. For the electrophoresis a Gellman cell was used with a Barbatol Barbitone buffer (pH of 8.6). The strips were stained with Ponceau S and cleared with methanol and acetic acid.

Experimental Procedure

This study consisted of four experiments. The ducks were transported to Wood Hall prior to each experiment and were exposed to the laboratory conditions for at least three days prior to the state of the experiment. During this period pretreatment blood samples were collected and analyzed for calcium, total protein, albumin and globulin to establish pretreatment levels.

Preseason Experiment 1

The first phase of this research was designed to investigate the effect of confinement and exogenous ACTH on the response of serum calcium, total protein and the albumin and globulin fractions of the total protein to exogenous 17-"B"-estradiol.[1] Twelve non-breeding (prenuptial) female mallard ducks were used in this experiment, which was conducted from December 7, 1966, to December 24, 1966. On day 1 the 12 ducks were weighed, banded and arbitrarily divided into six groups of two ducks each. Each of the ducks was given a daily subaxial injection of 2.5 mg 17-"B"-estradiol disolved in peanut oil. In addition, a stressor agent was applied to five of the six groups according to the

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[1]Estradiol provided by The Upjohn Company, Kalamazoo, Michigan.

schedule in Table 1. As listed in the table, the birds numbered S-29, S-30, S-33 and S-34 were treated with estradiol for one week prior to the application of the stressor. Birds numbered S-27, S-28, S-31 and S-32 were "stressed" concurrently with the estradiol treatment. The stressors in this experiment were confinement and administration of Depo-ACTH.[2] The selection of confinement was based on the observations of Smoes (1967) and the fact that it is a commonly used stressor in physiological work (Robert, Nezamis and Phillips, 1965). In this experiment estrogenized ducks were confined in 11" X 9.5" X 7" rat cages as compared to the larger cages to which the birds were acclimated. The dose of ACTH selected was based on the work conducted by Garren, Hill and Carter (1960). Garenn, et al. (1960) found that 6 U.S.P. units of ACTH was sufficient to initiate a stress response in the young chicken. When one bird received both estrogen and ACTH concurrently, they were injected into opposite axillary spaces to decrease the possibility of irritation and interaction.

At the termination of the experiment, one duck from each group was sacrificed; the reproductive tract and adrenal glands were removed and fixed for a permanent record. In addiiton, histological sections were made of the adrenal glands using the stains hemotoxylin and eosin.

Blood samples were collected on every third day from each bird and the weights of the birds were recorded at these times.

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[2]ACTH (Porcine) supplied by The Upjohn Company, Kalamazoo, Michigan.

Preseason Experiment Number 2

The second phase of the research was designed to re-examine the relationship between estrogen and ACTH, using different dosage levels of each. This experiment which began on January 14, 1967, and ended on February 1, 1967, required 12 ducks. On day 1 the ducks were banded, weighed and pretreatment blood samples were taken. Two ducks were placed in each cage and each pair served as a treatment group. The treatment was similar to that of Preseasonal Experiment 1 except that the dose of 17-"B"-estradiol was decreased to .25 mg per day and ACTH treatment was increased to 12 U.S.P. units per day. This level of estradiol was the minimal dosage which resulted in significant increases in the blood chemical constituents of the mallard duck (Hofman, 1966). ACTH and 17-"B"-estradiol were administered subaxially. Again, when these two substances were administered at the same time, opposite axillary spaces were used to decrease irritation and possible interaction. The birds were confined in the rat cages as in Preseasonal Experiment 1. The schedule of treatment appears in Table 2. Delay groups were included as before; birds numbered S-45, S-46, S-49 and S-50 were estrogenized for one week prior to application of "stress."

One duck from each treatment group was sacrificed at the end of the experiment; its reproductive tract was removed and fixed for a permanent record. Blood samples were collected at these times.

Seasonal Experiment 1

This experiment was designed to examine the stressors' influence on the blood constituents during the breeding season (nuptial) when

the birds were under natural forms of stimulation. Here and throughout this paper stimulation refers to increased estrogen blood titer. No exogenous 17-"B"-estradiol was administered during this experiment which was conducted from March 25, 1967, to April 12, 1967. The birds were stimulated only by endogenous estrogens from the developing ovarian follicles.

Fourteen birds were used in this experiment and again the birds were banded, weighed and two birds were placed in each cage. Pretreatment blood samples were collected as in the first two experiments.

A series of increasing doses of Depo-ACTH from 1 to 6 U.S.P. units were given according to the schedule in Table 3. ACTH was administered as in the first two experiments. Exposure to cold was used as the environmental stressor. Altogether four birds were placed in a cold room of minus 9.7 degrees centigrade with a photoperiod of 12 hours light and 12 hours dark. Two of this group were maintained under the same condition as the controls for one week and placed in the cold room for three weeks, and two were maintained under the same conditions as the controls for two weeks before exposure to cold. Each bird was placed in a separate 36" X 25" X 28" cage to eliminate any possible overlapping of stress responses from competition and crowding. Each bird was supplied with food and water ad libitum. The water was changed every four hours during the light period; no changes were made during the dark period.

Blood was collected on every third day as before and weights were recorded. All the birds were sacrificed and their reproductive tracts were removed and fixed for a permanent record.

Seasonal Experiment 2

This experiment was conducted twice, on April 11, 1967, and on April 15, 1967, to determine the time interval between injection of ACTH and the response. No exogenous 17-"B"-estradiol was administered to these birds. On each of the trials the birds were brought to Wood Hall the day before treatment to decrease the possibility of distorting the response to ACTH. The dose of ACTH selected was based on the observation in Preseasonal Experiment 2 that 12 U.S.P. units per day completely suppressed the bird stimulated with .25 mg of 17-"B"-estradiol per day. In each trial, pretreatment blood samples were collected, followed by administration of 12 U.S.P. units of ACTH. Blood samples were taken at 1/2, 11/2, 6 and 24 hours after injection on the first trial. The pretreatment blood samples were designated as 0. During the second trial, blood samples were collected at 3, 8 and 12 hours after injection. Again, the pretreatment sample was designated as 0. The response of the blood chemical constituents of the bird to 12 U.S.P. units of ACTH over a 24 hour period was constructed using a composite of all the samples.

Statistical Analysis

The data were treated statistically by analysis of variance and *t* tests of the means. The analysis of variance was performed on the differences among the treatment values of each day and the pretreatment means. Statistical *t* tests were performed between the pretreatment and posttreatment means of each bird and between the mean of the

means of each ACTH treated group and the control group in seasonal experiment. In addition, the values of each ACTH treated group and the control group were subdivided into two groups. The first consisted of the values on days 1 through 15 and the second on days 18 through 30. Statistical *t* tests were performed between each of the four ACTH treated groups and the control group using the means of these submeans.

Table 1: Treatment schedule for birds in the preseason experiment 1.

Bird number	Treatment on per day basis	Duration
S-23	2.5 mg estradiol	Dec. 10-24
S-24	2.5 mg estradiol	Dec. 10-24
S-27	2.5 mg estradiol and 6 U.S.P. units ACTH	Dec. 10-24
S-28	2.5 mg estradiol and 6 U.S.P. units ACTH	Dec. 10-24
S-29	2.5 mg estradiol	Dec. 10-16
	2.5 mg estradiol and 6 U.S.P. units ACTH	Dec. 17-24
S-30	2.5 mg estradiol	Dec. 10-16
	2.5 mg estradiol and 6 U.S.P. units ACTH	Dec. 17-24
S-31	2.5 mg estradiol confinement	Dec. 10-24
S-32	2.5 mg estradiol confinement	Dec. 10-24
S-33	2.5 mg estradiol	Dec. 10-16
	2.5 mg estradiol confinement	Dec. 17-24
S-34	2.5 mg estradiol	Dec. 10-16
	2.5 mg estradiol confinement	Dec. 17-24

Table 2: Treatment schedule for birds in the preseason experiment 2.

Bird number	Treatment on per day basis	Duration
S-39	12 U.S.P. units ACTH	Jan. 14-29
S-40	12 U.S.P. units ACTH	Jan. 14-29
S-41	confinement	Jan. 14-29
S-42	confinement	Jan. 14-29
S-43	.25 mg estradiol and 12 U.S.P. units ACTH	Jan. 14-29
S-44	.25 mg estradiol and 12 U.S.P. units ACTH	Jan. 14-29
S-45	.25 mg estradiol	Jan. 14-22
	.25 mg estradiol and 12 U.S.P. units ACTH	Jan. 23-29
S-46	.25 mg estradiol	Jan. 14-22
	.25 mg estradiol and 12 U.S.P. units ACTH	Jan. 23-29
S-47	.25 mg estradiol confinement	Jan. 14-29
S-48	.25 mg estradiol confinement	Jan. 14-29
S-49	.25 mg estradiol	Jan. 14-22
	.25 mg estradiol confinement	Jan. 23-29
S-50	.25 mg estradiol	Jan. 14-22
	.25 mg estradiol confinement	Jan. 23-29

Table 3: The treatment schedule for the birds in seasonal experiment 1.

Bird number	Treatment on per day basis	Duration
S-20	1 U.S.P. unit ACTH	March 19-April 12
S-11	1 U.S.P. unit ACTH	March 19-April 12
S-21	3 U.S.P. units ACTH	March 19-April 12
S-5	3 U.S.P. units ACTH	March 19-April 12
S-18	5 U.S.P. units ACTH	March 19-April 12
S-10	5 U.S.P. units ACTH	March 19-April 12
S-17	6 U.S.P. units ACTH	March 19-April 12
S-22	6 U.S.P. units ACTH	March 19-April 12
S-1	cold (-9.7 degrees C)	March 19-April 12
S-24	cold (-9.7 degrees C)	March 19-April 12
S-14	control conditions	March 19-March 26
	cold (-9.7 degrees C)	March 27-April 12
S-19	control conditions	March 19-March 26
	cold (-9.7 degrees C)	March 27-April 12
S-16	control conditions	March 13-April 12
S-4	control conditions	March 13-April 12

RESULTS AND DISCUSSION

As stated previously, the objective of this research was to determine whether the application of stressor agents would interfere with the use of the blood chemical constituents, calcium, total protein and the A/G ratio, as indices of the reproductive state of the mallard duck. Preseason Experiments 1 and 2 were performed to investigate the influence of two types of stressor agents (confinement and ACTH) on the response of the three blood chemical constituents to exogenous estradiol. In Preseason Experiment 1, each of the ducks was given a daily injection of estradiol. In addition, a stressor was applied to five of the groups (Table 1). In Preseason Experiment 2, two of the groups were treated with only a stressor agent and four of the groups were treated with a combination of estradiol and one stressor agent (confinement or ACTH) (Table 2). Seasonal Experiment 1 was performed to investigate the influence of cold as well as ACTH on the three blood chemical constituents of the naturally stimulated bird. Seasonal Experiment 2 was designed to determine the interval between injection of ACTH and the response, and the effect over a 24 hour period.

Preseason Experiment 1

In this experiment the serum levels of calcium, total protein, albumin, globulin and the A/G ratio of estradiol-injected birds, estradiol-ACTH-injected birds and estradiol-injected confined birds were analyzed and their reproductive tracts examined at the end of the experiment. Due to the inability of the American Optical T.S. meter

to measure total protein levels which exceeded 15 g%, the true total protein levels of the birds on days 9 through 15 were not determined. These were listed in Table 2, Appendix B as 15+. No posttreatment means were calculated for the total protein and the relative concentrations of albumin and globulin were not calculated because an exact value for the total protein was necessary for the calculations. In addition, no statistics were used to examine these three parameters.

The means of the results of the first experiment are presented in Table 4 and individual values in Table 2, Appendix B. An analysis of variance of the serum calcium levels and A/G ratios did not reveal any significant differences between the treatment groups on any blood sampling day (Table 5). This result indicates that, at these levels, the stressor agents had no significant effect on the response of the blood chemical constituents analyzed to exogenous estradiol. The lack of significant differences were probably due to the large amount of estradiol administered.

Examination of the blood chemical constituents variation of each bird was carried out to investigate any non-statistical differences which might be present. The results of the analysis of the blood chemical constituents of each bird are presented in Table 2, Appendix B and the pretreatment and posttreatment means of the serum calcium and the A/G ratio for each treatment group are presented in Table 4.

During the treatment period, the serum levels of calcium and total protein increased and the A/G ratio decreased (Table 2, Appendix B). Although it was not possible to calculate the relative concentrations of albumin and globulin during the treatment period, the

Table 4: Pretreatment (Pr) and posttreatment (Pt) means of serum calcium and the albumin to globulin ratio (A/G) of the groups in preseasonal experiment 1.

Group	Treatment on per day basis	No. of samples		Calcium (mg%)	A/G (ratio)
1		4	Pr	11.25	1.48
	2.5 mg estradiol	9	Pt	53.25	.36
2		4	Pr	10.75	1.31
	2.5 mg estradiol and 6 U.S.P. units ACTH	10	Pt	45.23	.43
3		4	Pr	10.80	2.66
	2.5 mg estradiol and 6 U.S.P. units ACTH-delay	10	Pt	55.27	.30
4		4	Pr	10.80	3.28
	2.5 mg estradiol and confinement	8	Pt	40.25	.45
5		4	Pr	11.05	2.68
	2.5 mg estradiol and confinement-delay	10	Pt	59.45	.45

Each group consists of 2 individuals.

Table 5: Analysis of variance results of the serum calcium and A/G ratios of all groups on each day after the initiation of treatment in preseason experiment 2.

Days after treatment start	Calcium	A/G ratio
3	F = .67 Df = 4,5	F = .52 Df = 4,5
6	F = 2.22 Df = 4,5	F = .38 Df = 4,5
9	F = 1.14 Df = 4,5	F = .57 D f = 4,5
12	F = .87 Df = 3,4	F = .06 Df = 3,4
15	F = .31 Df = 4,5	F = .26 Df = 4,5

electrophoretec pattern of serum samples on consecutive blood sampling days clearly indicated that the decrease of the A/G ratio was the result of an increase in the globulin fraction and a decrease in the albumin fraction. This result was in agreement with the work conducted by Hofman (1966) which also found a reversal of the A/G ratio after administration of estradiol to the non-breeding female mallard duck manifested in the same way. The increase in the globulin fraction in the present research was primarily due to increased beta globulins as well as some alpha globulins. Some increase in the gamma globulins was also noted. It will be shown in Seasonal Experiment 2 that estradiol increases the globulin fraction of the total protein by increasing the beta globulins. The response of all three globulin fractions might be due to the large dose of estradiol (2.5 mg/day) administered. One bird from each treatment group was sacrificed and its reproductive tract examined. The ovary of every examined bird was suppressed and the mean oviduct weight of the examined birds was 7.01 grams $\pm$ .18.

The levels of calcium, total protein and the A/G ratio of the ACTH delay birds (S-29, S-30, Table 2, Appendix B) were similar to the estradiol-injected birds (S-23, S-24, Table 2). Birds treated with ACTH and estradiol (S-27, S-28) from the outset had lower levels of serum calcium and a delay of 3 days in attaining the maximum response of this constituent (Figure 1). In bird S-28 there was a lower level to total protein (Table 2, Appendix B). It may be that these birds were initially suppressed but overcame the inhibitive influence of the ACTH. This phenomenon of "recovery" will be more clearly shown in Seasonal Experiment 1. The results also indicate that ACTH had a greater

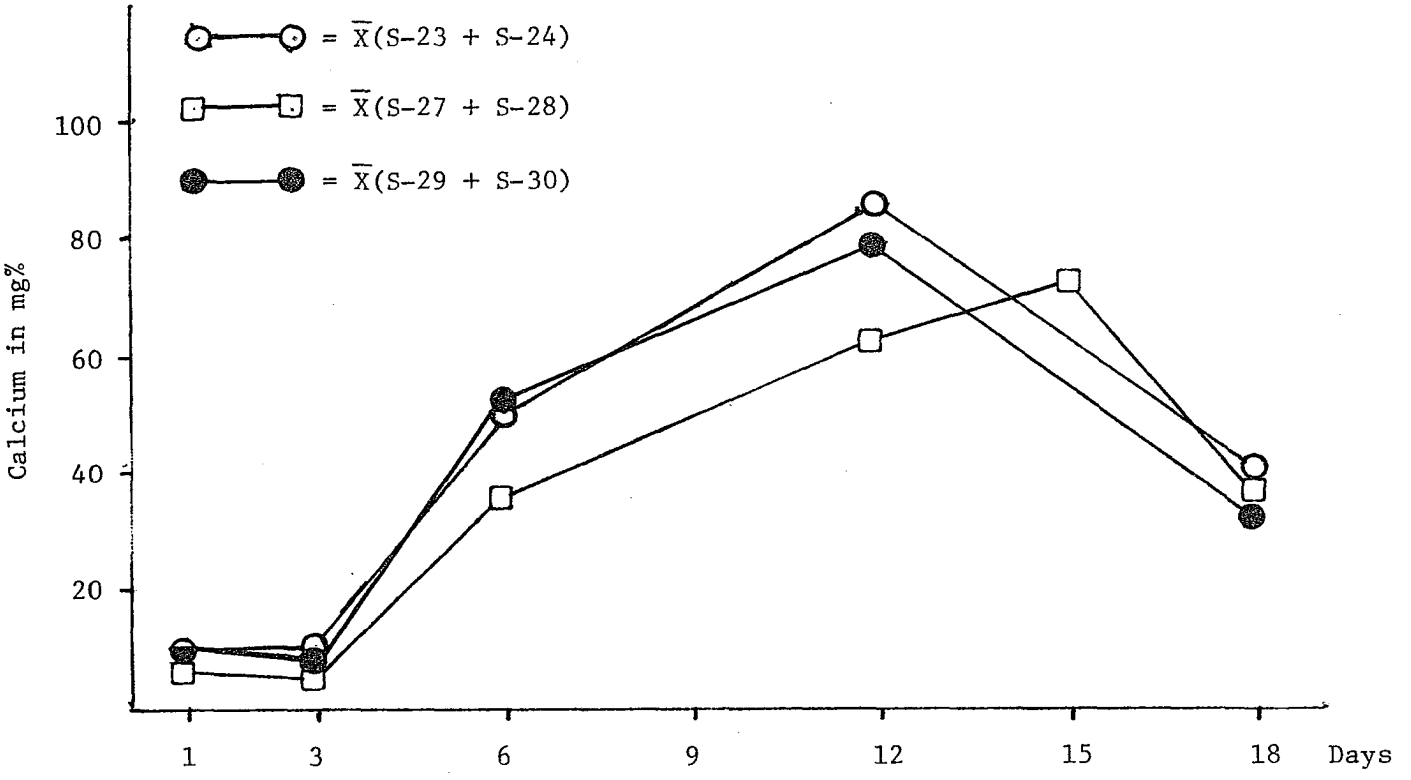

Figure 1: Serum calcium means of estradiol-treated birds (S-23, S-24), estradiol-ACTH-treated birds (S-27, S-28) and estradiol-ACTH-treated delay birds (S-29, S-30).

influence on the response of the blood chemical constituents to exogenous estradiol when both were administered to the untreated bird than when used on a previously estrogenized bird (Group 3 and Table 2, Appendix B).

From the results of the ACTH-injected birds in this experiment and the effect of ACTH in Preseasonal Experiment 2, some speculations were made. As will be shown, a daily dose of 12 U.S.P. units of ACTH per day inhibited the increase of serum calcium induced by a .25 mg dose of estradiol per day, either by acting directly on the estradiol levels or by some response mechanism. It appears to require more estradiol to initiate an increase in the serum calcium level than to maintain the increased level. If ACTH were able to only indirectly inhibit the estradiol induced hypercalcemia, the effect of ACTH might not be sufficient to decrease an existing high serum calcium level. Where as, if ACTH were administered with the estradiol to the previously untreated bird, the effect of the ACTH might be sufficient to result in a lower serum calcium level either by reducing the blood level of estradiol or through the response of the target organs to it.

Confinement appeared to have no influence on the response of the serum calcium, total protein or the A/G ratio to daily administration of 2.5 mg estradiol. The levels of these constituents during the treatment period were quite similar to the estradiol treated birds (Table 2, Appendix B) as were the posttreatment means (Table 4).

Preseason Experiment 2

This experiment was designed to examine further the influence of ACTH and confinement on the response of the same blood chemical constituents of mallards using different dose levels of ACTH and estradiol. In addition to the groups in the first experiment, an ACTH-injected group and a confined group were included without estradiol treatment to determine the effect of the stressor agents alone on the serum levels of calcium, total protein, albumin and globulin.

The estradiol injected birds employed for comparison were part of an experiment conducted simultaneously under identical conditions of light and temperature but were maintained on a different diet (Smyrnios, personal communication). The posttreatment means of the A/G ratio decreased but the decrease was the result of higher gamma and alpha globulins with little change in the albumin fraction, rather than the expected increase in the beta globulins and a decrease in the albumin fraction seen by Hofman (1966) and in the first experiment in this study. Because this difference indicated an abnormal response, the values of the albumin, globulin and the A/G ratio of the estradiol-injected birds (Group 7, Table 6) were not included in the statistical analysis of the groups in this experiment.

The results of this experiment are presented in Table 6 and individually in Table 2, Appendix C. An analysis of variance was performed on the levels of calcium, total protein, albumin and globulin and the A/G ratio. As stated above, the statistical analysis of calcium and total protein included the estradiol treated controls where the analysis

Table 6: Pretreatment (Pr) and posttreatment (Pt) means of serum calcium, total protein (TP), albumin (Alb), globulin (Glb) and the ratio (A/G) of the groups in preseasonal experiment 2.

Group	Treatment on per day basis	No. of samples		Calcium (mg%)	TP (g%)	A/G (ratio)	Alb (g%)	Glb (g%)
1		2	Pr	10.60	4.95	2.07	3.2	1.7
	12 U.S.P. units ACTH	10	Pt	9.95	3.91	1.00	2.2	2.6
2		2	Pr	10.49	4.00	1.45	2.3	1.7
	confinement	10	Pt	18.47	5.01	1.34	2.6	2.4
3		2	Pr	9.00	4.85	2.10	3.0	1.8
	.25 mg estradiol and 12 U.S.P. units ACTH	10	Pt	9.95	4.03	.76	2.7	3.1
4		2	Pr	11.00	4.84	1.92	3.0	1.6
	.25 mg estradiol and 12 U.S.P. units ACTH delay	10	Pt	17.65	5.40	1.12	3.1	2.7
5		2	Pr	10.75	4.40	3.20	3.2	1.2
	.25 mg estradiol and confinement	10	Pt	19.46	4.83	1.08	2.8	3.0
6		2	Pr	11.05	4.40	1.27	2.6	2.1
	.25 mg estradiol and confinement delay	7	Pt	26.54	4.83	.96	3.3	2.7
7		2	Pr	10.50	4.45	2.74	2.8	1.6
	.25 g estradiol	12	Pr	19.16	4.70	.75*	2.2*	3.3*

Each group consists of 2 individuals.

*Not a mean.

of the albumin, globulin and the A/G ratio was performed without the control group. A significant F ratio ($P < .05$) was found for the A/G ratio on the first blood sample after the initiation of the treatment and for serum calcium on the second blood sampling day after initiation of the treatment. These significant F ratios indicate that at least one group was significantly different from another on that day. The group (or groups) having an A/G ratio most different from the others was not readily determined by inspection. The two groups which had a calcium level most different from the other groups were Group 1 which was treated with 12 U.S.P. units of ACTH per day and Group 3 which was treated with 12 U.S.P. units of ACTH and .25 mg estradiol per day (Table 6). The fact that the ACTH-estradiol-treated birds (Group 3, Experiment 2) were similar to the ACTH-treated birds (Group 1, Experiment 2) and one or both were significantly different ($P < .05$) from the other groups (Table 7) further indicated that ACTH has the ability to suppress the estradiol-induced hypercalcemia.

In Group 1, essentially no increase in the serum levels of calcium and total protein were recorded during the treatment period. However, there was a slight increase in the levels of these two constituents after the tenth day of treatment in bird S-39 (Table 2, Appendix C). In both birds, there was an increase in the globulin fraction of the total protein due to an increase in the gamma and alpha globulins. The concentration of the beta globulins was comparatively low in these birds. These two responses coupled with a minor decrease in the albumin fraction resulted in a decrease in the A/G ratio. As indicated previously, there were no significant differences between the values of albumin and

Table 7: Analysis of variance results of the serum calcium, total protein, albumin, globulin and A/G ratio during the treatment period in preseasonal experiment 2.

Days after treatment	Calcium	Total Protein	A/G	Albumin	Globulin
3	F = 1.20 Df = 5,6	F = 1.91 Df = 6,7	F = 4.74* Df = 5,6	F = 1.65 Df = 5,6	F = 1.89 Df = 5,6
6	F = 3.93* Df = 6,7	F = 2.21 Df = 6,7	F = 3.34 Df = 5,6	F = 2.89 Df = 5,5	F = 3.94 Df = 5,5
9	F = 1.33 Df = 6,7	F = 1.04 Df = 6,7	F = 2.70 Df = 5,6	F = .46 Df = 5,6	F = 3.49 Df = 5,6
12	F = 1.17 Df = 5,6	F = .42 Df = 6,7	F = 3.26 Df = 5,6	F = .098 Df = 5,5	F = 2.37 Df = 5,5
15	F = .87 Df = 6,7	F = .37 Df = 6,7	F = 1.94 Df = 5,6	F = .65 Df = 5,5	F = 1.88 Df = 5,5
18	F = 2.45 Df = 6,7	F = 2.76 Df = 6,7	F = 1.00 Df = 5,6	F = 2.68 Df = 5,5	F = 1.34 Df = 5,5

*P < .05

globulin, and the A/G ratios on any blood sampling day during the treatment period (Table 7).

Many atretic follicles were present in the ovary of the ACTH-injected bird (S-39). All of the follicles that had begun to develop were atretic. The ovarian follicles of bird S-40 were not atretic but all were very small. The mean oviduct weight of these birds was .77 g $\pm$ .23 as compared with that of 34.66 g $\pm$ 1.24 for the laying bird. Flickinger (1966) reported that ACTH treatment of laying hens induced ovarian atrophy and a significant decrease in the mean oviduct weight.

Histological examination of the adrenal glands of the ACTH-treated birds (Group 1, Experiment 2) showed increased vascularization and a spreading of the chromaffin cells into the center of the gland as compared to the estradiol-treated birds (Group 1, Experiment 1). Involvement of the adrenal glands in reproductive suppression has been reported by Flickinger (1966).

In these birds low serum levels of calcium and total protein were accompanied by reproductive suppression. Because treatment with ACTH alone (Group 1, Table 6) did not increase the serum calcium as did estradiol treatment (Group 7, Table 6) the increase found in the ACTH-estradiol-treated birds (Group 3, Table 6) was probably due to the estradiol. The decrease in the A/G ratio may seem to introduce some doubt concerning the reliability of this ratio as to an index to the reproductive state of the bird. However, even with administration of 12 U.S.P. units of ACTH per day, which was sufficient to suppress the response of the bird to .25 mg estradiol per day, the posttreatment mean of the A/G ratio was still 1.00. A ratio this high still reflected

the reproductive condition (unstimulated) of the bird. More important, the ACTH induced decrease in the A/G ratio was primarily the result of an increase in the gamma and alpha globulins, with some decrease in the albumin fraction. The decrease in the A/G ratio as a result of an increase in the beta globulins and a greater decrease in the albumin fraction is an estradiol response (Szego and Rogerts, 1953). Thus, even if A/G ratio should be similar, examination of the relative levels of the three groups in the globulin fraction and the level of the albumin fraction should indicate whether the decrease in the A/G ratio was the result of some stressor or of stimulation.

Group 2 was confined throughout the experiment. No estradiol was administered to these birds. Some posttreatment stimulation (light induced) occurred in both these birds, as indicated by the levels of calcium, total protein and the A/G ratio during the treatment period (Table 2, Appendix C). Interpretation of the data from these birds may be misleading since we were testing confinement alone and the levels of the three blood chemical constituents seemed to indicate that some estrogens were present. The A/G ratio of the more stimulated birds (S-41) decreased from 1.2 to .53 and .93 on days 9 and 15 respectively (Table 2, Appendix C) as a result of an increase in the beta globulins. The ratio of the less stimulated decreased from 1.7 to 1.1 but as a result of higher gamma and alpha globulins. The ratio of bird S-42 remained above 1.00 on every blood sampling day during the experiment. The appearance of the ovaries of these birds indicated that some stimulation (light-induced) had been present but that this was followed by inhibition. The calcium level of bird S-41 increased from 10.9 mg% to

16.3 mg% on day 6 and bird S-42 increased from 10.1 mg% to 12.1 mg% on day 9. Sufficient stimulation had been present to initiate the development of yolk-filled follicles but these were being reabsorbed rather than ovulated. This reabsorbtion may have been caused by confinement.

The presence of high gamma and alpha globulins in the electrophoretic pattern of the serum of bird S-42 after nine days of confinement resembled that of the ACTH treated birds (Group 1, Table 6) only three days after initiation of treatment. Thus, confinement of this bird had the same effect on the globulin fraction of the total protein as ACTH but a longer period was required. It may be that confinement resulted in the release of ACTH but the lack of immediate suppression of the estradiol influence indicates that the response to confinement was manifested in a different way or simply at a different rate. Conn et al. (1954) reported that, in man, the effect of the stressor agent was slow to develop but a single injection of ACTH was prompt and short-lived. We will show in the Seasonal Experiment 2 that the effect of a single injection of ACTH is also prompt and short-lived in laying ducks.

When comparing the Preseasonal Experiments 1 and 2, Group 3 of Experiment 2 was treated with one-tenth the dose of estradiol and twice the dose of ACTH. The posttreatment means of serum calcium and total protein of these estradiol-ACTH-treated birds (Group 3, Table 6) resembled those of the ACTH-treated birds (Group 1, Table 6) rather than the estradiol-treated birds (Group 7, Table 6). The posttreatment mean of the A/G ratio of the ACTH-estradiol-treated birds was lower than that of the estradiol-treated birds (Group 7, Table 7). This may be the result of an interaction of the estradiol and the ACTH since both have been shown to lower the A/G ratio alone.

Inspection of the values of the estradiol-ACTH-treated birds (Table 2, Appendix C) indicates that bird S-43 was less influenced by the administration of ACTH than was bird S-44, as evidenced by the higher serum calcium of the former. In the more stimulated bird (S-43) the decrease in the A/G ratio was associated with an increase in the beta globulins where as the decrease in the less stimulated bird (S-44) was associated with high gamma and alpha globulins.

The reproductive tract of the estradiol-ACTH treated bird (Group 3, Experiment 2) was suppressed. No appreciable development of the ovarian follicles were present. Since the ovary of the ACTH-treated birds (Group 1, Experiment 2) showed some follicular development (light-induced) even though atresia had occurred, it might be that the lack of ovarian development in the estradiol-ACTH-treated birds (Group 3, Experiment 2) was due to an estradiol-ACTH interaction. Group 4 was treated with estradiol for the first week, and both estradiol and ACTH during the second. The serum levels of calcium and total protein increased during the first week and continued seemingly uneffected by the addition of ACTH. In both birds, there was a temporary decrease in the A/G ratio after the initial injection of estradiol, and a precipitous drop after injection of both estradiol and ACTH. In both birds (S-45, S-46), there was an increase in the globulin fraction (S-45, 1.5 g% - 3.4 g%; S-46, 1.5 g% - 2.1 g%) while the albumin fraction of only bird S-46 decreased (2.7 g% - 2.4 g%). The albumin fraction of bird S-45 fluctuated around the pretreatment value but the posttreatment mean was not greater than the pretreatment value. The increase in the globulin fraction of these two birds was the result of increases in

all three groups (gamma, beta and alpha). It might be that the gamma and alpha globulins responded to the ACTH while the beta globulins responded to the estradiol. The ovary of bird S-46 was suppressed and its oviduct was not developed.

Group 5 was confined while treated with estradiol. Bird S-47 seemed to be more stimulated than S-48, as evidenced by its higher serum levels of calcium and total protein, as well as lower A/G ratios on day 6 through 15. It is possible that we were dealing with two populations, one in which photoperiodic stimulation of ovarian development had occurred and one in which it had not. The lower A/G ratio was the result of an increase in the globulin fraction with little decrease in the albumin fraction. The A/G ratios of bird S-47 were less than 1.00 from day 6 through 18 while those of bird S-48 were less than 1.00 only on days 9 and 18. As in Group 4, the increase in the globulin fraction was associated with higher levels of all three globulins. Again, it is possible that the gamma and alpha globulins responded to the confinement. Examination of the reproductive tract of S-47 indicated that this bird was indeed stimulated but that earlier suppression had also occurred. Yolk-filled atretic follicles were present in the ovary and, in addition, an egg was found in the oviduct. Apparently neither confinement nor the level of estradiol (.25 mg/day) administered were sufficient to completely suppress follicular development. The presence of atretic yolk-filled follicles may indicate temporary suppression and the fact that the egg was not ovulated until the end of the treatment period might indicate that this bird overcame the effects of the treatment ("recovery"). The stimulation in this case was probably light induced.

Group 6 was treated with estradiol for the first week and, in addition, was confined during the second. The serum levels of calcium and total protein increased during the first week as expected, but decreased during the second while still receiving estradiol. The A/G ratio of bird S-50 decreased from 2.1 to .76 on the sixth day of estradiol treatment while that of bird S-49 decreased from 4.1 to .87 on day 15. Both decreases were the result of an increase in the globulin fraction (S-49, .9 g% - 3.6 g%; S-50, 1.5 g% - 3.2 g%) and a decrease in the albumin fraction (S-49, 3.7 g% - 3.2 g%; S-50, 2.7 g% - 2.3 g%). As in the two previous groups, the increase in the globulin fraction was the result of increases in all three globulins. A normal ovary has follicles of continuous graded size (Sturkie, 1965:467). Examination of the ovary of bird S-47 indicated that sufficient stimulation had been present for the development of yolk-filled follicles but that these had become atretic. In addition, there was no regular sequence of follicular gradations.

Summary of Preseason Results

The suppressive influence of ACTH on the response of the three blood chemical constituents seemed to be a function of the relative concentrations of ACTH and estradiol. Administration of 6 U.S.P. units of ACTH per day was not sufficient to suppress completely the response of 2.5 mg estradiol per day (Group 2, Experiment 1) but the administration of 12 U.S.P. units of ACTH per day was sufficient to completely suppress the response of the blood chemical constituents to .25 mg of estradiol per day (Group 3, Experiment 2). ACTH alone did not increase

the serum calcium level. ACTH was shown to increase the total protein level and decrease the A/G ratio (Group 1, Experiment 2) as a result of increased gamma and alpha globulins rather than increasing the beta globulins as did estradiol. Because of this difference in globulin response, it seems to be possible to differentiate between a "stressed" bird and a stimulated one, even if the total protein and A/G values are similar.

The effect of confinement on the response to estradiol treatment was ambiguous. The increase in gamma and alpha globulins and the presence of atretic follicles seemed to indicate a suppressive action but moderately high serum calcium levels were present. In addition, an egg was present in the oviduct of one of the birds treated with estradiol (.25 mg/day) while confined. Apparently neither this low level of estradiol nor confinement was sufficient to suppress the ovarian development.

The birds seemed to exhibit the ability to "recover" from the application of a stressor agent, as indicated by the presence of an egg in the oviduct of the bird S-48 at the end of the experiment and by the delayed increase in the blood chemical constituents (Group 2, Experiment 1). Even the birds treated with 12 U.S.P. units of ACTH showed some increase in serum calcium after nine days of treatment.

The electrophoretic pattern of the serum from birds treated with .25 mg of estradiol per day and a stressor agent seemed to reflect the influence of both (stimulant and stressor agent). In these groups, the globulin fraction increased as a result of higher levels of the three globulins (gamma, beta and alpha).

Seasonal Experiment 1

The birds in this phase of the study were stimulated by endogenous estrogens released from their developing ovarian follicles rather than by exogenous estradiol. The object of this experiment was to investigate the influence of increasing levels of ACTH or cold exposure on the blood chemical constituents of the naturally stimulated bird.

The results of this experiment are presented in Table 8. No significant differences were found between the overall means of any ACTH treated group and control birds for calcium, total protein or the A/G ratio. However, there were significant differences between the A/G ratio means on days 18 through 30 of the groups treated with 1 U.S.P. and 5 U.S.P. units of ACTH and the control birds. No significant difference was found between either of the submeans for any chemical constituents of the birds treated with 3 U.S.P. units of ACTH and the controls (Table 9). Inspection of Table 2, Appendix D will show that in each group 16.6% of the A/G ratios were less than 1.00 on day 1 through 15 while 80% of the A/G ratios of the control birds and none of the ratios of birds treated with 1 U.S.P. unit of ACTH and only 20% of the A/G ratios of the birds treated with 5 U.S.P. units of ACTH were less than 1.00 on days 16 through 30. It seems probable that this difference caused the significance.

This lack of decrease in the later A/G ratio would seem to refute the proposed ability of these birds to "recover" from the inhibitive influence of ACTH but all birds that were treated with ACTH while unstimulated (S-20, S-21, S-18, S-17) showed an increase in the serum calcium

Table 8: Pretreatment (Pr) and posttreatment (Pt) means of serum calcium (Ca), total protein (TP), albumin (Alb), globulin (Glb) and the A/G ratio for seasonal experiment 1.

Bird number	Treatment on per day basis	No. of samples		Ca (mg%)	TP (g%)	Alb (g%)	Glb (g%)	A/G (ratio)
S-20	1 U.S.P. unit ACTH	3	Pr	12.00	4.40	2.9	1.5	4.76
		8	Pt	19.95	5.52	2.8	2.7	1.06
S-11	1 U.S.P. unit ACTH	3	Pr	20.46	4.76	3.4	1.4	2.53
		8	Pt	25.35	6.25	3.3	2.7	1.23
S-21	3 U.S.P. units ACTH	3	Pr	12.76	5.00	3.3	1.6	2.32
		8	Pt	17.08	6.21	2.9	3.1	.95
S-5	3 U.S.P. units ACTH	3	Pr	27.33	4.83	3.1	1.7	1.94
		8	Pt	22.73	5.07	2.4	2.7	.92
S-18	5 U.S.P. units ACTH	3	Pr	11.20	3.80	2.5	1.3	1.94
		8	Pt	13.96	5.13	2.7	2.5	1.12
S-10	5 U.S.P. units ACTH	3	Pr	17.16	4.83	2.9	1.9	1.56
		8	Pt	22.43	5.58	2.9	2.7	1.06
S-17	6 U.S.P. units ACTH	3	Pr	10.23	4.16	2.8	1.4	1.97
		8	Pt	10.27	4.40	2.6	1.9	1.27
S-22	6 U.S.P. units ACTH	3	Pr	19.73	4.70	2.9	1.8	1.86
		8	Pt	20.50	6.33	3.0	3.3	1.01
S-1	cold exposure 3 wks.	3	Pr	24.60	6.10	3.2	2.9	1.13
		8	Pt	27.26	5.88	2.9	3.1	.88

Table 8: Continued

Bird number	Treatment on per day basis	No. of samples		Ca (mg%)	TP (g%)	Alb (g%)	Glb (g%)	A/G (ratio)
S-24	cold exposure 3 wks.	3	Pr	18.33	4.46	2.6	1.9	1.53
		8	Pt	26.78	5.91	3.5	3.1	1.19
S-14	cold exposure 2 wks.	3	Pr	19.96	6.13	2.9	2.6	4.30
		7	Pt	32.97	5.76	2.6	3.5	.77
S-19	cold exposure 2 wks.	3	Pr	12.90	4.43	2.9	2.6	1.02
		7	Pt	24.34	5.43	2.8	3.5	.99
S-16	control	3	Pr	13.26	4.36	2.5	1.8	1.38
		8	Pt	27.42	6.95	3.1	3.8	.80
S-4	control	3	Pr	17.53	4.30	2.5	1.8	1.56
		8	Pt	29.98	5.26	2.9	3.2	.90

Table 9: Statistical difference between the means of the means of all the samples (t^1), of days 1 through 15 (t^2), and of days 16 through 30 (t^3) of serum calcium (Ca), total protein (TP) and the A/G ratio for the ACTH-injected groups versus the control groups in seasonal experiment 1.

Treatment on per day basis	Ca	TP	A/G
1 U.S.P. unit ACTH	t^1 = 1.12, Df = 3 t^2 = -.85, Df = 3 t^3 = -.74, Df = 3	t^1 = -.19, Df = 3 t^2 = -.25, Df = 3 t^3 = -.07, Df = 3	t^1 = 1.76, Df = 3 t^2 = 1.43, Df = 3 t^3 = 3.51, Df = 3*
3 U.S.P. units ACTH	t^1 = -1.30, Df = 3 t^2 = -.84, Df = 3 t^3 = -.97, Df = 3	t^1 = -.36, Df = 3 t^2 = -.55, Df = 3 t^3 = -.10, Df = 3	t^1 = 1.33, Df = 3 t^2 = 1.00, Df = 3 t^3 = 1.68, Df = 3
5 U.S.P. units ACTH	t^1 = -2.17, Df = 3 t^2 = -1.43, Df = 3 t^3 = -1.60, Df = 3	t^1 = -1.18, Df = 3 t^2 = -1.07, Df = 3 t^3 = -.72, Df = 3	t^1 = 2.12, Df = 3 t^2 = .79, Df = 3 t^3 = 3.81, Df = 3*
6 U.S.P. units ACTH	t^1 = -2.60, Df = 3 t^2 = -2.00, Df = 3 t^3 = -1.72, Df = 3	t^1 = -1.23, Df = 3 t^2 = -1.07, Df = 3 t^3 = .97, Df = 3	t^1 = 1.86, Df = 3 t^2 = 1.02, Df = 3 t^3 = 1.73, Df = 3

*P < .05

levels during the treatment period. The length of time between the start of treatment and the "recovery" was variable. In addition, the electrophoretic pattern of the ACTH-treated bird (S-18) resembled the saddle shaped pattern (high gamma, low beta, high alpha) found in the ACTH-treated birds (Group 1, Experiment 2) until day 21 after which a small beta globulin peak appeared. The electrophoretic pattern of the serum samples in birds S-20 and S-21 were similar to that found in the bird S-18 but not as distinct. This may be further indication of the ability of these birds to "recover" from the suppressive effect of ACTH.

There was a significant difference between the calcium means of the birds treated with 6 U.S.P. units of ACTH and the controls. However, the differences between the total protein and the A/G ratio means of these birds and the controls were not significant. Statistical analysis of the submeans did not indicate any significant difference. The lack of significance here might be due to the effect of ACTH on the globulin fraction of the total protein.

Even though some of the values of the birds were not statistically different from those of the controls there were apparent physical differences as indicated by the condition of the reproductive tracts. For example, statistically there was no difference between the blood constituents of the birds treated with 1 U.S.P. unit of ACTH and the controls. However, the ovaries of the ACTH-treated bird contained no yolk-filled follicles while those of the more typical control bird (S-4) contained both yolk-filled follicles and ovulated follicles. The interpretation of the statistical results in this experiment may

be misleading because one bird in each group was stimulated and the other was not at the outset of the treatment. On the other hand, since each group contains both stimulated and unstimulated birds the comparison may be valid. Administration of 3 U.S.P. units of ACTH was sufficient to suppress the unstimulated bird (S-21) as indicated by the presence of atretic follicles and the absence of yolk-filled follicles, but was insufficient to suppress the already stimulated bird (S-5) as indicated by the presence of an egg in the oviduct of this bird. Some atresia was present in the ovary of this bird. While only the difference between the calcium means of the birds treated with 6 U.S.P. units of ACTH and the controls was significant, examination of the reproductive tract of these birds showed that they were severely suppressed, as indicated by the mass atresia.

In Table 10 are presented the t values for the pretreatment means versus the posttreatment means of each experimental bird. Only the increase in the total protein and the decrease in the A/G ratio was significant ($P < .05$) in the controls. However, the calcium levels did increase (S-16, 13.26 mg% - 27.42 mg%; S-4, 14.53 mg% - 29.98 mg%) during the treatment period. Two eggs were recorded for bird S-4, one on day 16 and the other on day 29 (Table 11). No eggs were recorded for the other control bird (S-16) during the experiment. The oviduct of this bird was somewhat developed as indicated by its size, and some atretic follicles were present in the ovary. It was noted that this bird reacted in a more threatening manner when approached than did the other birds.

There were significant increases in the serum levels of calcium and total protein and a significant decrease ($P < .05$) in the A/G ratio of the bird S-20 which was treated with 1 U.S.P. unit ACTH. In bird S-11, which also received 1 U.S.P. unit of ACTH, only the increase in the total protein and the decrease in the A/G ratio were significant. This result might be due to the fact that this bird (S-11) was stimulated at the start of the treatment. No eggs were recorded for either of these birds, however, the presence of many normally developing follicles indicated that these birds were overcoming the inhibitive influence of ACTH.

As in the previous group, one of the birds treated with 3 U.S.P. units of ACTH (Group 2, Table 8) was stimulated and one was not as evidenced by their respective calcium values (Table 2, Appendix D). In both the stimulated bird (S-5) and the unstimulated bird (S-21), the increase in the total protein and the decrease in the A/G ratio were significant ($P < .05$) while the increase in the serum calcium was not (Table 10). Administration of ACTH to bird S-21 seemed to cause a leveling off of the serum levels of calcium and total protein for a limited period ("plateau effect"). Prior to treatment, the serum calcium level was 13.2 mg%. On the next four blood sampling days (9, 12, 15 and 18), the range of the serum calcium was from 13.6 mg% to 14.5 mg%. On days 12, 24, 27 and 30 the calcium levels were 28.0 mg%, 26.6 mg% and 20.9 mg%. The level of total protein remained about the pretreatment level (5.4 g%) for two blood sampling days after the start of treatment. On days 15 through 30, the level of this constituents ranged from 6.1 g% to 7.0 g%. Thus, ACTH seemed to induce a

Table 10: Statistical differences between the pretreatment and posttreatment means of serum calcium (Ca), total protein (TP) and A/G ratio for each bird in seasonal experiment 1.

Bird number	Treatment on per day basis	Ca	TP	A/G
S-20	1 U.S.P. unit ACTH	t = -3.20*	t = 27.93*	t = 6.35*
S-11	1 U.S.P. unit ACTH	t = -1.47	t = 43.40*	t = 89.89*
S-21	3 U.S.P. units ACTH	t = -.76	t = 18.48*	t = 31.83*
S-5	3 U.S.P. units ACTH	t = .90	t = 29.13*	t = 78.95*
S-18	5 U.S.P. units ACTH	t = -2.06	t = 18.03*	t = 50.51*
S-10	5 U.S.P. units ACTH	t = -1.80	t = 52.49*	t = 123.8 *
S-17	6 U.S.P. units ACTH	t = -.01	t = .056	t = 15.42*
S-22	6 U.S.P. units ACTH	t = -.21	t = 54.67*	t = 61.51*
S-1	cold exposure 3 wks.	t = -.60	t = 25.41*	t = 201.4 *
S-24	cold exposure 3 wks.	t = -1.68	t = 18.27*	t = 65.07*
S-14	cold exposure 2 wks.	t = -4.88*	t = 9.54*	t = 9.96*
S-19	cold exposure 2 wks.	t = -1.55	t = 5.26*	t = 74.66*
S-16	control (no treatment)	t = -1.05	t = 8.52*	t = 76.66*
S-4	control (no treatment)	t = -1.58	t = 7.61*	t = 75.85*

*$P < .05$

Df = 10

Table 11: Egg production of each bird during seasonal experiment 1.

Bird number	Day 1-9	10	11	12	13	14	15	16	17-27	28	29	30	31
S-20													
S-11		X											
S-21													
S-5				X								X	X
S-18													
S-10													
S-17													
S-22													
S-1					X	X	X	X		X	X	X	X
S-24							X	X		X	X	X	X
S-14													
S-19													
S-16													
S-4								X		X			

X denotes an egg

leveling (plateau effect") of serum calcium and total protein. In addition, bird S-21 seemed to partially overcome ("recovery") the inhibitive influence of ACTH as evidenced by the second higher level of calcium and total protein values (Table 2, Appendix D). Administration of 3 U.S.P. units of ACTH to the stimulated bird (S-5) did not completely suppress this bird but did cause a leveling off of the serum calcium and total protein values ("plateau effect") followed by an increase in these two constituents on days 27 and 24 respectively. The range of the A/G ratio after treatment was .70 to 1.22 (Table 2, Appendix D). No eggs were recorded for this bird during the study. Atretic follicles were present in the ovary and the oviduct was somewhat developed. On the other hand, eggs were recorded for the stimulated bird on days 12, 30 and 31. This result was not surprising since the addition of ACTH to the previously estrogenized birds did not seem to exert a suppressive effect.

In Group 3 (treated with 5 U.S.P. units of ACTH), one of the birds was stimulated (S-10) and one was not (S-18) as in Group 1 and 2 previously mentioned. The difference between the pretreatment and posttreatment means of the total protein and the A/G ratio are significant ($P < .05$) while the increase in the serum calcium was not. In the unstimulated bird, the pretreatment calcium mean was 11.20 mg% as compared to the posttreatment mean of 13.96 mg%. In the stimulated bird the pretreatment and posttreatment calcium means were 14.16 mg% and 22.43 mg% respectively (Table 8). It is interesting to note that the serum levels of calcium and total protein of the unstimulated and stimulated birds were at approximately the same level at the end of

the treatment period. This indicated that this level of ACTH was able to suppress the unstimulated bird as well as the stimulated bird. As shown previously, administration of 3 U.S.P. units of ACTH was insufficient to inhibit the stimulated bird (S-5). The serum calcium and protein compositon of birds S-18 and S-10 exhibited "plateau effects" during the six sample days after initiation of the treatment. The serum calcium and total protein values of the stimulated bird S-10 remained relatively constant during the treatment period (Table 2, Appendix D).

Group 4 which was treated with 6 U.S.P. units of ACTH per day also contained one stimulated bird (S-22) and one unstimulated bird (S-17) at the outset of the experiment. In the unstimulated bird (S-14) there was a significant decrease in the A/G ratio. The increase in the serum levels of calcium and total protein were not significant. The posttreatment calcium mean was 10.27 mg% as compared to the pretreatment mean of 10.23 mg% and the pretreatment and posttreatment means of the total protein were 4.16 g% and 4.4 g% respectively. The posttreatment means of calcium (10.27 mg%), total protein (4.4 g%) are less than those expected in a stimulated bird (Ca = 30 mg% and TP = 6 g%) and the A/G ratio (1.27) was greater than that expected in a stimulated bird (.6) (Hofman, 1966). In the stimulated bird (S-22) the increase in the total protein and the decrease in the A/G ratio were significant at the .05 level (Table 10). The decrease in the calcium was not significant. The posttreatment calcium mean was 20.50 mg% as compared to the pretreatment mean of 19.73 mg%. The maintenance of constant levels of serum calcium ("plateau effect") indicates that suppression was not

complete and some stimulation (estrogens) was still present. The phenomena of "plateau" and "recovery" were exhibited by virtually all the non-laying birds in this experiment.

The birds maintained under the same conditions as the controls for one week and exposed to a temperature of minus 9.7 degrees centigrade for a period of three weeks were not suppressed by the low temperatures. Eggs were recorded for bird S-24 on days 15, 16, 28, 29, 30 and 31 and for bird S-1 on days 13, 14, 15, 16, 28, 29, 30 and 31 (Table 11).

The birds maintained under the same conditions as the controls for the two weeks and placed in the cold room for two weeks seemed to be influenced by the low temperature. No eggs were recorded for these birds during the treatment period (or during the previous week). The increase in the serum calcium and the total protein and the decrease in the A/G ratio fo bird S-14 and the increase in the total protein and the decrease in the A/G ratio of bird S-19 were significant at the .05 level (Table 10). In bird S-19 the A/G ratios of less than 1.00 (days 15, 24 and 30) were associated with gamma and alpha globulins as well as beta globulins and a high albumin fraction 2.7 g%, 2.5 g% and 3.0 g% respectively. In bird S-14 the A/G ratio of less than 1.00 on days 15, 18, 21, 24, 27 and 30 were associated with gamma and alpha globulins as well as beta globulins and albumin fraction levels of 2.9 g%, 2.1 g%, 1.8 g%, 3.1 g%, 2.5 g% and 3.5 g% respectively. If, in fact the gamma and alpha globulins do increase in response to stress, the low A/G ratios here were partially the result of "stress" (cold) and partly an estrogen response.

The ovary of bird S-14 contained no yolk-filled follicles but atretic follicles were present. The ovary of bird S-19 contained yolk-filled atretic follicles. It appeared that this bird may have been stimulated but that suppression had occurred which resulted in the absorption of the yolk-filled follicles. The size of the oviducts in these birds indicated that some growth had occurred.

Seasonal Experiment 2

The first three experiments were designed to investigate the influence of extended administration of ACTH whereas this one was designed to examine the time and duration of the response to a single injection of 12 U.S.P. units of ACTH. The results of this experiment are presented in Table 12. The single injection of ACTH seemed to exert its effect within one-half hour as indicated by the decrease in serum calcium and the increase in the A/G ratio within this period. The effect of the ACTH on the total protein was minimal. The birds seemed to begin recovery after 11/2 hours and attained the pretreatment level by three hours. Normal fluctuation of these three blood chemical constituents was observed in the 6, 8, 12 and 24 hours samples. It should be noted that these are the responses of a stimulated duck. It has been shown that unstimulated and stimulated ducks respond differently to ACTH.

Table 12: Time and duration of the effect of a single injection of 12 U.S.P. units of ACTH on the serum levels of calcium (Ca), total protein (TP) and the A/G ratio of seasonal experiment 2.

Bird number		Hours 0	1/2	11/2	3	6	8	12	24
S-64	Ca (mg%)	22.6	19.7	20.8	--	26.6	--	--	21.6
	TP (g%)	5.7	5.1	5.1	--	6.2	--	--	5.9
	A/G	1.20	1.36	1.40	--	1.35	--	--	1.33
S-66	Ca (mg%)	42.4	29.6	41.0	--	38.4	--	--	30.2
	TP (g%)	5.7	5.7	5.6	--	6.1	--	--	4.6
	A/G	1.20	1.36	1.40	--	1.35	--	--	1.33
S-74	Ca (mg%)	30.8	--	--	23.9	--	27.2	16.5	--
	TP (g%)	6.9	--	--	7.7	--	6.2	6.3	--
	A/G	.46	--	--	1.00	--	.53	.62	--
S-78	Ca (mg%)	24.0	--	--	37.2	--	28.6	28.6	--
	TP (g%)	7.6	--	--	7.3	--	8.6	8.8	--
	A/G	.73	--	--	.53	--	.94	1.09	--

No blood samples were taken from S-64 and S-66 on hours 3, 8 and 12.

No blood samples were taken from S-74 and S-78 on hours 1/2, 11/2 and 6.

SUMMARY

Two preseason (prenuptial) experiments were carried out to determine the influence of the stressors (ACTH or confinement) on the response of blood chemical constituents of exogenous estradiol. One seasonal (nuptial) experiment was carried out to determine the influence of the stressors (ACTH or cold) on the blood chemical constituents of the duck under the influence of endogenous estrogens only. Estradiol increased the serum levels of calcium and total protein and decreased the A/G ratio. With the proper concentrations of ACTH and estradiol, ACTH suppressed the estradiol induced hypercalcemia but the total protein and the A/G ratio responded as if only estradiol were administered. ACTH alone did not increase the serum calcium but did increase the total protein and decrease the A/G ratio. Ducks with low serum calcium levels also exhibited lack of oviductual development. Thus, the serum calcium level reflected the reproductive condition of the mallard even under stressful conditions. Since the total protein and A/G ratio responded to ACTH in the same manner as to estradiol, these two parameters alone can not be used to indicate the reproductive state of the mallard. However, examination of the subdivisions of the globulin fraction of the serum protein did indicate whether the total protein and A/G ratio were responding to "stress" or estradiol. Administration of ACTH resulted in an increase in the gamma and alpha globulins with little influence on the albumin fraction whereas administration of estradiol resulted in an increase in the beta globulins

and a decrease in the albumin fraction. In the Seasonal Experiment 1, statistical differences between the ACTH-treated groups and the controls were variable. All the ACTH-treated groups differed physically from the controls. Birds placed in the cold room (minus 9.7 degrees centigrade) after one week under the same conditions as the controls did not seem to be suppressed by the low temperature. The birds maintained under the same conditions as the controls for two weeks before placement in the cold room were suppressed by the low temperature. Within the limits of our ability to determine, all cases where the reproductive tract was examined the levels of the blood chemical constituents reflected the reproductive condition (stimulated or unstimulated) of the bird. Administration of ACTH resulted in a leveling off ("plateau effect") of the serum calcium, total protein, albumin, globulin and the A/G ratio. Towards the end of the experiment the birds overcame the inhibitive influence of ACTH ("recovery") as indicated by the increase in the levels of serum calcium, total protein and the nature of the protein response. Only the serum calcium and A/G ratio of the stimulated bird seemed to be influenced by a single injection of 12 U.S.P. units of ACTH. The serum calcium decreased and the A/G ratio decreased. The response of these two constituents occurred within one-half hour and had returned to the pretreatment level three hours after injection. Normal fluctuation of these constituents was observed at 6, 8 12 and 24 hours after injection.

LITERATURE CITED

Brimblecomb, R. I., D. K. Halkerston and M. Reiss. 1954. The effect of various ACTH extracts on the gonads. J. Endocrinol. 10:291-304.

Chancellor, L. and B. Glick. 1960. The effect of temperature as a stressor on white blood cells, adrenal and fabricus of chicks. Am. J. Physiol. 198:1346.

Chowers, I., H. T. Hammel, S. B. Stromme and S. M. McCan. 1964. Comparison of the effect of environmental and preoptic cooling on plasma cortisol levels. Am. J. Physiol. 207:577.

Christian, J. J. 1955. Effect of population size on the weights on the reproductive organs of white mice. Am. J. Physiol. 181:477-498.

Christian, J. J. 1956. Adrenal and reproductive responses to population size in mice from a freely growing population. Ecology 37: 258-273.

Christian, J. J. 1964. Actions of ACTH in intact and corticoid-maintained adrenalectomized female mice with emphasis on the reproductive tract. Endocrinol. 75:653.

Conn, J., S. Fajans, L. Louise, H. Seltze and H. Kane. 1954. A comparison of steroidal excretion and metabolic effects induced in man by stress and ACTH. Recent Prog. in Hormone Res. X:482-484.

Decker, E. H. 1965. A study of the fluctuations of some blood chemical constituents in relation to the reproductive cycle of the mallard duck. Unpublished master's thesis, Western Michigan University, Kalamazoo, Michigan, v + 69 pp.

Flickinger, G. L. 1961. Effect of grouping on adrenals and gonads of chickens. Gen. Comp. Endocrinol. 1:332-340.

Flickinger, G. L. 1966. Effect of prolonged ACTH administration on the gonads of sexually mature chickens. Poultry Sci. 45:753-761.

Garren, H. W., C. H. Hill and M. W. Carter. 1961. Adrenal responses of young chickens to adrenocorticotropic hormone as influenced by dosage and frequency of injection. Poultry Sci. 40:446-453.

Greenburg, D. M., C. E. Larson, P. P. Pearson and B. R. Burnster. 1936. The state and partition of the calcium and inorganic phosphorus in the serum of the fowl: Effect of growth and ovulation. Poultry Sci. 15:483.

Hofman, W. E. 1966. The effects of estrogen and gonadotrophic hormones on normal levels of some blood chemical constituents in the non-breeding female mallard duck. Unpublished master's thesis, Western Michigan University, Kalamazoo, Michigan, i + 88.

Hurwitz, S. 1964. Bone Composition and Ca^{45} retention in fowl as influenced by egg formation. Am. J. Physiol. 206:198-204.

Jarrett, R. J. 1965. Effects and mode of action of adrenocorticotropic hormone upon the reproductive tract of the female mouse. Endocrinol. 76:434.

Katsh, S., G. G. Katsh and P. Osher. 1954. Adrenal, pituitary and urinary ascorbic acid levels in rats subjected to hypothermic environment. Am. J. Physiol. 178:457-461.

Kepner, B. and D. Hercules. 1963. Fluorometric determination of calcium in blood serum. Anal. Chem. 35:1238-1240.

Keys, P. and T. S. Potter. 1934. Physiological marrow ossification in female pigeons. Anat. Rec. 60:377.

Laudauer, W., C. A. Pfeiffer, W. U. Garner and B. C. Shaw. 1941. Blood serum and skeletal changes in two breeds of ducks receiving estrogens. Endocrinol. 28:458.

Levin, L. 1945. The effects of several varieties of stress on the cholesterol content of the adrenal glands and of this serum of rats. Endocrinol. 37:34-43.

Long, C. N. H. 1947. Recent studies on the function of the adrenal cortex. Bull. New York Acad. Med. 23:260-282.

Lorenz, F. W., R. E. Burger, E. B. Bennet and W. Reimann. 1962. Hepatic and renal effects of potency of estrogenic stilbene derrivatives in chickens. Endocrinol. 71:649.

Mason, H. L., M. H. Power, E. H. Rynearson, L. C. Ciarnelli, C. H. Li and H. M. Evans. 1948. Results of Administration of anterior pituitary adrenocorticotropic hormone to a normal human subject. J. Clin. Endo. 8:1.

Ranney, R. E. and I. L. Chaikoff. 1951. Effect of functional hepatectomy upon estrogen-induced lipemia in the fowl. An. J. Physiol. 165:600.

Riddle, O. and L. B. Dotti. 1934. Action of parathyroid hormone in normal and hypophysectomized pigeons. Proc. Soc. Exp. Biol. and Med. 32:507.

Riddle, O. and W. Reinhart. 1926. Studies on the physiology of reproduction in birds: Blood calcium changes in the reproductive cycle. Am. J. Physiol. 76:660.

Roberts, A., J. E. Nezamis and J. P. Phillips. 1965. Ulcers and hydrothorax by restraint in the rat. Fed. Proc. 24:714.

Sayers, G. and M. A. Sayers. 1969. The pituitary-adrenal system. Ann. N. Y. Acad. Sci. 50:522-539.

Siegel, H. S. 1968a. Effects of increased population density on the adrenals of cockerels. Poultry Sci. 37:1242-1243.

Siegel, H. S. 1959b. Egg production characteristics and adrenal gland function in white leghorns confined at different floor space levels. Poultry Sci. 38:893-898.

Smoes, R. L. 1967. Blood constituent levels associated with transition into reproduction and during egg formation in the female mallard duck. Unpublished master's thesis, Western Michigan University, Kalamazoo, Michigan, vi + 76 pp.

Sturkie, P. D. 1965. Avian Physiology, 2nd ed., Cornell Univ. Press, New York, xxx + 765 pp.

Szego, C. M. and S. Roberts. 1953. Steroid action and interaction in uterine metabolism. Recent Prog. in Hormone Res. 8:419-460.

Turner, C. D. 1960. General Endocrinology, 4th ed., W. B. Saunders Co., Philadelphia and London, xi + 511 pp.

Urist, M. R. 1959. The effects of calcium deprivation upon the blood adrenal cortex, ovary and skeleton in domestic fowl. Recent Prog. in Hormone Res. 15:455-477.

Urist, M. R. and N. M. Deutsch. 1960. Influence of ACTH upon avian species and osteoporosis. Proc. Soc. Ex. Biol. and Med. 104:35.

Varon, H. H. and J. J. Christian. 1963. Effects of adrenal androgens on immature female mice. Endocrinol. 72:210.

Wallack, D. F. and T. L. Steck. 1963. Fluorescent techniques in the microdetermination of metals in biological materials. Anal. Chem. 35:1037-1044.

Wilson, H. J., J. Boris and R. C. Bahn. 1958. Steroids in the blood and urine of female mice bearing an ACTH-producing pituitary tumor. Endocrinol. 62:135-149.

Wilson, W. D., E. H. McNallay and H. Ota. 1957. Temperature and calorimetric studies on hens in individual cages. Poultry Sci. 36:254.

Zarrow, M. X., D. L. Grennman and L. E. Peters. 1961. Inhibition of the bursa of fabricus and the stilbesterol stimulated oviduct of the domestic chick. Poultry Sci. 40:87.

APPENDIX A

Ingredients of Napiana Duck Developer Pellets

Ingredients of Napiana Duck Developer Pellets

Ground Yellow Corn, Pulverized Oats, Meat and Bone Meal, Soybean Meal, Dehydrated Alfalfa Meal preserved with Ethoxyquin, Corn Gluten Feed, Wheat Middlings, Wheat Red Dog, Folic Acid, Corn Molasses, Vitamin A Palmitate, D-Activated Animal Sterol (Source of Vitamin K), Vitamin B12 Supplement, Riboflavin Niacin, Phosphate, Salt, Trace Amounts of Matnesium Carbonate, Monaganous Oxide, Iron Carbonate, Iron Oxide, Copper Oxide, Cobalt Carbonate, Zinc Oxide and Calcium Iodate.

Manfactured by

Nappanee Milling Company, Inc.

Nappanee, Indiana

APPENDIX B

Raw data for the ducks in the Preseason Experiment 1

Table 1: Pretreatment (Pr) and posttreatment (Pt) mean weights of the birds in preseason experiment 1.

Bird number	Group	Treatment on per day basis	Pr (g)	Pt (g)
S-23	1	2.5 mg estradiol	1329	1154
S-24	1	2.5 mg estradiol	1300	1246
S-27	2	2.5 mg estradiol 6 U.S.P. units ACTH	1557	1354
S-28	2	2.5 mg estradiol 6 U.S.P. units ACTH	1314	1131
S-29	3	2.5 mg estradiol 6 U.S.P. units ACTH delay	1157	1000
S-30	3	2.5 mg estradiol 6 U.S.P. units ACTH delay	1043	1034
S-31	4	2.5 mg estradiol confinement	1257	1005
S-32	4	2.5 mg estradiol confinement	1271	1097
S-33	5	2.5 mg estradiol confinement delay	1029	1017
S-34	5	2.5 mg estradiol confinement delay	1371	1257

Table 2: Values of serum calcium (Ca), total protein (TP), albumin (Alb), globulin (Glb) and the A/G ratio.

Bird number		Treatment period 1	3	6	9	12	15	18	Day
S-23	Ca	9.3	10.7	51.0	81.9	82.0	78.5	---	g%
	TP	5.8	5.3	11.6	15.+*	15.+	15.+	8.2	g%
	Alb	2.9	2.4	3.4	---**	---	---		g%
	Glb	2.7	2.5	8.2	---	---	---	---	g%
	A/G	1.07	.96	.42	.38	---	.08	---	
S-24	Ca	12.3	10.9	50.5	71.5	89.5	74.5	38.0	
	TP	5.6	5.1	11.3	15.+	15.+	15.+	8.2	
	Alb	3.4	4.3	3.9	---	---	---	2.4	
	Glb	1.8	.8	7.3	---	---	---	5.8	
	A/G	1.89	5.4	.53	.28	.15	.15	.41	
S-27	Ca	10.0	8.6	39.4	71.9	72.0	81.0	61.5	
	TP	5.3	6.5	9.8	15.+	15.+	15.+	15.+	
	Alb	2.9	2.4	2.9	---	---	---	3.2	
	Glb	2.2	3.8	6.7	---	---	---		
	A/G	1.32	.63	.43	.31	---	.07	.42	
S-28	Ca	10.5	10.0	35.0	45.5	50.5	52.5	14.4	
	TP	5.4	5.0	6.5	13.3	13.3	15.+	4.8	
	Alb	3.0	2.8	3.2	3.1	2.4	---	1.6	
	Glb	2.3	1.8	6.3	10.2	10.9	---	3.2	
	A/G	1.3	1.5	.51	.30	.24	.23	.50	
S-29	Ca	10.9	10.0	51.5	76.0***	83.5	84.5	26.0	
	TP	5.3	5.7	11.4	15.+	14.8	15.+	6.7	
	Alb	3.4	2.7	3.3	---	2.8	---	1.7	
	Glb	1.3	2.1	8.1	---	12.0	---	4.5	
	A/G	2.6	1.3	.41	.19	.23	---	.35	
S-30	Ca	10.7	10.4	56.5	73.0***	82.0	82.5	28.0	
	TP	5.9	5.5	12.9	15.+	15.+	15.+	6.7	
	Alb	4.3	3.2	3.5	---	---	---	1.8	
	Glb	1.6	1.7	9.4	---	---	---	4.5	
	A/G	2.7	1.9	.37	---	.19	.25	.40	
S-31	Ca	10.3	11.5	53.0	68.0	51.4	---	42.8	
	TP	6.0	5.2	11.3	15.+	15.+	15.+	6.9	
	Alb	2.8	2.8	2.6	---	---	---	1.6	
	Glb	1.9	3.2	8.7	---	---	---	5.0	
	A/G	1.48	.87	.30	.39	.24	---	.30	

Table 2: Continued

Bird number		Treatment Period 1	3	6	9	12	15	18 Day
S-32	Ca	11.3	12.0	54.5	67.9	90.0	No	32.0
	TP	5.2	5.2	10.1	15.+	15.+	Sp.	6.1
	Alb		2.8	2.5	---	---		1.7
	Glb		1.9	7.6	---	---		4.2
	A/G	5.8	1.4	.33	.29	.34		.40
S-33	Ca	10.8	10.4	68.0	91.0***	83.0	83.2	49.0
	TP	4.9	4.9	11.7	15.+	15.+	15.+	7.1
	Alb	2.8	2.4	2.5	---	---	---	---
	Glb	1.8	2.0	9.2	---	---	---	
	A/G	1.5	1.2	.27	.15	.16		.88
S-34	Ca	11.3	10.8	36.2	83.1***	88.0	79.2	31.6
	TP	5.3	6.1	8.9	15.+	15.+	15.+	7.1
	Alb	4.2	3.9	2.6	---	---	---	1.9
	Glb	1.1	2.2	6.3	---	---	---	4.8
	A/G	3.8	1.8	.41	.18		.11	.40

*15.+ represents the protein level when it exceeded 15 mg%.

**No values for albumin and globulin were calculated when the total protein exceeded 15 mg%.

***This represents the day on which the delay group was started.

Table 3: Analysis of variance of serum calcium on each sampling day during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
6	Between group	4	349.66	87.41	.67
	Within group	5	650.94	130.18	
	Total	9	1000.60		
9	Between group	4	859.27	214.81	2.22
	Within group	5	483.65	96.73	
	Total	9	1342.92		
12	Between group	4	909.15	227.48	1.14
	Within group	5	991.15	198.23	
	Total	9	1901.10		
15	Between group	3	299.94	99.98	.87
	Within group	4	454.72	113.68	
	Total	7	754.66		
18	Between group	4	429.18	107.29	.31
	Within group	5	1705.01	341.00	
	Total	9	2134.20		

Table 4: Analysis of variance of the albumin to globulin ratio in each sampling day during the experiment.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
6	Between group	4	2.17	.54	.42
	Within group	5	6.43	1.28	
	Total	9	8.61		
9	Between group	4	2.70	.67	.38
	Within group	5	8.77	1.75	
	Total	9	11.48		
12	Between group	4	4.50	1.12	.57
	Within group	5	9.85		
	Total	9	14.25		
15	Between group	3	.56	.18	.06
	Within group	4	12.33	3.08	
	Total	7	12.90		
18	Between group	4	1.99	.49	.26
	Within group	5	9.32	1.86	
	Total	9	11.32		

APPENDIX C

Raw data for the ducks in the Preseason Experiment 2

Table 1: Pretreatment (Pr) and posttreatment (Pt) mean weights of the birds in preseason experiment 2.

Bird number	Group	Treatment on per day basis	Pr (g)	Pt (g)
S-39	1	12 U.S.P. units ACTH	1229	1186
S-40	1	12 U.S.P. units ACTH	771	1028
S-41	2	confinement	1086	1043
S-42	2	confinement	1114	1000
S-43	3	.25 mg estradiol 12 U.S.P. units ACTH	1057	706
S-44	3	.25 mg estradiol 12 U.S.P. units ACTH	1143	928
S-45	4	.25 mg estradiol 12 U.S.P. units ACTH delay	1371	1480
S-46	4	.25 mg estradiol 12 U.S.P. units ACTH delay	1142	1137
S-47	5	.25 mg estradiol confinement	1057	1057
S-48	5	.25 mg estradiol confinement	1371	1343
S-49	6	.25 mg estradiol confinement delay	1457	1395
S-50	6	.25 mg estradiol confinement delay	1085	1090

Table 2: Values of serum calcium (Ca), total protein (TP), albumin (Alb), globulin (Glb) and the A/G ratio.

Bird number		Treatment period 1	3	6	9	12	15	18	Day
S-39	Ca	10.5	10.6	10.1	10.3	11.1	11.2	11.6	g%
	TP	4.0	4.0	4.8	4.6	5.6	5.6	4.8	g%
	Alb	2.9	2.4	2.5	2.5	2.9	2.5	2.1	g%
	Glb	1.1	1.6	2.3	2.1	2.7	3.1	2.7	g%
	A/G	1.2	2.1	1.4	.53	1.1	.93	1.4	
S-40	Ca	10.7	11.2	12.8	19.5	10.5	10.5		
	TP	5.9	4.8	7.2	4.1	4.1	4.6		
	Alb	3.5	3.7	2.0	1.6	1.5	1.6		
	Glb	2.4	1.1	5.2	2.5	2.6	3.0		
	A/G	1.7	1.3	1.2	1.3	1.1	1.3	2.3	
S-41	Ca	10.7	10.9	16.3	31.8	33.0	30.4	29.0	
	TP	3.5	5.2	4.3	5.8	5.4	6.0	5.1	
	Alb	1.9	3.4	2.5	2.0	2.8	2.9	3.0	
	Glb	1.6	1.8	1.7	3.8	2.6	3.1	2.1	
	A/G	1.2	2.1	1.4	.53	1.1	.93	1.41	
S-42	Ca	10.1	11.7	10.3	12.1	11.2	11.1	15.1	
	TP	4.5	4.9	4.4	4.5	4.8	4.5	5.3	
	Alb	2.8	2.7	2.4	2.5	2.5	2.4	3.7	
	Glb	1.7	2.2	2.0	2.0	2.3	2.1	1.5	
	A/G	1.7	1.3	1.2	1.3	1.1	1.3	2.3	
S-43	Ca	9.6	11.6	11.9	13.6	*	13.1	12.1	
	TP	4.3	4.4	4.6	4.7		5.6	5.6	
	Alb	3.2	2.9	2.7	2.4		1.7	1.8	
	Glb	1.1	1.5	1.9	2.3		3.9	3.8	
	A/G	3.0	1.9	1.5	1.9		.46	.50	
S-44	Ca	8.4	11.9	*	16.6	23.8	16.6	12.0	
	TP	5.4	5.6		6.2	8.8	6.2	5.5	
	Alb	2.9	3.2		3.5	3.3	2.1	1.7	
	Glb	2.5	2.4		2.7	5.5	4.0	3.8	
	A/G	1.2	1.3		1.3	.61	.51	.46	
S-45	Ca	12.2	14.1	19.4**	25.2	14.7	21.8	13.5	
	TP	5.4	5.6	5.9	6.4	7.6	8.8	5.6	
	Alb	3.4	3.3	4.1	2.7	3.4	3.8	2.5	
	Glb	1.6	2.3	1.8	3.7	4.2	5.0	3.1	
	A/G	2.1	1.5	2.2	.72	.81	.80	.80	

Table 2: Continued

Bird number		Treatment period 1	3	6	9	12	15	18 Day
S-46	Ca	9.8	15.4	20.4**	21.3	23.3	*	12.8
	TP	4.3	4.2	4.8	5.7	5.9		4.3
	Alb	2.7	2.3	2.9	2.8	2.5		3.0
	Glb	1.5	1.9	1.9	2.9	3.4		1.3
	A/G	1.7	1.2	1.5	.94	.74		2.3
S-47	Ca	12.5	13.2	29.2	55.0	44.4	50.0	26.6
	TP	5.0	5.1	4.9	10.1	6.4	8.6	5.0
	Alb	2.5	3.2	2.3	4.9	2.9	3.4	2.4
	Glb	2.5	1.9	2.6	5.2	3.5	5.2	2.6
	A/G	1.0	1.5	.91	.68	.84	.73	.83
S-48	Ca	9.6	14.2	17.9	19.6	19.9	15.4	13.1
	TP	4.5	3.9	4.2	5.2	5.3	5.3	5.2
	Alb	2.3	2.3	2.4	2.4	2.8	3.7	2.4
	Glb	2.2	1.6	1.8	2.8	2.5	1.5	2.8
	A/G	1.5	1.7	1.3	.96	1.14	2.3	.95
S-49	Ca	10.6	12.9	22.6**	31.2	26.0	28.4	16.8
	TP	4.6	4.5	5.1	6.1	5.7	6.8	4.3
	Alb	3.7	2.3	3.1	4.3	2.9	3.2	2.6
	Glb	.9	2.2	2.0	1.8	2.8	3.6	2.6
	A/G	4.2	1.1	1.6	2.4	1.0	.87	1.1
S-50	Ca	10.9	15.4	18.7**	27.0	26.8	19.6	14.2
	TP	4.2	4.9	5.6	6.6	6.0	5.1	4.1
	Alb	2.7	2.5	2.3	2.6	2.7	2.1	2.1
	Glb	1.6	2.4	3.2	4.0	3.3	3.0	2.1
	A/G	2.1	1.0	.76	.66	.78	.70	1.1
S-1	Ca	10.4	10.1	16.1	26.0	25.6	25.0	30.4
	TP	4.7	5.0	5.8	5.8	5.7	6.2	6.3
	Alb	3.1	2.8	2.9	2.2	2.6	1.9	2.5
	Glb	1.6	2.2	2.9	3.6	3.1	4.3	3.8
	A/G	1.8	1.3	1.0	.62	.64	.46	.66
S-2	Ca	10.6	10.4	13.1	17.3	20.6	22.2	22.6
	TP	4.0	3.7	4.2	5.1	5.5	7.0	6.1
	Alb	2.5	1.9	1.8	2.2	2.1	2.1	1.6
	Glb	1.5	1.8	1.8	2.9	3.4	4.9	4.5
	A/G	1.6	1.4	.98	.75	.63	.35	.42

*No sample.

**Day on which stressor was applied in the delay group.

Table 3: Analysis of variance of serum calcium on each sampling day during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
3	Between group	5	19.19	3.83	1.20
	Within group	6	19.05	3.17	
	Total	11	38.24		
6	Between group	6	258.15	43.02	3.93*
	Within group	7	76.53	10.93	
	Total	13	334.68		
9	Between group	6	880.01	146.66	1.33
	Within group	7	769.69	109.95	
	Total	13	1649.71		
12	Between group	5	462.43	92.48	1.17
	Within group	6	472.75	78.48	
	Total	11	935.18		
15	Between group	6	584.82	97.47	.87
	Within group	7	780.75	111.53	
	Total	13	1365.57		
18	Between group	6	387.71	64.61	2.45
	Within group	7	184.29	26.32	
	Total	13	572.00		

*$P < .05$

Table 4: Analysis of varinace of the total protein on each sampling during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
3	Between group	6	3.08	.51	1.91
	Within group	7	1.88	.26	
	Total	13	4.97		
6	Between group	6	2.04	.34	2.21
	Within group	7	1.08	.15	
	Total	13	3.21		
9	Between group	6	14.11	2.35	1.04
	Within group	7	15.81	2.25	
	Total	13	29.92		
12	Between group	6	5.04	.84	.42
	Within group	7	13.04	1.96	
	Total	13	18.80		
15	Between group	6	6.33	1.05	.37
	Within group	7	19.80	2.82	
	Total	13	26.13		
18	Between group	6	4.99	.83	2.76
	Within group	7	2.11	.30	
	Total	13	7.10		

Table 5: Analysis of variance of the albumin to globulin ratio on each blood sampling day during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
3	Between group	5	14.64	2.92	4.74*
	Within group	6	3.70	.61	
	Total	11	18.34		
6	Between group	5	5.27	1.05	3.34
	Within group	6	1.89	.31	
	Total	11	7.16		
9	Between group	5	2.21	.44	2.70
	Within group	6	.98	.16	
	Total	11	3.19		
12	Between group	5	6.17	1.23	3.26
	Within group	6	2.27	.37	
	Total	11	8.44		
15	Between group	5	7.72	1.54	1.94
	Within group	6	4.77	.79	
	Total	11	12.49		
18	Between group	5	4.85	.97	1.00
	Within group	6	5.87	.96	
	Total	11	10.63		

*P < .05

Table 6: Analysis of variance of the albumin values on each blood sampling day during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
3	Between group	5	2.76	.55	1.65
	Within group	6	1.99	.33	
	Total	11	4.74		
6	Between group	5	2.08	.42	2.89
	Within group	5	.71	.14	
	Total	10	2.79		
9	Between group	5	2.76	.55	.46
	Within group	6	7.11	1.18	
	Total	11	9.87		
12	Between group	5	.70	.14	.098
	Within group	5	7.65	1.53	
	Total	10	8.34		
15	Between group	5	8.13	2.59	.65
	Within group	5	12.47	1.62	
	Total	10	20.60		
18	Between group	5	6.39	1.28	2.68
	Within group	5	2.38	.48	
	Total	10	8.77		

Table 7: Analysis of variance of the globulin values on each blood sampling day during the treatment period.

Day	Source of variance	Df	Sum of squares	Mean squares	F Ratio
3	Between group	5	3.23	.64	1.89
	Within group	6	2.04	.34	
	Total	11	5.27		
6	Between group	5	5.94	1.19	3.94
	Within group	5	1.50	.30	
	Total	10	7.44		
9	Between group	5	2.57	.51	3.49
	Within group	6	9.04	1.51	
	Total	11	11.61		
12	Between group	5	6.63	1.33	2.37
	Within group	5	2.80	.56	
	Total	10	9.43		
15	Between group	5	5.99	1.99	1.88
	Within group	5	3.17	.63	
	Total	10	9.16		
18	Between group	5	4.41	.88	1.34
	Within group	5	3.27	.65	
	Total	10	7.68		

APPENDIX D

Raw data for the ducks in the Seasonal Experiment 1

Table 1: Pretreatment (Pr) and posttreatment (Pt) mean weights of the bires in seasonal experiment 1.

Bird number	Group	Treatment on per day basis	Pr (g)	Pt (g)
S-20	1	1 U.S.P. unit ACTH	1177	1164
S-11	1	1 U.S.P. unit ACTH	1121	1103
S-21	2	3 U.S.P. units ACTH	899	874
S-5	2	3 U.S.P. units ACTH	1186	1103
S-18	3	5 U.S.P. units ACTH	1134	1283
S-10	3	5 U.S.P. units ACTH	1206	1200
S-17	4	6 U.S.P. units ACTH	1137	1025
S-22	4	6 U.S.P. units ACTH	850	877
S-1	5	cold exposure 3 wks.	1171	1082
S-24	5	cold exposure 3 wks.	1093	1188
S-14	6	cold exposure 2 wks.	1157	1239
S-19	6	cold exposure 2 wks.	1049	1017
S-16	7	no treatment	1045	1146
S-4	7	no treatment	1143	1107

Table 2: Values of serum calcium (Ca), total protein (TP), albumin (Alb), globulin (Glb) and the A/G ratio.

Bird number		Treatment period 1	3	6	9	12	15	18	21	24	27	30	Day
S-20	Ca	11.9	11.7	12.4	13.9	17.8	18.4	17.3	18.8	20.0	25.4	27.0	g%
	TP	4.6	4.0	4.6	5.9	5.0	5.5	5.4	5.3	5.3	5.9	5.9	g%
	Alb	2.9	2.1	3.6	2.6	2.9	2.7	2.8	2.9	2.8	2.9	3.1	g%
	Glb	1.7	1.9	1.0	3.3	2.1	2.8	2.6	2.4	2.5	3.0	2.8	g%
	A/G	1.7	1.1	1.6	.81	1.4	.97	1.1	1.0	1.1	1.0	1.1	
S-11	Ca	13.9	16.3	31.2	25.2	24.2	28.8	26.6	25.0	20.8	25.2	27.0	
	TP	4.8	4.6	4.9	5.5	5.7	6.4	6.3	6.6	6.4	7.2	5.9	
	Alb	3.5	3.4	3.2	3.2	3.2	3.3	4.1	3.6	3.4	3.2	2.3	
	Glb	1.3	1.2	1.7	2.3	2.5	3.1	2.2	2.0	2.0	4.0	3.6	
	A/G	2.8	2.8	2.0	1.4	1.3	1.1	1.8	1.2	1.1	1.0	1.1	
S-21	Ca	10.9	14.2	13.2	14.5	13.2	14.4	13.6	28.0	25.8	26.6	20.9	
	TP	5.1	4.6	5.3	5.4	5.5	6.1	6.3	6.7	6.6	7.0	6.1	
	Alb	3.9	3.0	3.2	2.9	2.6	3.0	2.7	3.1	2.7	3.2	3.4	
	Glb	1.2	1.6	2.1	2.5	2.9	3.1	3.5	3.6	3.9	2.8	2.7	
	A/G	3.6	1.9	1.5	1.1	.90	.18	.76	.88	.70	1.0	1.2	
S-5	Ca	33.4	24.0	24.6	23.0	18.7	23.0	17.9	14.8	16.1	38.6	29.8	
	TP	5.7	4.6	4.2	4.9	4.3	4.2	4.2	3.7	7.1	6.1	6.1	
	Alb	4.2	2.2	2.9	2.0	2.0	1.9	2.4	1.9	3.8	2.5	2.7	
	Glb	1.5	2.4	1.3	2.9	2.3	2.3	1.8	1.8	3.3	3.6	3.4	
	A/G	2.8	.89	2.2	.68	.89	.82	1.3	1.0	1.2	.70	.77	

Table 2: Continued

Bird number		Treatment period 1	3	6	9	12	15	18	21	24	27	30 Day
S-18	Ca	11.3	10.0	12.3	12.5	10.7	12.0	13.5	15.1	16.6	14.7	16.6
	TP	4.0	3.7	3.7	4.2	4.8	4.9	5.0	5.2	5.7	5.3	6.0
	Alb	2.6	2.6	2.3	2.4	1.8	2.5	2.9	2.6	3.1	3.1	2.9
	Glb	1.4	1.1	1.4	1.8	3.0	2.4	2.1	2.6	2.6	2.2	3.1
	A/G	1.8	2.3	1.7	1.4	.65	1.1	1.5	1.0	1.4	.96	1.0
S-10	Ca	14.6	18.0	18.9	30.2	26.8	20.1	18.4	27.0	18.3	19.4	19.3
	TP	4.5	4.6	5.4	5.6	6.0	5.5	5.9	5.6	5.5	5.2	5.4
	Alb	2.8	2.9	3.0	2.7	2.8	2.7	3.0	3.1	3.1	2.6	3.1
	Glb	1.7	1.7	2.4	2.8	3.2	2.8	2.9	2.5	2.4	2.6	2.3
	A/G	1.8	2.3	1.7	1.4	.65	1.1	1.5	1.0	1.4	.96	1.0
S-17	Ca	10.2	9.8	10.7	11.6	11.0	11.9	10.5	*	11.4	12.8	13.0
	TP	4.0	4.5	4.0	4.5	4.6	4.6	4.5		4.8	4.5	4.4
	Alb	2.7	2.8	2.8	2.8	2.0	2.5	2.1		3.6	2.8	2.4
	Glb	1.3	1.7	1.2	1.7	2.6	2.1	2.4		1.2	1.7	2.0
	A/G	2.0	1.7	2.3	1.7	.78	1.2	.91		2.9	1.7	1.2
S-22	Ca	27.6	14.1	17.5	15.5	16.2	16.3	22.0	23.0	26.4	26.8	17.8
	TP	4.7	4.6	4.8	5.6	6.6	6.1	6.4	6.7	6.8	6.5	6.0
	Alb	2.5	3.4	2.9	2.5	3.1	3.3	3.5	3.4	2.7	2.7	2.9
	Glb	2.2	1.2	1.9	3.1	3.5	2.8	2.9	3.3	4.1	3.8	3.1
	A/G	1.2	2.9	1.6	1.3	.87	1.2	1.2	1.0	.87	.73	.94
S-1	Ca	20.0	29.6	24.2	36.0	27.8	24.2	27.2	18.9	38.6	24.4	21.0
	TP	6.8	5.7	5.8	6.4	4.7	5.3	5.0	6.9	8.1	5.9	4.8
	Alb	3.3	2.9	3.4	4.1	2.5	2.1	2.3	3.4	3.4	3.2	3.2
	Glb	3.5	2.9	2.4	3.3	2.2	3.2	2.7	3.5	4.7	2.7	1.6
	A/G	.92	1.0	1.4	.94	.93	.68	.87	.88	.71	1.1	.97

Table 2: Continued

Bird number		Treatment period 1	3	6	9	12	15	18	21	24	27	30 Day
S-24	Ca	16.4	25.2	13.4	16.2	16.4	26.0	35.4	35.6	33.0	23.9	27.8
	TP	4.5	4.2	4.7	4.9	4.9	6.5	5.8	6.9	7.7	4.4	6.2
	Alb	2.9	2.7	2.1	2.8	2.7	4.0	3.0	3.6	3.0	2.6	3.6
	Glb	1.6	1.5	2.6	2.1	2.2	2.5	2.8	3.3	4.7	1.8	2.6
	A/G	1.9	1.9	.84	1.0	1.3	1.6	1.1	1.1	.63	1.5	1.4
S-14	Ca	26.0	16.1	17.8	27.0	32.8**	34.6	30.6	39.0	34.0	33.8	32.0
	TP	6.3	4.6	7.5	7.3	6.7	6.0	6.2	6.4	7.4	6.5	6.9
	Alb	3.4	4.0	2.3	2.6	3.5	2.9	2.1	1.8	3.1	2.5	3.5
	Glb	2.9	3.5	5.2	3.7	3.2	3.1	4.1	4.6	4.2	4.0	3.4
	A/G	1.2	1.2	.46	.55	1.1	.93	.92	.64	.70	.64	.70
S-19	Ca	11.3	12.2	15.2	18.1	25.0**	25.0	35.8	18.6	35.8	*	35.4
	TP	4.3	4.1	4.9	5.8	5.9	5.9	7.2	4.6	7.0		7.1
	Alb	2.5	1.9	2.2	2.1	3.2	2.7	3.2	2.6	2.5		3.0
	Glb	1.8	2.2	2.7	3.7	2.7	3.2	4.0	2.0	4.5		4.1
	A/G	1.4	.87	.81	.98	1.2	.83	1.3	1.4	.73		.74
S-16	Ca	11.5	11.8	16.5	16.8	17.4	58.0	18.4	35.0	42.0	48.0	58.8
	TP	4.1	4.3	4.7	5.3	5.5	6.6	6.9	7.3	8.1	6.9	9.0
	Alb	2.6	2.3	2.7	2.8	2.5	3.5	3.0	2.6	3.1	2.9	3.5
	Glb	1.5	2.0	2.0	2.5	3.0	3.1	3.9	4.7	5.0	4.0	5.5
	A/G	1.7	1.2	1.3	1.1	.85	1.1	.78	.56	.62	.75	.63

Table 2: Continued

Bird number		Treatment period 1	3	6	9	12	15	18	21	24	27	30 Day
S-4	Ca	14.9	21.0	16.7	19.5	30.4	36.8	28.2	28.6	52.4	27.8	36.2
	TP	4.2	4.0	4.7	4.7	5.8	9.0	5.4	4.5	8.0	4.7	15.+
	Alb	2.5	2.4	2.5	2.3	3.1	3.7	2.5	2.5	3.0	2.5	3.4
	Glb	1.7	1.6	2.2	2.4	2.7	5.3	2.9	2.0	5.0	2.2	11.6
	A/G	2.1	1.4	1.2	1.1	1.0	.70	.87	1.1	.61	1.1	.57

*No samples.

**The day on which the treatment was initiated for this group (delay group).

Table 3: Statistical comparison (t tests) between the mean of the means of (A) all values of calcium, total protein and the albumin to globulin ratio, (B) of values on days 1 through 15 and (C) of values on days 18 through 30 for each ACTH-treated group versus the control group.

Group 1 versus the control group

Analysis		$\bar{X}$	n	s /	$\bar{X}$	n	s	t	Df
A	calcium	20.90	2	5.93	26.07	2	16.34	-1.12	3
	total protein	5.53	2	.78	5.62	2	2.03	-.19	3
	A/G	1.83	2	2.11	1.02	2	.39	1.76	3
B	calcium	18.89	2	6.80	22.60	2	13.34	-.85	3
	total protein	5.12	2	.68	5.24	2	1.41	-.25	3
	A/G	2.39	2	2.77	1.23	2	.37	1.43	3
C	calcium	23.31	2	3.69	28.04	2	19.69	-.74	3
	total protein	6.02	2	.61	6.08	2	2.60	-.07	3
	A/G	1.15	2	.24	.78	2	.24	3.51	3

Group 2 versus the control group

Analysis		$\bar{X}$	n	s /	$\bar{X}$	n	s	t	Df
A	calcium	19.95	2	8.64	25.07	2	16.34	-1.30	3
	total protein	5.44	2	1.01	5.62	2	2.03	-.36	3
	A/G	1.26	2	.74	1.02	2	.39	1.36	3
B	calcium	18.97	2	6.65	22.60	2	13.34	-.84	3
	total protein	5.12	2	.63	5.24	2	1.41	-.55	3
	A/G	1.52	2	.91	1.23	2	.37	1.00	3
C	calcium	21.12	2	10.84	28.04	2	19.69	-.97	3
	total protein	5.99	2	1.14	6.08	2	2.60	-.10	3
	A/G	.96	2	.23	.78	2	.24	1.68	3

Group 3 versus the control group

Analysis		$\bar{X}$	n	s /	$\bar{X}$	n	s	t	Df
A	calcium	17.10	2	5.41	25.07	2	16.34	-2.17	3
	total protein	5.07	2	.70	5.62	2	2.03	-1.18	3
	A/G	1.27	2	.39	1.02	2	.39	2.12	3

Table 3: Continued

Group 3 versus the control group

Analysis		$\bar{X}$	n	s /	$\bar{X}$	n	s	t	Df
B	calcium	16.45	2	6.57	22.60	2	13.34	-1.43	3
	total protein	4.74	2	.76	5.24	2	1.41	-1.07	3
	A/G	1.37	2	.48	1.23	2	.37	.79	3
C	calcium	17.89	2	3.77	28.04	2	19.69	-1.60	3
	total protein	5.48	2	.32	6.08	2	2.60	-.72	3
	A/G	1.16	2	.20	.78	2	.24	3.81	3

Group 4 versus the control group

Analysis		$\bar{X}$	n	s /	$\bar{X}$	n	s	t	Df
A	calcium	15.27	2	15.27	25.07	2	16.34	-2.60	3
	total protein	4.96	2	1.45	5.62	2	2.03	-1.23	3
	A/G	1.35	2	.73	1.02	2	.39	1.86	3
B	calcium	14.36	2	4.95	22.60	2	13.34	-2.00	3
	total protein	4.88	2	.80	5.24	2	1.41	-.76	3.
	A/G	1.47	2	.72	1.23	2	.37	1.02	3
C	calcium	16.37	2	8.42	28.04	2	19.69	-1.72	3
	total protein	5.06	2	2.03	6.08	2	2.60	-.97	3
	A/G	1.22	2	.76	.78	2	.24	1.73	3

Table 4: Statistical comparison (t tests) of the pretreatment and posttreatment means of serum calcium (Ca), total protein (TP) and the albumin to globulin ratio (A/G) for each bird.

Bird number		Pretreatment $\bar{X}$	n	s	/	Posttreatment $\bar{X}$	n	s	t	Df
S-20	Ca	12.00	3	.13		19.95	8	17.16	-3.20	10
	TP	4.40	3	.12		5.52	8	.11	27.93	10
	A/G	4.76	3	28.92		1.06	8	.03	6.35	10
S-11	Ca	20.46	3	87.84		23.35	8	5.48	-1.46	10
	TP	4.76	3	.02		6.25	8	.29	43.20	10
	A/G	2.53	3	.21		1.23	8	.06	89.89	10
S-21	Ca	12.76	3	2.86		17.08	8	87.88	-.76	10
	TP	5.00	3	.13		6.21	8	.31	18.48	10
	A/G	2.32	3	1.24		.95	8	.03	31.83	10
S-5	Ca	27.33	3	27.69		22.73	8	64.05	.90	10
	TP	4.83	3	.60		5.07	8	1.46	29.13	10
	A/G	1.94	3	.91		.92	8	.05	79.95	10
S-18	Ca	11.20	3	1.33		13.96	8	4.65	-2.06	10
	TP	3.80	3	.03		5.13	8	.30	18.03	10
	A/G	1.94	3	.10		1.12	8	.08	50.51	10
S-10	Ca	17.16	3	5.14		22.43	8	22.57	-1.80	10
	TP	4.83	3	.24		5.58	8	.06	52.49	10
	A/G	1.56	3	.05		1.06	8	.03	123.81	10
S-22	Ca	19.73	3	49.30		20.50	8	21.63	-.21	10
	TP	4.70	3	.01		6.33	8	.16	54.67	10
	A/G	1.86	3	.77		1.01	8	.03	61.51	10
S-17	Ca	10.23	3	.30		10.37	7	17.94	-.01	9
	TP	4.16	3	.08		3.98	7	2.60	6.44	9
	A/G	1.97	3	.09		1.27	8	.92	15.42	10
S-1	Ca	24.60	3	23.16		27.26	8	47.41	-.60	10
	TP	6.10	3	.37		5.88	8	1.41	25.41	10
	A/G	1.13	3	.07		.88	8	.01	201.40	10
S-24	Ca	18.33	3	37.61		26.78	8	60.05	-1.68	10
	TP	4.46	3	.06		5.91	8	1.27	18.27	10
	A/G	1.53	3	.36		1.19	8	.09	65.07	10
S-14	Ca	19.96	3	28.02		32.97	8	11.85	-4.88	10
	TP	6.13	3	2.12		5.76	8	5.60	9.54	10
	A/G	4.30	3	36.32		.77	8	.03	9.96	10

Table 4: Continued

Bird number		$\bar{X}$	n	s	/ $\bar{X}$	n	s	t	Df
S-19	Ca	12.90	3	4.17	24.34	7	150.07	-1.55	9
	TP	4.43	3	.17	5.43	7	5.49	5.26	9
	A/G	1.02	3	.10	2.13	7	13.72	4.86	9
S-16	Ca	13.26	3	7.86	27.42	8	507.88	-1.05	10
	TP	4.36	3	.09	6.95	8	1.51	8.52	10
	A/G	1.38	3	.08	.80	8	.04	76.66	10
S-4	Ca	17.53	3	9.82	29.98	8	170.69	-1.58	10
	TP	4.30	3	.13	5.26	8	7.23	7.61	10
	A/G	1.56	3	.23	.90	8	.06	75.84	10

APPENDIX E

Raw data for the ducks in the Seasonal Experiment 2

Table 1: Time and duration of the effect of a single injection of 12 U.S.P. units of ACTH on the serum levels of calcium (Ca), total protein (TP) and the A/G ratio of seasonal experiment 1.

Bird number		Hours 0	1/2	11/2	3	6	8	12	24
S-64	Ca (mg%)	22.6	19.7	20.8	---	26.6	---	---	21.6
	TP (g%)	5.7	5.1	5.1	---	6.2	---	---	5.9
	A/G	1.20	1.36	1.40	---	1.35	---	---	1.33
S-66	Ca (mg%)	42.4	29.6	41.0	---	38.4	---	---	30.2
	TP (g%)	5.7	5.7	5.6	---	6.1	---	---	4.6
	A/G	1.20	1.36	1.40	---	1.35	---	---	1.33
S-74	Ca (mg%)	30.8	---	---	23.9	---	27.2	16.5	---
	TP (g%)	6.9	---	---	7.7	---	6.2	6.3	---
	A/G	.46	---	---	1.00	---	.53	.62	---
S-78	Ca (mg%)	24.0	---	---	37.2	---	28.6	28.6	---
	TP (g%)	7.6	---	---	7.3	---	8.6	8.8	---
	A/G	.73	---	---	.53	---	.94	1.09	---

No blood samples were taken for S-64 and S-66 on hours 3, 8 and 12.

No blood samples were taken for S-74 and S-78 on hours 1/2, 11/2 and 6.